Ameliorative Potential of Sitagliptin and/or Calcipotriol on Lipopolysaccharide-Induced Alzheimer's Disease

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Abstract

Background: Alzheimer's disease (AD) is a progressive neuropsychiatric disorder that causes dementia. It mostly affects people older than 65 years. The exact mechanisms of AD are fully understood but affection of apoptosis, oxidative stress and neuroinflammation are contributing factors. Aim: To evaluate the ability of sitagliptin and/or calcipotriol to attenuate lipopolysaccharide (LPS)-induced AD in mice and to elucidate their possible mechanisms of action. Methods: Sixty male Balb/c mice were divided into 6 equal groups: Control; LPS; + carboxymethyl cellulose; LPS + Sitagliptin; LPS + Calcipotriol; and LPS + Sitagliptin + Calcipotriol group. Behavioral tests, tissue catalase (CAT), superoxide dismutase (SOD) and thiobarbituric acid derivatives (TBARS) were assessed. Also, tissue transforming growth factor beta-1 (TGF-β1), tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) were determined. Parts of the hippocampus were subjected to histopathological, immunohistochemical and electron microscopic examination. Results: Administration of sitagliptin and/or calcipotriol prior to LPS injection induced significant increase in recognition index, tissue CAT and SOD associated with significant decrease in tissue TBARS and TNF-α, IL-6 and TGF-β1 and significant improvement of the histopathological immunohistochemical and electron microscopic picture compared to LPS group. These changes were significant in sitagliptin/calcipotriol combination group compared to the use of each of these drugs alone. Conclusion: Sitagliptin/calcipotriol combination might represent a therapeutic modality for amelioration of Alzheimer’s disease.

Keywords
• Sitagliptin
• Calcipotriol
• Lipopolysaccharide
• Alzheimer's disease
• Mice

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INTRODUCTION

Alzheimer’s disease (AD) is a progressive neuropsychiatric disorder characterized by formation of senile plaques following neurofibrillary tangles which cause neuronal degeneration and synaptic loss. These senile plaques are formed in brain regions such as the entorhinal cortex, basal forebrain, hippocampus, and amygdale which can impair learning and memory functions [1]. The brains of AD also exhibit a number of pathological abnormalities, including significant loss of synapses, reactive gliosis and flaring up of the inflammatory process [2].

Recent studies have indicated that neuroinflammation contributes to the pathogenesis of AD. It was suggested that neuroinflammation plays a crucial role in deposition of cerebral amyloid [3]. It has been shown that inflammatory cytokines such as interleukin (IL)-1β, IL-6, tumor necrosis factor-α (TNF-α) or transforming growth factor-β (TGF-β) can augment APP expression and Aβ formation [4]. Moreover, it was reported that cytokines are able to upregulate the transcription of β-secretase mRNA, protein and enzymatic activity. β-secretase is a key rate-limiting enzyme that initiates Aβ formation [5]. Also, the inflammatory mediators frequently encountered in AD were suggested to stimulate the underlying key events of the inflammatory cascade leading to increased Aβ production with activation of the microglial cells [6].

It was reported that intraperitoneal injection of lipopolysaccharide (LPS) induces cognitive impairment in mice [7]. However, the mechanisms underlying LPS induced cognitive impairment are not yet fully understood. To investigate the impact of systemic inflammation on memory impairment and its role in cortical amyloid formation and deposition, LPS was an ideal agent to induce systemic inflammation and cognitive impairment and to investigate the effect of different drugs on AD [8].

Sitagliptin is one of dipeptidyl peptidase-4 (DPP-4) inhibitors that are used for treatment of type II diabetes mellitus. They act by increasing certain hormones that stimulate the pancreas to produce more insulin and inhibit hepatic gluconeogenesis [9]. Sitagliptin was reported to possess pleiotropic antioxidant and anti-inflammatory properties which may have beneficial effects on various body tissues [10]. Moreover, sitagliptin was proven to have potent antifibrotic effects, possibly through its action on TGF-β1 which is the primary cytokine responsible for induction of the inflammatory cascade in different body organs [11]. Taken together, these properties may make sitagliptin to be a promising agent for amelioration of LPS-induced AD.

