A Review of Medicinal Herbs with Antioxidant Properties in the Treatment of Cerebral Ischemia and Reperfusion

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ABSTRACT

BACKGROUND AND OBJECTIVE: Stroke is the second most common cause of death and a major cause of disability in individuals aged over 65 years, worldwide. Considering the complex pathological process of ischemia, use of one single agent does not seem ideal for treatment. Therefore, studies in search of effective compounds and methods are under way. In recent years, increased attention has been paid to medicinal plants as potential sources for the treatment of ischemia and reperfusion. This study aimed to introduce the pathogenesis of cerebral ischemia and review the effects of medicinal plants and their mechanisms of action in cerebral ischemia.

METHODS: In this study, articles indexed in scientific databases including ISI, SID, PubMed, PubMed Central, Scopus, and Web of Science were evaluated, using the following keywords in Farsi and English: "Ischemia and reperfusion", "medicinal herbs", and "antioxidant properties".

FINDINGS: The review of conducted studies showed that medicinal plants and their compounds are capable of reducing infarct volume, cerebral edema, neuronal damages, sensory problems, and motor disorders through reducing oxidative and nitrative stress. Moreover, these plants are able to decrease the expression of inflammatory mediators, inhibit DNA fragmentation and oxidative DNA damage, reduce microglial and astrocyte activities, increase the expression of mitochondrial genes, inhibit apoptotic protein expression, reduce eicosanoids, and boost anti-apoptotic protein expression.

CONCLUSION: Medicinal herbs and their compounds are able to diminish the damages caused by cerebral ischemia through several pathways; therefore, they can be used as new sources against cerebral ischemia.

KEY WORDS: Antioxidant Properties, Ischemia, Medicinal Herbs, Reperfusion.

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**Introduction**

Stroke is a major cause of disability and the third leading cause of mortality. In recent decades, the incidence of stroke has significantly increased, particularly in developing countries (1). Approximately 20% of these patients die within the first month after stroke and those who survive for more than six months are highly dependent on others. These patients suffer from complications such as partial body paralysis, memory disorders, and cognitive/lingual impairments (2).

Factors increasing the risk of stroke include high blood pressure, diabetes mellitus, smoking, alcohol abuse, dyslipidemia, and hypercholesterolaemia (3). Overall, 15% of strokes are caused by hemorrhage, while 85% of these cases occur due to ischemia (2). Cerebral ischemia leads to neurological disorders (e.g., motor, sensory, and visual disorders), speech disorders, and neuropsychiatric defects such as reduced cognitive function, apraxia, cognitive impairment, anterograde amnesia, and impaired spatial learning and memory (4).

The brain requires 25% of cardiac output for its metabolic requirements. Therefore, any decline in cerebral blood flow may lead to ischemia and neurodegenerative disorders (5). Ischemia is caused by factors such as thrombosis, embolism, and reduced systemic hypoperfusion (2).

During ischemia, temporary or permanent decline in blood flow to the brain leads to the limited transfer or loss of glucose and oxygen, required for cellular homeostasis. At this stage, events such as stimulus-induced cytotoxicity, acidosis, ion imbalance, oxidative stress, lipid peroxidation, inflammation, and apoptosis lead to cell death. In the next stage, reperfusion causes mitochondrial dysfunction, release of glutamates and inflammatory mediators, production of reactive oxygen species (ROS), and lipid peroxidation (1). Due to the complex pathological process of ischemia, use of one single agent does not seem ideal for treatment (1). To date, except for recombinant tissue plasminogen activator (R-TPA), no effective treatment has been proposed for ischemia to alleviate the symptoms within less than three hours following their onset (2). Given the fact that apoptotic death occurs in the late phase of ischemia at low rates, use of apoptosis inhibitors is the next phase of treatment (6). In recent years, use of medicinal herbs and their active constituents has been emphasized as a potential source for the treatment of ischemia-reperfusion (7). Significant attention has been paid to these herbs due to their recognition as safe substances, cost-effectiveness, availability, long-term use, consumers’ preference, and limited side-effects (8).

So far, several studies have been conducted on the neuroprotective effects of medicinal plants. Also, a number of studies have investigated the effects of medicinal herbs and their effective compounds on cerebral ischemia and have identified their mechanisms of action. In the present study, in addition to introducing cerebral ischemia and its pathogenesis, we aimed to review the literature on the protective effects of medicinal herbs on cerebral ischemia and their mechanisms of action.

