INTRODUCTION

Alzheimer’s disease (AD) is a progressive degenerative disorder. The main neuropathological hallmarks of AD are the formation of senile plaques (SPs) following neurofibrillary tangles (NFTs) which causes neuronal degeneration and synaptic loss. In particular, the senile plaques are extracellular aggregates of the amyloid beta-peptide (Aβ) that is cleaved from the amyloid precursor protein (APP).¹

Neuropathological studies in the human brains have proved that the activation of glial cells excessively releases proinflammatory mediators and cytokines, oxidative stress which followed by triggering a neurodegenerative cascades via neuroinflammation.²

Several theories might explain pathogenesis of AD, including cholinergic hypothesis, tau protein hypothesis and amyloid hypothesis.³

Deposition of beta amyloid (Aβ) plaques may activate the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex, resulting in increased rate of ROS formation.⁴

Amyloid-β proteins (Aβ) of 42 (Aβ42) accumulate as senile plaques (SP) and cerebrovascular amyloid protein deposits that are defining diagnostic features of Alzheimer’s disease (AD) so Aβ accumulation is therefore a key pathological event and a prime target for the prevention and treatment of AD.⁵

Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) is a member of a family of nitroxide compounds that has been studied extensively in animal models of increased reactive oxygen species and its effects on hypertension and endothelial function.⁶

Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine- N-oxyl) is an antioxidant that works as a superoxide dismutase mimic, directly reacts with both carbon-centered and peroxy radicals, and prevents the reduction of hydrogen peroxide to the hydroxyl radical. Furthermore, this nitroxide can oxidize reduced transition metals that would otherwise serve to catalyze the formation of the hydroxyl radical via the Fenton reaction.⁷

Tempol was well-known as a ROS scavenger.⁸⁻¹⁰ It was selected as a reference treatment as oxidative stress was believed not only to be a result of Aβ plaque deposition but also a causative factor, triggering brain Aβ formation and deposition.¹¹⁻¹³

The aim of the present study aims to study the possible protective effects of tempol on lipopolysaccharide induced amyloidogenesis, neuroinflammation and oxidative stress.

MATERIALS AND METHODS

Animals

Adult male mice, 25±2 g, were purchased from the National Research Center, Giza, Egypt.

Animal handling and experimental procedures were carried out according to the guidelines of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).
**Drugs, Chemicals and Reagent kits**

Tempol, LPS, thiobarbituric acid, 1,1,3,3-tetramethoxypropane, 5,5′-dithio-bis-(2-nitrobenzoic acid), and glutathione (GSH) standard were purchased from Sigma Chemicals Co. (USA).

ELISA kits of murine Aβ1-42 was obtained from MyBioSource Co. All other chemicals, solvents and reagents were of analytical grade.

**Experimental Design**

Animals were divided into three groups: a normal control group received saline intraperitoneal (i.p) injection, Alzheimer control group received lipopolysaccharide (0.8 mg/kg) for seven days and third group received tempol (100 mg/kg) plus LPS (0.8 mg/kg).

Twenty-four hours later, animals were sacrificed and brain tissue withdrawn for biochemical studies.

**Methodology**

**Induction of amyloidogenesis, neuroinflammation**

Amyloidogenesis, neuroinflammation induction was performed by a single i.p. dose of LPS (0.8 mg/kg), suspended in 1% tween 80 solution in saline. Tempol was administered once daily via the i.p. route for 7 days starting from the day of LPS administration.

**Tissue preparation**

Animals were sacrificed by decapitation with the brains removed rapidly in ice-cold saline twenty-four hours after the last dose of test agents or vehicles then they were homogenized in cold saline using a homogenizer (yellow line, Di18 basic, Germany), centrifuged at 4000 rpm at 4°C for 15 min using a cooling centrifuge (Sigma 3-30k, USA), and the supernatant was withdrawn and kept in a deep freezer at -80°C (Als Angelantoni Life Science, Italy) till the time of assay of Aβ, Super oxide dismutase (SOD), reduced glutathione (GSH) and Malondialdehyde (MDA).

**Biochemical Estimation**

**Alzheimer marker**

Group received LPS (0.8 mg/kg) showed significant increase of Aβ content when compared with normal control group, while group received tempol (100 mg/kg) plus LPS showed significant decrease of Aβ content when compared with LPS received Alzheimer group. (Table 1).