Calcipotriol is a synthetic derivative of calcitriol (Vitamin D) which is widely used for treatment of psoriasis and alopecia areata [12]. Vitamin D analogues such as calcipotriol were proven to have potent antioxidant and anti-inflammatory properties that may provide protection to various body tissues against oxidative stress and inflammation [13]. Also, calcipotriol was reported to protect from inflammation through antagonizing TGF-β1 signaling via vitamin D receptor/Smad3 genomic crosstalk. This blocks Smad residency on chromatin and inhibits acetylation of histone H3.
leading to suppression of gene expression of the pro-inflammatory cytokines [14]. The present study was undertaken to evaluate the ability of sitagliptin and/or calcipotriol to attenuate LPS-induced AD in mice and to elucidate their possible mechanisms of action.

2. Materials and Methods

2.1. Drugs and chemicals

LPS (Escherichia coli, serotype 055:B5) was obtained from Sigma Aldrich chemical Co. (St. Louis, MO, USA). Calcipotriol was obtained from Hölzel Diagnostika (Hohenzollernring, Germany). Sitagliptin was obtained from Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany. Carboxymethylcellulose (CMC) was obtained from Sigma Pharmaceutical Company, Quesna, Egypt. Both sitagliptin and calcipotriol were suspended in 1.5 % CMC solution. All other chemicals and reagents were purchased from Sigma Aldrich chemical Co. (St. Louis, MO, USA).

2.2. Experimental animals

In this study, we used sixty male Balb/c mice weighing about 18–25 grams. They were allowed to acclimatize for two weeks before starting the experiment. The animals were kept in a special room at a constant temperature of 23 ± 3 °C with relative humidity of 60 ± 10%, exposed to 12h light/dark cycles with free access to food and tap water. All the experiments were conducted according to the National Research Council’s guidelines. This study was approved by the Research Ethics Committee of faculty of medicine, Tanta University, Egypt (Approval code 1235/10/16). Animal handling was followed according to Helsinki declaration of animal ethics.

2.3. Animals grouping:

Mice were randomized into six equal groups (10 mice/group) as follows:

Group (1): Control group, received a single intraperitoneal injection of 0.9% saline solution 4 hrs prior to behavioral testing.

Group (2): Mice received a single intraperitoneal injection of 250 μg/kg of lipopolysaccharide (LPS) 4 hrs prior to behavioral testing [15].

Group (3): Vehicle control group; received 1.5% CMC solution daily by oral gavage for 2 weeks before LPS injection.

Group (4): Mice were treated with sitagliptin (10 mg/kg/day) once daily by oral gavage for 2 weeks before LPS injection [10].

Group (5): Mice received calcipotriol by oral gavage in a dose of 20 μg/kg/day for 2 weeks before LPS injection [16].

Group (6): Mice were treated with sitagliptin (10 mg/kg/day) once daily by oral gavage concomitantly with calcipotriol (20 μg/kg/day) once daily by oral gavage for 2 weeks before LPS injection.

2.4. Behavioral tests (Novel object recognition task)

A novel object recognition task was carried out as described by Gomes et al. [17]. The task was performed in a 30 × 30 × 30 cm wooden chamber, with walls painted black, a front wall made of
Plexiglas and a floor covered with ethyl vinyl acetate sheet. A light bulb, hanging 60 cm above the behavioral apparatus, provided constant illumination of about 40 lux, and an air-conditioner provided constant background sound isolation. The objects used were plastic mounting bricks, each with different shapes and colors, but the same size. Throughout the experiments, objects were used in a counterbalanced manner. Animals had not previously displayed a preference for any of the objects. Chambers and objects were cleaned with 30% ethanol immediately before and at the end of each behavioral evaluation. The task consisted of habituation, training, and testing sessions, each lasting 8 minutes. In the first session, mice were individually habituated to the behavioral apparatus and then returned to their home cages. Twenty-four hours later, the animals were subjected to a training session in which the animals were exposed to two of the same objects (object A), and the exploration time was recorded with two stopwatches. Exploration was recorded when the animal touched or reached the object with the nose at a distance of less than 2 cm. Climbing or sitting on the object was not considered exploration. The test session was carried out 24 hours after training. Mice were placed back in the behavioral chamber and one of the familiar objects (object A) was replaced by a novel object (object B). The times spent exploring the familiar and the novel objects were recorded. The discrimination index was then calculated according to Frühauf et al. [15]. The discrimination index was used as a memory parameter.