**Pathogenesis of cerebral ischemia and reperfusion:**

During a stroke, two cellular responses occur in brain tissues:

1. **Severe response to ischemia, reduced energy, and production of free radicals:** During ischemia, due to decreased oxygen concentration, the brain starts to consume anaerobic glycolysis, although this process is inefficient in terms of energy supply (9). During anaerobic glycolysis, pyruvate converts into lactate by lactate dehydrogenase, and thus lactate concentration in ischemic tissues increases, leading to intracellular acidosis (10). Free radicals, which are produced by the breakdown of adenosine triphosphate (ATP) metabolites via xanthine oxidase, along with prostaglandins, attack unsaturated fatty acids (via plasma membrane polyunsaturated fatty acids), causing damage and increased permeability.

Mitochondrial respiratory chain is the main source for ROS production. Necrotic cell death occurs, following mitochondrial dysfunction. Endothelial cell death destroys the blood-brain barrier and results in brain edema (11). Damage to energy-dependent ion pumps leads to cellular depolarization and release of glutamates into the extracellular space. Afterwards, N-methyl-D-aspartate receptors (NMDAs) are activated, resulting in the increased level of intracellular calcium, which is responsible for the production of ROS and nitric oxide. A nitrite proxy is produced by the combination of nitric oxide and superoxide, leading to lipid peroxidation (12).

2. **Delayed response to ischemia, inflammation:** The second wave of cell death in response to ischemia is caused by inflammatory neuronal mediators. During ischemia, the activated microglia can produce inflammatory cytokines such as tumor necrosis factor-α, interleukin-1 beta, and other cytotoxic molecules
such as nitric oxide and ROS (13). Similar to microglia, astrocytes are able to produce inflammatory factors such as cytokines, chemokines, and nitric oxide. Cytokines increase the expression of cell adhesion molecules. Through this process, leukocytes adhere to arterial walls, migrate to brain tissues, start to secrete inflammatory mediators, and cause secondary brain injury within four to six hours following the onset of ischemia (14).

Inflammatory changes of neurons eventually destroys the blood-brain barrier, causes cerebral edema, and induces cell death (2). Several biochemical, molecular, and electrophysiological cascades are responsible for cell death after ischemia. In the early stage of ischemia, apoptosis is the major form of cell death, i.e., a simple and rapid death, followed by the release of inflammatory cellular contents. This type of cell death mainly occurs in the absence of energy (e.g., the early stage of ischemia); in fact, necrotic death requires less energy and facilitates ischemia (6).

Apoptosis is identified with processes such as the swelling of cytoplasmic organelles and nucleus, mitochondrial destruction, and disintegration of plasma membranes (15). In the late phase of ischemia, apoptotic cell death is characterized with the contraction of nuclear chromatin, DNA fragmentation, shrinkage of cytoplasm, and bubble-shape of the membrane (16). Apoptotic death prevents the release of organelle contents (e.g., lysosomes) into the extracellular matrix, resulting in secondary damage to the tissues through inflammation. Apoptotic death normally occurs when there is sufficient energy supply, e.g., during reperfusion (6).

Oxidative stress, as a major cause of neuronal injury during cerebral ischemia, arises when the physiological balance between oxidants and antioxidants is disturbed in favor of the former, causing serious injuries to the tissues. Oxidative stress leads to the formation of ROS and reactive nitrogen species and causes multiple adverse outcomes including mitochondrial dysfunction, increased calcium level, reperfusion injury, and inflammation (17). Due to the significant presence of oxygen, ROS production is more probable in aerobic tissues such as brain tissues. Brain cells are responsible for almost 20% of the total oxygen consumption, despite the fact that the brain only accounts for 2% of body weight (18). Increased ROS production causes oxidative damage to cellular components, changes nucleic acid bases, induces single- and double-strand breaks in DNA structure, and destroys the glycosylated bonds between the ribose and bases (19). The brain is protected against these free radicals by endogenous free radicals (e.g., ascorbate and alpha-tocopherol) or endogenous antioxidant enzymes (e.g., superoxide dismutase, glutathione peroxidase, and catalase).

