Shilajit, a Natural Phytocomplex Acts as a Neuroprotective Agent Against Amyloid Beta-induced Cytotoxicity and Inflammation

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Background: Shilajit is a natural phytocomplex known for centuries in Ayurveda traditional medicine for its antioxidant, immunomodulatory, and neuroprotective properties. However, there is little published scientific evidence to support these acclaimed properties.

Methods: The safety, regarding the heavy metal content, component analysis, the neuroprotective effects and amyloid beta (Aβ)–induced cytotoxicity and inflammation of 3 samples of Shilajit derived from different geographical origins were assessed. Neuroprotective effects of Shilajit were examined using neuroblastoma cell lines (SH-SY5Y and IMR-152, Suji-gu, Yongin, 16890, Republic of Korea) examining the biological activity of Shilajit, sourced from Altai, Himalayas at an altitude of 1,000–5,000 m above sea level [1]. It is characterized as a product that has been mixed with rock minerals via progressive accumulation and decomposition of substances (such as plants, fungi, and lichens) over centuries [2]. Comprising 60%–80% organic matter, 20%–40% minerals, and 5% trace elements, Shilajit predominantly contains humin, humic acid, and fulvic acid. Traditionally, Shilajit has been utilized across Asia to treat a variety of diseases [3,4]. To date, it is administered as a dietary supplement for health benefits. Preclinical studies have demonstrated a range of various biological activities associated with Shilajit, including anti-inflammatory, antioxidant, immunomodulatory, and neuroprotective effects [5]. In addition, the extract, and the physiologically active compounds from Shilajit have shown promise for therapeutic applications in various neurodegenerative diseases [6,7]. Preclinical assays examining the biological activity of Shilajit, sourced
from various geographical regions, are lacking in the context of Alzheimer’s disease (AD), the most common form of age-related dementia [8]. Due to the variations of natural products, the chemical composition of Shilajit is fundamentally influenced by certain properties such as the type of plant species involved, geological properties of the rock, ambient temperature, and altitude and humidity of a particular area [9]. Hence, the efficacy of Shilajit will vary across regions, depending on the location or its extraction method [10]. Furthermore, concerns about the safety of ingesting Shilajit has been raised due to heavy metal content, molds, and other allergens. It is necessary to compare the differences in composition, safety, and physiological activity according to the region of the collection of Shilajit.

The present study aimed to investigate the safety and efficacy of Shilajit in conferring neuroprotection in a preclinical model based on amyloid beta (Aβ)-induced cytotoxicity and inflammation, using Shilajit from different geographic origins. Ultimately, we aimed to identify candidate components in Shilajit that demonstrate potential neuroprotective effects in AD.

Materials and Methods

1. Preparation of Shilajit

1.1. Preparation of 3 types of Shilajit sourced from different regions

In the analysis, samples of Shilajit derived from 3 different geographic origins were used. Shilajit A, B, and C samples were obtained from the Altai Mountains in Russia, and the Gilgit Mountains in Pakistan, and the Pamir mountains in Tajikistan in the Himalayas, respectively. Shilajit is locally traded as a supplementary food in the form of resin, which is dissolved in purified water, and used without any further purification. To prepare the aqueous extract, all Shilajit materials were diluted with warm water. The stock solution was diluted to 10 mg/mL with water before the experiment. The stock solution (10 mg/mL) was further diluted in culture medium to obtain the selected concentrations. Before treatment, the samples were incubated for 15 minutes at 37°C in a water bath. According to the manufacturer’s instructions, a pea-sized amount of Shilajit can be dissolved in liquid and administered up to 3 times daily. The recommended dose for Shilajit is 300-500 mg per day.

2. Component analysis of Shilajit

2.1. High-performance liquid chromatography analysis of Shilajit

Shilajit samples were dissolved in distilled water (10 mg/mL) and filtered using a 0.45-μm syringe filter. Subsequently, 10 μL aliquots were subjected to high-performance liquid chromatography (HPLC). The aliquots were separated using a Capcell Pak C18 MGII column (4.6 × 250 mm, 5 μm; Agilent Technologies). The mobile Phases A and B comprised water and acetonitrile, respectively, along with 0.05% (v/v) trifluoroacetic acid. The solvent gradient conditions for Phase B were as follows: 0%-100% at 0-60 minutes, and 100% at 60-70 minutes. The UV absorption of each compound was detected at 210 nm.