At the end of the object recognition test session, animals were transferred to a 30 cm × 30 cm open field, with the floor divided into four squares. During the 5-min open field session, the number of crossing and rearing responses was recorded. The open field was used to identify motor disabilities, which might influence the object recognition performance [15].

After the behavioral tests, mice were anaesthetized with 50 mg/kg pentobarbital sodium given by intraperitoneal injection and brain tissues were extracted. The hippocampus was divided into two parts; one for the histopathological and immunohistochemical examination and the other part was homogenized, centrifuged and the supernatant was used for determination of the biochemical parameters.

2.5. Assessment of tissue oxidative stress parameters

Tissue catalase (CAT) was determined according to Sinha method [18]. Tissue superoxide dismutase (SOD) was assessed according to Marklund and Marklund [19]. Tissue thiobarbituric acid derivatives (TBARS) were determined using ELISA kits supplied by Cell Biolabs, Inc., San Diego, USA according to the manufacturer's instructions.

2.6. Assessment of tissue tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6) and transforming growth factor beta 1 (TGF-β1)

Tissue TNF-α was determined by using TNF-α ELISA kits of Ray Biotech, Inc. according to the manufacturer's instructions. Tissue IL-6 was measured using ELISA kits supplied by Sigma Aldrich Co. according to the instructions of the
manufacturer. Tissue TGF-β1 was measured using kits supplied by Uscn Life Science Inc. Wuhan, according to the manufacturer's instructions.

2.7. Histopathological and immunohistochemical examination

Sections from the hippocampus were immediately fixed in 10% neutral buffered formalin. Then, paraffin sections were prepared and stained with hematoxylin and eosin (H & E) stain and examined under light microscope. Immunohistochemistry for caspase 3 was performed in sections prepared from formalin-fixed, paraffin-embedded tissues using the avidin–biotin immunodetection complex method according to manufacturer’s instruction (Labvision, USA). Interpretation of results was done semiquantitatively by evaluating both intensity and distribution of positive cells. The intensity of caspase-3 immunostaining was assessed as follows: none = 0, mild = 1, moderate = 2 and strong = 3 [20].

Sections from the hippocampus were immersed in 3% hydrogen peroxide in methanol for 15 min to block endogenous peroxidase activity. Slides were then incubated with universal blocking reagent (BioGenex, San Ramon, CA, USA) for 10 min at room temperature. Then, the excess serum was drained and sections were incubated with primary antibodies mouse anti-NF-κB (p65) antibody (RayBiotech, USA, Code No. 168-10007) and the slides were visualized under light microscope. The NF-κB was determined by detecting the activated subunit p65 in the examined tissues. Nuclear staining of NF-κB (p65) was considered positive when nuclear staining percentage was 10% or more in the studied field at ×100 magnification. Five different fields for each specimen were evaluated, and the mean was calculated. Scoring of immunostaining was done according to the intensity of staining as (+1) weak when nuclear staining is visible at (×200) magnification, (+2) when visible at (×100) magnification and (+3) strong when visible at (×40) magnification. Calculation of the percentage of positive nuclear staining of NF-κB (p65) was carried out via IHC profiler tool in image J software (1.49v) National Institute of Health, USA [21].

2.8. Electron microscopic examination

The specimens of the hippocampus were fixed in 4% gluteraldehyde in 0.1 M cacodylate buffer (pH 7.4). Then, they were kept in gluteraldehyde solution for 24-48 hrs at 4°C. Then, they were cut into small pieces and washed with distilled water then fixed in 1% osmium tetroxide with 15 mg/ml of potassium ferrocyanide for 1-2 hr at 4°C. Then, the specimens were cut using an ultramicrotome (JEOL-JUM-7) and stained with uranyl acetate and lead. Finally, the specimens were examined and photographed using a JEOL, JEM 1010 electron microscope (Jeol Ltd, Tokyo, Japan) [22].