Nuclear factor-erythroid 2-related factor-2 (Nrf2)/antioxidant responsive element (ARE) pathway is one of the main pathways against oxidative stress, which regulates the cellular redox status. Under normal homeostatic conditions, Nrf2 is inhibited by Keap1. However, in the presence of ROS, Nrf2 is abandoned by Keap1 and is transferred into the nucleus, where it connects to ARE in the promoter region of genes, encoding antioxidant enzymes, and induces the production of antioxidant enzymes (20).

Researchers have shown that Nrf2 expression and production of antioxidant enzymes increase during cerebral ischemia; however, due to their limited capacity, the concentration of free radicals starts to rise (17). According to previous research, numerous compounds (such as polyphenols) with antioxidant properties, which are found in some food sources, are able to increase the expression of transcription factor Nrf2, followed by the increased production of antioxidant enzymes in brain cells (17, 21).

High concentrations of iron (which catalyzes the production of free radicals) and unsaturated fatty acids in the brain are among other factors, which increase brain sensitivity to oxidative stress (18). It has been reported that during ischemia, the concentration of free fatty acids, particularly arachidonic acids, significantly increases (13).

In fact, the increase in intracellular calcium during ischemia activates phospholipase C and A, resulting in the hydrolysis of phospholipids and release of fatty acids. Re-synthesis of phospholipids requires the presence of ATP. As a result, following the increase in intracellular calcium concentration and decreased ATP level in ischemia, free fatty acids are released and the membrane is damaged (22). Release of free fatty acids is followed by various adverse effects such as the inhibition of oxidative phosphorylation, conversion of arachidonic acids to eicosanoids (e.g., thromboxane and prostaglandin) by cyclooxygenase (COX), lipid peroxidation by free radicals, mediated chain reactions by lipid peroxidation, and cytotoxicity induced by lipid peroxide products (e.g., hydroxynonenal), which might be followed by apoptosis (17).
As recently demonstrated, impaired mitochondrial gene expression during cerebral ischemia damages neurons in the CA1 region of the brain (23). Induction of global cerebral ischemia in rats reduces the expression of cytochrome oxidase, encoded by mitochondrial DNA; this process disturbs the energy metabolism and causes oxidative phosphorylation (24). It has been reported that Ginkgo biloba and its active compound, i.e., bilobalide, are able to regulate COX expression and reduce ischemia-induced neuronal damages (24).

In addition to intracellular pathological events, disruption in signaling pathways in the cellular space, involved in cell-to-cell or cell-to-matrix interactions, contributes to neuronal damages during ischemia. It has been indicated that the induction of ischemia-reperfusion in rats increases the expression of matrix metalloproteinase-2 and metalloproteinase-9. Therefore, the activated enzymes facilitate the destruction of components in the basement membrane matrix and disintegrate brain capillaries. Consequently, genetic and pharmacological inhibition of these enzymes has been considered in the treatment and prevention of cerebral ischemia (25). Considering the abovementioned points, new strategies for the treatment of ischemia and stroke include the identification of ROS-neutralizing compounds, chelating metal ions, non-steroidal, anti-inflammatory drugs, anti-apoptotic agents, and bioenergetic medicines (used either solely or in combination) (8).

The scavenging effects of free radicals, chelating effect of metal ions, and anti-inflammatory properties of medicinal plants have been revealed in animal and human models. Therefore, recent research has focused on the use of medicinal plants and their active compounds in the treatment of nervous system disorders and possibly ischemia (26).

The studied medicinal plants for the treatment of ischemia-reperfusion: In this section, we present a summary of studies on the protective effects of medicinal plants and their active compounds. The scientific name, local name, the used parts, studied concentrations, and the mechanisms of influence in these plants are also discussed (table 1).