2.2. Purification and identification of compounds in Shilajit

Preparative HPLC was performed on a Thermo Ultimate 300 (HPG-3200BX pump and DAD-3000 diode array detector) platform equipped with a Capcell Pak C18 MGII column (30 × 250 mm, 5 μm; Agilent Technologies) using a mixed solvent system of MeCN-H2O at a flow rate of 10 mL/minute. The 1D and 2D (H-1 COSY, HSQC, and HMBC) nuclear magnetic resonance (NMR) spectroscopy spectra of the isolated compounds were analyzed using a Bruker Ascend III 700 spectrometer (Rheinstetten, Germany). Liquid chromatography (LC) / mass spectrometry [MS; (LC/MS)] analyses were performed using an LTQ Orbitrap XL (Thermo Electron Co., USA) coupled with an Accela ultra-high-pressure liquid chromatography system (Thermo Electron Co., USA). Chromatographic separation of the compounds was conducted using an ACQUITY UPLC® BEH C18 column (2.1 × 150 mm, 1.7 μm) operated at 40°C using mobile Phases A (water with 0.1% formic acid) and B (acetonitrile with 0.1% formic acid). The solvent gradient conditions were as follows: 5% B for 0-1 minute, 5%-70% B for 1-20 minutes, 70%-100% B for 20-24 minutes, and 100% B for 24-27 minutes. Each compound was detected using a photodiode array at a wavelength ranging from 200 nm to 500 nm. The MS analysis was performed with polarity switching, and the MS/MS scan was performed with the following parameters: m/z range (150-1,500); collision-induced dissociation energy of 45%; and data-dependent scan mode. High-resolution mass spectra were acquired using the LTQ Orbitrap XL and analyzed using XCALIBUR software (Thermo Fisher Scientific, Waltham, MA, USA).

2.3. Heavy metal analysis

The concentrations of arsenic (As), mercury (Hg), lead (Pb), and cadmium (Cd) in the Shilajit samples were determined using inductively coupled plasma-MS (ICP-MS). The samples were placed in a polytetrafluoroethylene digestion vessel, and 65% nitric acid and hydrogen peroxide were added. The container was then incubated at 15-25 °C for 2 hours and placed in a microwave oven. The digestion was performed according to the manufacturer’s instructions. Thereafter, the digested solution was dried by heating at 100 °C to remove nitric acid, redissolved in 20 mL of 5% nitric acid (v/v, in water), and filtered using hydrophilic polyvinylidene
fluoride filters (Millipore Millex-HV, Billerica, Massachusetts, USA). Pb and Cd were analyzed using the inductively coupled plasma-MS instrument (Elan DRC II, PerkinElmer Inc., Waltham, MA, USA).

3. Examination of neuroprotective effects of Shilajit

The neuroprotective effects of Shilajit were evaluated in undifferentiated human neuroblastoma cell lines (SH-SY5Y and IMR-32). The SH-SY5Y and IMR-32 cell lines have been widely used in the study of neurodegenerative disorders because they exhibit the characteristics of various types of functional neurons.

3.1. Cell lines

SH-SY5Y and IMR-32 were obtained from the Korean Cell Line Bank (Seoul, Republic of Korea). The cells were maintained in RPMI medium supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum at 37 °C in an incubator containing 5% CO2. The cells were subcultured and removed with 0.05% trypsin-ethylenediaminetetraacetic acid and phosphate-buffered saline.

3.2. Preparation of Aβ oligomers and fibrils

Aβ oligomers were prepared as previously described [11]. Briefly, synthetic human Aβ1-42 peptides were dissolved in 0.1% ammonia solution and further diluted in 0.1× phosphate-buffered saline to form a 45 μM stock solution (monomers, pH = 7.4). The monomers were incubated at temperatures ranging from 15-25 °C for 14 hours to obtain Aβ oligomers, which were then used for in vitro experiments.