2.9. Statistical analysis

The statistical analysis of the results was performed using Graph Pad Prism version 5. Multiple comparisons were performed using multiple measures analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Data were presented as mean ± standard error of mean (SEM). Differences between the means of the different groups were considered significant at a level of p-value less than 0.05.
3. Results

3.1. Effect of different treatments on the novel object recognition task

Administration of LPS induced significant decrease in the recognition index compared to the control group. Administration of CMC prior to LPS injection induced non-significant effect on the recognition index compared to LPS group. Administration of sitagliptin and/or calcipotriol prior to LPS injection induced significant increase in the recognition index compared to LPS group. This increase was significant in sitagliptin/calcipotriol combination group compared to the use of each of these drugs alone (Fig. 1).

3.2. Effect of different treatments on tissue oxidative stress parameters

Administration of LPS induced significant decrease in tissue CAT and SOD with significant increase in tissue TBARS compared to the control group. Administration of CMC prior to LPS injection induced non-significant effect on tissue CAT, SOD and TBARS compared to LPS group. Administration of sitagliptin and/or calcipotriol prior to LPS injection induced significant increase in tissue CAT and SOD associated with significant decrease in tissue TBARS compared to LPS group. These changes were significant in sitagliptin/calcipotriol combination group compared to the use of each of these drugs alone (Table 1).

3.3. Effect of different treatments on tissue TNF-α, IL-6 and TGF-β1

Administration of LPS induced significant increase in tissue TNF-α, IL-6 and TGF-β1 compared to the control group. Administration of CMC prior to LPS injection induced non-significant effect on tissue TNF-α, IL-6 and TGF-β1 compared to LPS group. Administration of sitagliptin or calcipotriol prior to LPS injection induced significant decrease in tissue TNF-α, IL-6 and TGF-β1 compared to LPS group but the combination had the upper hand over the use of each of these drugs alone (Table 2).

Figure 1: Effect of the different treatments on the recognition index (%) in the studied groups. Values were represented as mean ± S.E.M. * Significant compared to the control group (p-value less than 0.05); # Significant compared to LPS group (p-value less than 0.05); ● Significant compared to sitagliptin + LPS group (p-value less than 0.05); $ Significant compared to calcipotriol + LPS group (p-value less than 0.05).
Table 1: Effect of different treatments on tissue CAT, SOD and TBARS in the studied groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>LPS</th>
<th>CMC + LPS</th>
<th>Sitagliptin + LPS</th>
<th>Calcipotriol + LPS</th>
<th>Sitagliptin + Calcipotriol + LPS</th>
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<td>Tissue CAT</td>
<td>28.5±1.42</td>
<td>11.75±0.85*</td>
<td>12.6±0.72</td>
<td>16.64±0.88#</td>
<td>17.96±0.97#</td>
<td>22.95±1.32**</td>
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<td>(U/mg tissue)</td>
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<tr>
<td>Tissue SOD</td>
<td>209.4±12.1</td>
<td>135.7±7.4*</td>
<td>131.8±8.6</td>
<td>171.6±8.81*</td>
<td>183.64±9.5*</td>
<td>196.42±10.7**</td>
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<td>(U/g/min)</td>
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<tr>
<td>Tissue TBARS</td>
<td>8.34±0.29</td>
<td>52.34±3.1*</td>
<td>49.92±2.9</td>
<td>38.9±2.1*</td>
<td>37.53±2.21*</td>
<td>23.54±1.7**</td>
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<td>(μM/g tissue)</td>
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Values were represented as mean ± S.E.M.
* Significant compared to the control group (p-value less than 0.05); # Significant compared to LPS group (p-value less than 0.05); * Significant compared to sitagliptin + LPS group (p-value less than 0.05); ● Significant compared to calcipotriol + LPS group (p-value less than 0.05).