**Oxidative stress markers**

Lipopolysaccharide (0.8 mg/kg) received group showed significant increase of MDA and significant decrease of GSH and SOD when compared with normal control group, while tempol (100 mg/kg) plus LPS received group showed significant decrease of MDA and significant increase of SOD and GSH when compared with LPS received Alzheimer disease. (Table 2).

**RESULTS**

**Biochemical Estimation**

**Table 1:** Effect of Tempol on brain levels of Aβ in mice with LPS induced amyloidogenesis, oxidative stress

<table>
<thead>
<tr>
<th>Group Parameter</th>
<th>Normal Control</th>
<th>LPS Control</th>
<th>Tempol (100 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ (pg/g)</td>
<td>3.2 ± 0.21</td>
<td>15.7 ± 0.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.13 ± 0.31&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
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</table>

Each value represents a mean of 10-15 values ± SEM.

<sup>a</sup> significantly different from normal control value at p < 0.05.

<sup>b</sup> significantly different from LPS control value at p < 0.05.

Aβ: beta amyloid, LPS: lipopolysaccharide (0.8 mg/kg), SEM: standard error of the mean.

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</thead>
<tbody>
<tr>
<td>SOD (u/g)</td>
<td>8.23 ± 0.38</td>
<td>2.9 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.4 ± 0.24&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>MDA (nmol/g)</td>
<td>11.2 ± 0.57</td>
<td>67.5 ± 2.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.6 ± 0.95&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSH (μmol/g)</td>
<td>5.45 ± 0.20</td>
<td>0.85 ± 0.035&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.33 ± 0.053&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value represents a mean of 10-15 values ± SEM.

<sup>a</sup>Significantly different from normal control value at p < 0.05.

<sup>b</sup>Significantly different from LPS control value at p < 0.05.

LPS: lipopolysaccharide (0.8 mg/kg), GSH: glutathione reduced, MDA: Malondialdehyde, lipopolysaccharide, SEM: standard error of the mean, SOD: superoxide dismutase.

**DISCUSSION**

In the present study, the possible protective effects of tempol, as well as the underlying protective mechanisms, were investigated on LPS-induced amyloidogenesis and neuroinflammation in mice. In this study, memory impairment in mice was associated with brain Aβ deposition accompanied with massive neurodegenerative changes. In support, LPS administration was reported to enhance brain Aβ accumulation through pro-
inflammatory potential or through affecting brain permeability.\textsuperscript{25,26}

The deposited Aβ was reported to be included in activation of free radical generation through activation of NADPH oxidase complex, causing increased rate of ROS formation and lipid peroxidation.\textsuperscript{9,11}

Additionally, iron and copper ions attached to Aβ plaques act as catalysts for further ROS formation.\textsuperscript{27,28}

Immunologically, microglia cells recognize and attack Aβ plaques, again increasing cytokine production and causing excessive ROS formation\textsuperscript{29} as evident in our results, where brain tissue MDA was significantly increased while brain tissue GSH was significantly depleted, which also came in agreement with our results. In support,\textsuperscript{10} reported that tempol could decrease cerebral amyloid angiopathy and Aβ deposition in animal models, Tempol was reported to possess antioxidant effect, being a ROS scavenger\textsuperscript{15,16} the antioxidant effect of tempol in the present investigation was evidenced by suppressed tissue MDA level, coupled with elevated GSH levels. The recorded ability of tempol in the current study to upgrade SOD activity in brain tissue may explain partly its antioxidant potential. Support of antioxidant status was claimed by many authors to suppress AD progression in different models.\textsuperscript{30-32} Similarly, oxidative stress in brain tissue was considered as a result of Aβ deposition\textsuperscript{33} and also as a pathogenic factor enhancing neuronal damage and AD progression.\textsuperscript{34} The antioxidant effect of tempol and telmisartan may therefore represent another explanation to the beneficial anti-Alzheimer effect of tempol evident in the current work.

**CONCLUSION**

Lipopolysaccharide (0.8 mg/kg) induced amyloidogenesis and neuroinflammation and neuronal degeneration including oxidative stress. Tempol at dose of 100 mg/kg could improve neuroinflammation and neuronal degeneration through significant decrease of amyloid β deposition in neurons and significant decrease oxidative stress parameters through significant decrease of MDA and significant increase of GSH and SOD.

**REFERENCES**


5. Lombardo S, Masksos U, Role of the nicotinic acetylcholine receptor in Alzheimer’s disease pathology and treatment, *Neuropsychopharmacology* 2014.