3.3. Cell viability assay

The 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2(4-sulfophenyl)-2H-tetrazolium (MTS) assay was performed to evaluate the cytotoxicity of the Shilajit samples by treating the cells with Shilajit A, B, and C at concentrations ranging from 50 to 1,000 μM. Cell viability was determined using the MTS assay (Promega, Madison, WI, USA) according to the manufacturer’s protocol as previously described [12].

4. Aβ-induced inflammation in vitro

4.1. Macrophage differentiation

Human primary bone marrow mononuclear cells were purchased from American Type Culture Collection (Manassas, Virginia, USA, catalog no. PCS-800-013) and differentiated into macrophages on a 12-well plate as previously described [13].

4.2. Measurement of Aβ-induced formation of the mature form of interleukin (IL)-1β in macrophages

The differentiated cells were primed with lipopolysaccharide (LPS, 0.5 μg/mL) in Roswell Park Memorial Institute (RPMI) 1640 containing 10% fetal bovine serum. After 2 hours of 5% CO2 incubation, the cells were washed with serum-free RPMI 1640 and were either left untreated or pretreated for 1 hour with the indicated concentrations of Shilajit. Aβ oligomers were then added to the culture at a final concentration of 9 μM, followed by 5% CO2 incubation for 4 hours. The conditioned media were concentrated approximately 5× using an Amicon centrifugal filter device (Millipore, Danvers, MA, USA; 3 kDa MWCO) and then used for western blot analysis, as previously described [13].

5. Animal care and experimental model for Aβ-induced inflammation in vivo

5.1. Animal care

Seven-week-old male ICR mice were purchased from Daehan Biolink (Republic of Korea) and maintained under pathogen-free conditions for 1 week. All animal procedures were approved by the Animal Care and Use Committee at Dankook University and performed in accordance with the guidelines issued by the Korean Ministry of Food and Drug Safety, and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

5.2. Experimental model for Aβ-induced inflammation in vivo

Aβs were administered via intracerebroventricular (i.c.v.) injection (200 pmole per 5 μL), as previously described [14]. Sham control mice were administered phosphate-buffered saline by i.c.v. injection. Separate groups of mice (n = 7) were orally administered vehicle or Shilajit at 0.5 mg/ml and 16 hours after the i.c.v. injection of Aβ. A selective nucleotide oligomerization domain (NOD)-like receptor protein-3 inflammasome inhibitor MCC950 (10 mg/kg, intraperitoneal injection) was used as a reference compound to block the release of IL1β induced by NOD-like receptor protein-3 activators.

5.3. Enzyme-linked immunosorbent assay

At 24 hours after Aβ injection, the whole brain was homogenized in ProtinEX tissue lysis solution (GeneAll, Republic of Korea) and centrifuged at 10,000× g for 15 minutes. The supernatants were maintained at -80 °C until further analysis of IL1β and tumor necrosis factor-alpha (TNF-α) levels. Enzyme-linked immunosorbent assay was
performed using Invitrogen IL-1β and TNF-α mouse enzyme-linked immunosorbent assay kits.

6. Statistical analysis

Statistical significance was determined via one-way analysis of variance, followed by Tukey’s post hoc test with the aid of GraphPad Prism 5.0. A p value of less than 0.05 was considered statistically significant.

Results

1. Components of Shilajit

1.1. HPLC analysis of Shilajit

The HPLC analysis showed that Shilajit samples derived from the 3 different geographic origins showed similar chromatographic profiles. However, the peak areas for each compound amongst the 3 samples in the HPLC analysis appeared to be different (Supplementary Figure 1 and Supplementary Table 1). To identify the chemical constituents of Shilajit, the major ingredients were purified and NMR spectroscopy was used to determine the structures of the isolated compounds. Based on 1D and 2D (1H-1H COSY, HSQC, and HMBC) NMR spectroscopy spectra, together with high-resolution MS data (MS and MS/MS), 3 compounds with m/z values of 194.0833, 180.0662, and 121.0296 were tentatively identified. In comparison with previously published data [15-20], their identities were: (1) salicyluric acid; (2), hippuric acid; and (3) benzoic acid (Supplementary Figure 1D).