Table 2: Effect of different treatments on tissue TNF-α, IL-6 and TGF-β1 in the studied groups

<table>
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<tr>
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<th>Control</th>
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<th>CMC + LPS</th>
<th>Sitagliptin + LPS</th>
<th>Calcipotriol + LPS</th>
<th>Sitagliptin + Calcipotriol + LPS</th>
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<tr>
<td>Tissue TNF-α</td>
<td>42.6±2.7</td>
<td>453.2±12.2*</td>
<td>446.8±11.3</td>
<td>328.2±7.8*</td>
<td>311.5±7.3*</td>
<td>251.5±4.7**</td>
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<td>(pg/mg protein)</td>
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<tr>
<td>Tissue IL-6</td>
<td>221.3±4.42</td>
<td>671.2±16.3*</td>
<td>688.3±17.4</td>
<td>515.7±13.4*</td>
<td>481.6±12.3*</td>
<td>391.7±10.3**</td>
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<td>(pg/mg protein)</td>
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<tr>
<td>Tissue TGF-β1</td>
<td>21.56±1.3</td>
<td>69.2±3.7*</td>
<td>71.2±3.8</td>
<td>58.3±3.2*</td>
<td>62.8±3.3*</td>
<td>35.9±2.4**</td>
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Values were represented as mean ± S.E.M.
* Significant compared to the control group (p-value less than 0.05); # Significant compared to LPS group (p-value less than 0.05); * Significant compared to sitagliptin + LPS group (p-value less than 0.05); ● Significant compared to calcipotriol + LPS group (p-value less than 0.05).

3.4. Effect of different treatments on the histopathological, immunohistochemical and electron microscopic picture

Administration of LPS induced marked disorganization and degeneration of the pyramidal cells (Fig. 2b) with significant increase in tissue caspase 3 expression (Fig. 3b) and NF-κB (P 65) immunostaining (Fig. 4) compared to the control group. These changes were associated with significant decrease in the size of the nuclei of the pyramidal cells with apparently normal mitochondria compared to the control group (Fig. 5b). These changes were ameliorated with administration of sitagliptin and/or calcipotriol with restoration of the normal size and arrangement of the pyramidal cells (Fig. 2d, 2e) with significant decrease in tissue caspase 3 expression (Fig. 3d,e) and NF-κB (P 65) immunostaining (Fig. 4) compared to the control group. These changes were associated with restoration of the normal size of the nuclei of the pyramidal cells with apparently normal mitochondria compared to the control group (Fig. 5d,e). These changes were significant in sitagliptin/calcipotriol combination group compared to the use of each of these drugs alone (Fig. 2f, 3f, 4, 5f). Administration of CMC prior to LPS injection induced non-significant effect on the histopathological, immunohistochemical and electron microscopic picture compared to LPS group (Fig. 2c, 3c, 4, 5c).
Figure 2: Sections in the hippocampus of a) control group showing 5-6 compact layers of pyramidal cells with vesicular nuclei. Stratum moleculare shows many glial cells (G) among neuronal processes and scattered nerve cells. Pyramidal cells are characterized with large, rounded vesicular nuclei (N) with dispersed chromatin and prominent nucleoli. Blood capillaries (B.C) are lined with simple squamous epithelium; b) LPS group showing decrease in row number of pyramidal cells (1-2 rows). The molecular layer shows excess in glial cells (G), disorganization of pyramidal cells (some having pale nuclei and other dark). Some pyramidal cells have degenerated and deeply stained nuclei (double arrow); c) CMC + LPS group showing that the pyramidal cells are arranged into 1-2 rows of cells, shrunken and distorted pyramidal cells with condensation of chromatin. Some cells are surrounded with pericellular halo (double arrow); d) sitagliptin + LPS group showing reduction in the pyramidal cell layer into 3-4 rows of cells. There is apparent decrease in the size of pyramidal cells. Some pyramidal cells have degenerated and deeply stained nuclei (double arrow); e) calcipotriol + LPS group showing that the pyramidal cells are arranged into 3-4 rows of cells. There is marked increase of the glial cells (G) in the molecular layer, with a decrease in the pyramidal cells size. Some pyramidal cells have degenerated and deeply stained nuclei (double arrow). The pyramidal cells nuclei vary in the shape and size; f) sitagliptin + calcipotriol + LPS group showing that the pyramidal cells are arranged into 3-4 rows of cells with dilated blood capillaries (B.C) lined with squamous epithelium. Pyramidal cells are characterized with large, rounded and euchromatic nucleus (N), with areas of pyramidal layer distortion (H&E X 1000).