1.2. Heavy metals in Shilajit

Shilajit A, B, and C samples contained heavy metals to a certain degree (Table 1). Considering these results, it was hypothesized that Shilajit may exhibit different compositions and biological efficacies depending on the harvest region. In this study, Shilajit showed low levels of heavy metals per 500 mg (Table 1). In addition, all Shilajit samples met the established US Food and Drug Administration limits for adult exposure (130, 20, 75, and 55 μg/d for As, Hg, Pb, and Cd, respectively).

2. Evaluation of the neuroprotective effect of Shilajit

2.1. Evaluation of Shilajit cytotoxicity in neuronal cells

When SH-SY5Y cells were exposed to Shilajit sample A (1,000 μg/mL), weak cytotoxic effects were detected (control: 100% cell viability, 1,000 μg/mL Shilajit A: 81% cell viability; Figure 1A). However, no significant difference in cell viability was detected at lower concentrations of 50 and 100 μg/mL. When IMR-32 cells were exposed to Shilajit sample A there was no significant difference in cell viability after 24 hours. Shilajit sample B did not show a significant difference in cell viability at 24 hours. Shilajit sample B did not show a significant difference in cell viability at 24 hours. Shilajit sample B did not show a significant difference in cell viability at 24 hours. Shilajit sample B did not show a significant difference in cell viability at 24 hours. Shilajit sample B did not show a significant difference in cell viability at 24 hours. Shilajit sample B did not show a significant difference in cell viability at 24 hours. Shilajit sample B did not show a significant difference in cell viability at 24 hours. Shilajit sample B did not show a significant difference in cell viability at 24 hours. Shilajit sample B did not show a significant difference in cell viability at 24 hours. Shilajit sample B did not show a significant difference in cell viability at 24 hours. Shilajit sample B did not show a significant difference in cell viability at 24 hours. Shilajit sample B did not show a significant difference in cell viability at 24 hours.
cell viability after 24 hours at concentrations of 50, 100, 250, 500, and 1,000 μg/mL in SH-SY5Y and IMR-32 cells. Moreover, while Shilajit sample C did not exhibit specific cytotoxicity, a slightly higher level in cell viability was observed at a concentration of 1,000 μg/mL (Figure 1C). No instances of neurotoxicity attributable to each Shilajit sample were observed. Taken together, no cytotoxicity was observed with Shilajit samples B and C, but mild cytotoxicity was observed with Shilajit sample A at 250, 500, and 1,000 μg/mL (Figure 1).

2.2. Neuroprotective activity of Shilajit against Aβ-induced cytotoxicity

To test the applicability of Shilajit to the treatment of AD, neuroprotective activity was evaluated using an Aβ1-42-induced cytotoxicity model (a representative and reliable in vitro model for AD-related studies) in SH-SY5Y and IMR-32 cells. The cytotoxicity of the most commonly used peptide, Aβ1-42, was tested in SH-SY5Y and IMR-32 cells, using the MTS assay. Exposure of the cells to different concentrations of Aβ1-42 for 24 hours resulted in a remarkable decrease in cell viability (Figures 2A and 2C). Compared with the control group with no Aβ1-42, cell viability in the 3 μM Aβ1-42 group was less than 50%. To evaluate the protective effects of Shilajit, the cells exposed to Shilajit A, B, and C samples and were cotreated with 3 μM Aβ1-42. The results showed that when the cells were cotreated with Shilajit A, B, or C and Aβ, Aβ-induced cell death in SH-SY5Y and IMR-32 cells was substantially reduced (Figures 2B and 2D). In addition, Shilajit A showed protective effects against Aβ-induced cell death at a concentration of 100 μg/mL after treatment for 48, 72, and 96 hours (Supplementary Figures 2A-2C).

3. Evaluation of Shilajit in in vitro and in vivo inflammation models

3.1. Shilajit inhibited Aβ-activated inflammasome in macrophages

The inhibitory effect of Shilajit on Aβ-induced inflammasome activation was investigated in vitro. The LPS-primed macrophages were preincubated with Shilajit for 1 hours before exposure to Aβ4 for 4 h. The protein levels of secreted IL-1β in the culture media were analyzed using western blotting. Shilajit samples B and C had little effect on the levels of secreted IL-1β (Figure 3A). Furthermore, the protein levels of the secreted IL-1β were lower in Shilajit A-treated cells than those in Aβ-treated cells. However, the protein levels of IL-1β in the cellular extracts remained unaffected by Shilajit. These results suggested that Shilajit inhibited Aβ-induced inflammasome activation in macrophages. We also investigated whether the compounds from Shilajit could inhibit Aβ-induced inflammasome activation in vitro. However, none of the isolated compounds exerted inhibitory effects on Aβ-induced IL-1β processing in macrophages (data not shown).

3.2. Shilajit attenuated Aβ-induced IL-1β production in a mouse model of neuroinflammation

The observed inhibition of Aβ-induced production of mature IL-1β by Shilajit A in vitro prompted further investigation into whether Shilajit could lead to the desired biological outcome in an animal model. The results showed that i.c.v. injection of Aβ substantially increased IL-1β levels in the brain (Figure 3B), which is consistent with a previous study [14]. In this established animal model, Shilajit sample A was orally administered at 0.5 mg/ml and 16 hours after i.c.v. injection of Aβ. MCC950 was used as the reference compound. Analysis of brain lysates 24 hours after the Aβ injection showed that the Aβ-induced increase in IL-1β level was markedly suppressed by MCC950 and Shilajit A (sham control), Aβ injection (vehicle), MCC950, Shilajit A 75 mg/kg, and Shilajit A 150 mg/kg at [(78.26 ± 3.23), (100.84 ± 46.33), (73.32 ± 9.29), (60.23 ± 11.79), and (58.55 ± 9.86)] pg per 100 mg of brain weight, respectively. Although i.c.v. injection of Aβ led to a slight increase in TNF-α levels in the brain, Shilajit A exhibited a minor effect on Aβ-induced TNF-α expression (Figure 3C).

Table 1. Heavy Metal Concentrations in 3 Shilajit Samples Derived from Different Geographical Origins

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>Shilajit A (mg/kg)</th>
<th>Shilajit B (mg/kg)</th>
<th>Shilajit C (mg/kg)</th>
<th>US FDA Daily limit (mg/kg)</th>
<th>Shilajit A (per 500 mg)</th>
<th>Shilajit B (per 500 mg)</th>
<th>Shilajit C (per 500 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>0.88</td>
<td>0.23</td>
<td>2.42</td>
<td>130</td>
<td>0.44</td>
<td>0.12</td>
<td>1.21</td>
</tr>
<tr>
<td>Hg</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>20</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Pb</td>
<td>1.52</td>
<td>0.18</td>
<td>1.89</td>
<td>75</td>
<td>0.815</td>
<td>0.09</td>
<td>0.943</td>
</tr>
<tr>
<td>Cd</td>
<td>0.22</td>
<td>0.02</td>
<td>0.31</td>
<td>55</td>
<td>0.11</td>
<td>0.01</td>
<td>0.15</td>
</tr>
</tbody>
</table>

The concentrations are calculated based on the daily intake amount of Shilajit (500 mg). FDA = Food and Drug Administration; ND = not detectable.
Figure 2. Neuroprotective effects of Shilajit against Aβ-induced cytotoxicity in SH-SYSY and IMR-32 cell lines. SH-SYSY (A) and IMR-32 cells (B) were treated with various concentrations of 3, 10, and 30 μM of Aβ1-42, and cell viability was measured using the MTS assay. SH-SYSY cells (C) and IMR-32 cells (D) were pretreated for 1 h with individual concentrations of Shilajit and then treated with 3 μM Aβ1-42 for 24 h. The protective effects of Shilajit on Aβ-induced cell death were measured using the MTS assay.

* p < 0.05.
** p < 0.01.
*** p < 0.001 [(A and B) compared with the control; (C and D) compared with the Aβ-treated group].

Data are shown as the mean ± SD.