Figure 3: A Photomicrograph of the hippocampus of a) control group showing negative caspase-3 immunoreactivity; b) LPS group showing strong positive caspase-3 immunoreactivity (arrow); c) CMC + LPS group showing strong positive caspase-3 immunoreactivity (arrow); d) sitagliptin + LPS group showing moderate positive caspase-3 immunoreactivity (arrow); e) calcipotriol + LPS group showing moderate positive Caspase-3 immunoreactivity (arrow); f) sitagliptin + calcipotriol + LPS group showing weak positive caspase-3 immunoreactivity (arrow) (Caspase-3 Immunostaining X 400)
Figure 4: Percentage of positive nuclear staining of NF-kB (p65) in the different studied groups. Values were represented as mean ± S.E.M. *Significant compared to the control group (p-value less than 0.05); # Significant compared to LPS group (p-value less than 0.05); ● Significant compared to sitagliptin + LPS group (p-value less than 0.05); $ Significant compared to calcipotriol + LPS group (p-value less than 0.05).

Figure 5: An electron micrograph of an ultrathin section in the hippocampus of a) control group showing pyramidal cell with central nucleus (N). Mitochondria (arrow) are normal and appear as oval or rounded electron dense masses scattered in the cytoplasm, the inner mitochondrial membrane forms the cristae through a series of in folding while the outer membrane is a smooth continuous envelope that is separate and distinct from the inner membrane. Myelinated axon (A) appears surrounded with concentric layers of myelin. Free ribosomes (R) appear as electron dense particles freely floating in the cytoplasm. b) LPS group showing pyramidal cell with small nucleus (N). The chromatin is peripherally condensed. Mitochondria (double arrow) show more vacuolation and damage of cristae. Free ribosomes (R) appear as electron dense particles. Axons (A) appear as oval masses surrounded with concentric layers of myelin; c) CMC + LPS group showing pyramidal cell with small size rounded nucleus (N). The chromatin is peripherally condensed. Mitochondria (arrow) show vacuolation and damage of cristae. Myelinated axon (A) is normal. Smooth endoplasmic reticulum (SER) shows dilatation. Free ribosomes (R) scattered in the cytoplasm. Axons (A) is surrounded with myelin which appear as electron dense layers; d) sitagliptin + LPS group showing pyramidal cell. Mitochondria (arrow) shows vacuolation or swelling, damage of cristae. Smooth endoplasmic reticulum (SER) shows dilatation. Free ribosomes (R) appear as electron dense particles scattered in the cytoplasm. The nuclear membrane is irregular with condensed chromatin peripherally along the nuclear membrane; e) calcipotriol + LPS group showing pyramidal cell with a centrally located, rounded nucleus (N) with condensed chromatin. Mitochondria (arrow) appear as oval masses scattered in the cytoplasm, vacuolatoin with destruction of its cisternae. Myelinated axon (A) is normal. Free ribosomes (R) appear as electron dense particles; f) sitagliptin + calcipotriol + LPS group showing pyramidal cell with euchromatic nucleus (N). There is a mild reduction of nuclear size. Mitochondria (arrow) appear as oval or rounded electron dense masses showing vacuolation with destruction of cristae. Free ribosomes (R) appear as electron dense particles (EM; X 2000).
4. Discussion
Alzheimer’s disease is one of degenerative brain disorders that markedly affects the patient’s perception, memory, judgement and cognition [2]. The exact mechanisms underlying this disorder are still not yet fully understood but accumulating data suggest that neuroinflammation, oxidative stress and apoptosis may play a crucial role in this disorder [23]. This was in the same line with the results of the present study where LPS injection induced significant deterioration of the recognition index associated with significant increase in the proinflammatory cytokines, induction of oxidative stress and increased caspase 3 expression.