Figure 3. Effects of Shilajit in in vitro and in vivo inflammation models. Bone marrow-derived mononuclear cells were differentiated into macrophages. The cells were primed with LPS for 2 hours and then pretreated with distilled water or Shilajit samples for 1 hour before exposure to Aβ (A). The conditioned media and total lysates were used for western blot analyses with antibodies against IL-1β cleaved form and IL-1β preform, respectively. (B and C) Shilajit was orally administered 0.5 and 16 hours after i.c.v. injection of Aβ, and total brain lysates were analyzed for IL-1β and TNF-α levels at 24 hours after Aβ i.c.v. injection. MCC950 was used as a reference compound.

*** p < 0.001 vs. sham group.
### p < 0.001 vs. Aβ + vehicle-administered group.

Data are expressed as the mean ± SD.
Discussion

Traditionally, Shilajit has been prescribed in Ayurvedic medicine as an antiaging memory-enhancing medication [24]. However, given its origin involving the accumulation and decomposition of rock minerals by weathering and various organisms, assessing the risk of heavy metal contamination in Shilajit is important [1]. In practice, some Shilajit supplements have been reported to contain high levels of heavy metals such as Pb [22]. However, a considerable variation in the components of Shilajit exist according to its geographical origin [9]. Interestingly, Shilajit is also reported to help eliminate free radicals and heavy metals such as Hg and Pb from the human body [23]. To address safety concerns, the levels of heavy metals, in daily dose samples of Shilajit A, B, and C, were tested and confirmed to be within the permissible limits established by the US Food and Drug Administration.

Shilajit may be beneficial in neurodegenerative diseases, and potentially of great value in AD where it is thought to protect neurons and inhibit proinflammatory signals in neurons and macrophages. Its efficacy is dependent on its origin. Senile plaques of Aβ protein and tau proteins are well-known substances involved in the pathological development of AD and are toxic to neurons [7]. The histological characteristics of AD include the presence of Aβ plaques and tau neurofibrillary tangles. Therefore, the accumulation of Aβ may be a key event in the pathogenesis of AD, which, in turn, may lead to neurodegeneration through a cascade of interactions between oxidative stress, inflammation, and apoptosis [25]. In particular, a positive correlation between neuroinflammatory cytokine release and AD progression has been reported, suggesting that these cytokines are involved in the pathology of AD [26]. The Aβ1-42 oligomers activate the NOD-like receptor protein 3 (NLRP3) inflammasome, resulting in the processing of pro-IL-1β into mature IL-1β in macrophages [21]. The present study showed that Shilajit A, B, and C samples exerted protective effects against Aβ-induced cytotoxicity in vitro, in addition to low cytotoxicity in SH-SYSY and IMR-32 cells. Only Shilajit A at 100 μg/mL protected against Aβ-induced cell death for up to 96 hours.

The anti-inflammatory effects of Shilajit were evaluated in an animal model of Aβ-induced acute inflammation and it was observed that 24 hour-treatment with Shilajit A reduced Aβ-induced increase in IL-1β levels in the brain compared with sham control. In the pathogenesis of AD, sustained activation of microglia (brain-resident macrophages) and other immune cells was reported to exacerbate Aβ and tau pathologies [27]. Furthermore, Aβ triggers an inflammatory cascade in the surrounding macrophages and exhibits indirect toxicity in nerve cells by releasing various proinflammatory cytokines [26]. IL-1β, a key mediator of the inflammatory response, exacerbates damage during chronic disease, and acute tissue injury [29]. Therefore, the findings of this current study provide preliminary evidence for the neuroprotective effects of Shilajit in the treatment of AD. In addition, as the 3 compounds identified from Shilajit had little effect on Aβ-induced processing of pro-IL-1β into mature IL-1β. The active compounds in the Shilajit sample were derived from the Altai Mountains (Shilajit A).

HPLC analyses of the components of the 3 Shilajit samples showed that the peak area of each compound differed depending on the origin of the Shilajit sample, despite their similar chromatographic profiles (Supplementary Figure 1). In particular, the amount of salicyluric acid (the principal metabolite of aspirin) was the highest in Shilajit A derived from the Altai Mountains compared with other samples. Only Shilajit sample A suppressed Aβ-induced processing of pro-IL-1β into mature IL-1β in macrophages. Therefore, it is conceivable that the efficacy of Shilajit in the treatment of AD depends on the concentration of salicyluric acid. The neuroprotective effects of salicyluric acid in AD may be mediated through various pathways and cellular mechanisms. Anti-inflammatory properties extend beyond reducing IL-1β levels, potentially impacting broader inflammatory signaling pathways implicated in neuroinflammation. Furthermore, antioxidant activity may involve scavenging reactive oxygen species, enhancing endogenous antioxidant defenses, and modulating redox-sensitive pathways to counteract oxidative stress. Furthermore, it may promote neuronal survival and function by influencing neurotrophic factors and synaptic plasticity mechanisms.

Additionally, investigating whether previously reported Shilajit samples exhibit similar chromatographic profiles in HPLC analyses [24,30,31] and neuroprotective effects as those observed in this current study would be of significant interest. Ensuring the consistency and quality of Shilajit preparations is crucial for translating these findings into clinical practice. Establishing standardized protocols for Shilajit extraction, characterization, and quality control will be essential to guaranteeing the reproducibility and efficacy of therapeutic interventions. Given the complex nature of AD pathology, exploring the potential synergies between Shilajit and other natural compounds or pharmacological agents holds promise. Combination therapies targeting multiple pathological mechanisms, such as Aβ aggregation, neuro-inflammation, and oxidative stress, may yield enhanced therapeutic efficacy and disease-modifying effects. Investigating the molecular targets and signaling pathways modulated by Shilajit in AD pathogenesis could uncover novel therapeutic targets and facilitate the development of more targeted interventions.

The main strength of this study lies in its comparative analysis of the 3 Shilajit samples derived from different regions, in contrast with previous studies that focus on
a single Shilajit sample. As a candidate component, we suggest that Shilajit may alleviate the pathology of AD via neuroprotective activity against Aβ-induced toxicity and anti-inflammatory activity by reducing IL-1β levels. Our study had several limitations. Firstly, the differences in Shilajit A, B, and C samples were inferred only based on their geographical origins. We could not estimate the extent to which the extraction process affected the components of each Shilajit. Secondly, the component analysis was performed using HPLC. Therefore, additional MS analyses are necessary to identify the specific metabolites in Shilajit. Further functional analysis of the active compounds and safety should be conducted. Shilajit predominantly contains humic substances including humin, humic acid, and fulvic acid. Fulvic acid, the main component of Shilajit, was reported to prevent the accumulation of tau protein in neurodegenerative diseases [32]. The assembly of the four-repeat microtubule-binding domain, a tau fragment, is inhibited by fulvic acid. The effect of fulvic acid on tau fragments has been investigated using a pyrolytic carbon called highly ordered pyrolytic graphite [32]. Therefore, additional in vivo experiments are necessary to verify this effect. Finally, detailed mechanistic studies are required to elucidate the effects of Shilajit on AD by linking its neuroprotective activity against Aβ-induced cytotoxicity and anti-inflammatory effects.

Conclusion

Shilajit samples derived from 3 different geographic origins have been evaluated and the protective effects of Shilajit against Aβ-induced cell death in human neuroblastoma SH-SY5Y and IMR-32 cell lines have been observed. Shilajit derived from the Altai Mountains contained safe levels of heavy metals and were shown to inhibit the production of Aβ-induced proinflammatory cytokine (IL-1β) in macrophages and in an animal model of acute inflammation. Mechanistic studies focusing on elucidating the molecular pathways and cellular mechanisms underlying Shilajit’s neuroprotective effects are essential for a deeper understanding of its therapeutic potential. Additionally, exploring the effects of Shilajit derivatives or novel formulations with enhanced bioavailability and therapeutic efficacy could lead to the development of potent therapeutic agents for AD.

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Author Contributions

SK and CS performed the experiments and drafted the manuscript. HJM and YC conceptualized and designed the project. SL administered the project through funding acquisition and edited the final manuscript. All authors contributed to the relevant sections and approved the final manuscript.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Supplementary Materials

Supplementary materials are available at: https://doi.org/10.56986/pim.2024.06.007

References