Oxidative stress was proven to play an important role in the pathogenesis of AD [24]. Increased production of reactive oxygen species and marked impairment of the activities of the antioxidant enzymes are prominent features of AD [25]. This was in agreement with the results of the present study where LPS induced significant decrease in the activities of tissue CAT and SOD with significant increase in tissue TBARS compared to the control group. These changes were ameliorated in the present study by administration of sitagliptin which was proven to have strong antioxidant and free radical scavenging activities [10]. Also, calcipotriol in the present study was able to restore the activities of the antioxidant enzymes and decrease the level of TBARS. Vitamin D analogues, including calcipotriol, have strong antioxidant properties which may be attributed to decreased production of reactive oxygen species and restoration of the protective mechanisms that protect the body against oxidative stress [12].

Neuroinflammation was considered as the cornerstone in the pathophysiology of AD [26]. NF-κB is a protein complex that controls DNA transcription, production of the proinflammatory cytokines and cell survival [10]. Freudsperger et al. [27] reported that the cross-talk that exists between NF-κB and TGF-β1 signaling pathways is the triggering event that induces the production of the proinflammatory cytokines such as TNF-α and IL-6 which may have potential effects on memory and cognition. This was in the same line with the results of the present study where LPS administration induced significant increase in the expression of NF-κB, TGF-β1, TNF-α and IL-6 associated with significant histopathological changes compared to the control group.

In the present study, sitagliptin was able to decrease the levels of NF-κB, TGF-β1 and the proinflammatory cytokines compared to LPS group which was attributed to the fact that sitagliptin has potent anti-inflammatory effects on both the nuclear and cytoplasmic levels [28]. Moreover, sitagliptin was reported to increase glucagon-like peptide 1 (GLP-1) which in turn inhibits the expression of both NF-κB and TGF-β1 at the nuclear level [29]. Also, calcipotriol in our study exerted potent anti-inflammatory effects, possibly due to the hypothesis that calcipotriol blocks smad residency on the chromatin and inhibits acetylation of histones leading to marked suppression of gene expression of the proinflammatory cytokines [16].

Recent studies suggest that there is a strong relationship between induction of apoptosis and the increased liability to AD [30]. Dickson [31] reported that selective loss of the neurons in neurodegenerative disorders such as AD may be due to activation of cysteine aspartyl proteases such as caspase 3 which initiates apoptosis. In AD,
both the extracellular and the intracellular amyloid proteins may activate caspases which in turn lead to cleavage of the intracellular proteins which strongly correlates with dementia [32]. This was in accordance with the results of the present study where LPS administration induced significant increase in caspase 3 expression compared to the control group.

Sitagliptin in our study was able to decrease caspase 3 expression, possibly due to the effect of GLP-1 on PI3K/Akt signaling pathway which significantly affects the pathways of apoptosis in which caspase 3 is directly involved [33]. Also, calcipotriol in the present study ameliorated caspase 3 expression compared to LPS group. This may be due to the inhibitory effect of calcipotriol on TGF-β Smad3 signaling pathway which is considered as one of the main regulators of apoptosis [34].

In the present study, sitagliptin/calcipotriol combination induced significant increase in the recognition index and tissue antioxidant enzymes associated with significant decrease in tissue TBARS, TGF-β1 and the proinflammatory cytokines associated with significant improvement of the histopathological, immunohistochemical and electron microscopic picture compared to the use of each of these drugs alone. This may be explained by the combined anti-inflammatory, antioxidant and anti-apoptotic effects of both drugs together with their ability to affect apoptosis and restore the behavioral tests to the normal levels. This combination might represent a new therapeutic modality for amelioration of AD.

5. Conclusion

Sitagliptin/calcipotriol combination had a better effect on LPS-induced AD than the use of each of these drugs alone. This may be attributed to the antioxidant and the anti-inflammatory properties of both drugs together with their ability to affect apoptosis and restore the behavioral tests to the normal levels. This combination might represent a new therapeutic modality for amelioration of AD.

Conflict of interest

The authors had no conflict of interest to declare.

References