<table>
<thead>
<tr>
<th>Local name (scientific name)</th>
<th>Family</th>
<th>The used parts</th>
<th>Concentration</th>
<th>Effects (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olive (Olea europaea)</td>
<td>Oleaceae</td>
<td>Olive leaf extract</td>
<td>Oral treatment with 50, 75, and 100 mg/kg of the extract in male Wistar rats with cerebral ischemia</td>
<td>Reduced LDL/HDL ratio, reduced infarct volume, cerebral edema, blood-brain barrier permeability, and neurological defects (27)</td>
</tr>
<tr>
<td>Ginkgo (Ginkgo biloba)</td>
<td>Ginkgoaceae</td>
<td>Extract and bilobalide</td>
<td>Oral treatment with 25, 30, and 100 mg/kg of the extracts and isolated terpene at 3 and 6 mg/kg in male rats with cerebral ischemia</td>
<td>Reduced death of neurons and increased expression of mitochondrial genes such as COX II (24)</td>
</tr>
<tr>
<td>Pomegranate (Punica granatum L.)</td>
<td>Punicaceae</td>
<td>Pomegranate extract</td>
<td>Oral treatment with the extract at 250 and 500 mg/kg in male Wistar rats with cerebral ischemia</td>
<td>Increased levels of cholesterol, cholesterol ester, and triglyceride in the brain (30)</td>
</tr>
<tr>
<td>Cherokee Rose (Rosa laevigata)</td>
<td>Rosaceae</td>
<td>Flavonoid-rich extracts</td>
<td>Oral treatment with 50, 100, and 200 mg/kg of the extracts in male Sprague-Dawley rats with cerebral ischemia</td>
<td>Increased survival of rats, reduced DNA fragmentation, decreased expression of apoptotic molecules, increased expression of anti-apoptotic proteins, and decreased expression of inflammatory mediators (e.g., iNOS, MMP-9, COX-2, TNF-α, IL-10, and IL-1β) (1)</td>
</tr>
<tr>
<td>Garlic (Allium sativum L.)</td>
<td>Alliaceae</td>
<td>Hydroethanolic extract of garlic peel and seeds</td>
<td>Oral treatment at a concentration of 360 mg/kg in male Wistar rats with cerebral ischemia</td>
<td>Decreased expression of NMDA subunits such as NR1 and NR2B, increased ATP production in the brain, and inhibition of the decline in neuronal nuclear antigens (31)</td>
</tr>
<tr>
<td>Absinthisum (Artemisia absinthium)</td>
<td>Asteraceae</td>
<td>Methanic extract of aerial parts</td>
<td>Oral doses (100 and 200 mg/kg) in male Swiss albino rats with focal cerebral ischemia</td>
<td>Reduced infarct volume, improved short-term memory and balance, reduced lipid peroxidation, and increased level of endogenous antioxidants (32)</td>
</tr>
<tr>
<td>Basil (Ocimum basilicum)</td>
<td>Lamiaceae</td>
<td>Ethyl acetate leaf extracts</td>
<td>Oral treatment with 100 and 200 mg/kg of the extracts in male Swiss albino rats with cerebral ischemia</td>
<td>Reduced infarct volume, reduced lipid peroxidation, increased glutathione in the brain, and increased short-term memory and balance (3)</td>
</tr>
</tbody>
</table>

Table 1. The studied medicinal plants for the treatment of cerebral ischemia-reperfusion
Discussion

So far, various studies have revealed the positive effects of a large number of medicinal plants on the devastating outcomes of ischemia-reperfusion. Based on multiple studies, the active constituents of medicinal plants are capable of reducing infarct volume, cerebral edema, neuronal damages, and sensory, motor, and neurological disorders induced by ischemia. The proposed mechanisms for the protective effects of medicinal plants are as follows: 1) reduced oxidative and nitrative stress; 2) reduced lipid peroxidation; 3) inhibition of DNA fragmentation and oxidative damage; 4) decreased activity of microglia and astrocytes; 5) inhibition of apoptotic protein expression; 6) increased expression of mitochondrial genes; 7) reduced levels of eicosanoids (including leukotrienes), prostaglandins, and thromboxane; 8) increased expression of anti-apoptotic proteins; and 9) reduced expression of inflammatory mediators. In the majority of conducted studies on the protective effects of medicinal plants and their compounds.
against cerebral ischemia, the toxic effects of these plants have been disregarded and the line between their therapeutic effects and toxicity has not been established yet.

In a study by Chandrashekhar and colleagues, basil extracts at 10-2000 mg/kg concentrations were fed to rats and the lethal dose 50 (LD$_{50}$) was determined after 24 hours. Finally, 10%, 20%, and 30% extracts were studied for LD$_{50}$ in the ischemic model (50). Moreover, Buch et al. investigated the acute toxicity of palmarosa extracts (500 mg/kg) in rats. Eventually, 10% and 20% doses were studied against ischemia in rats (42).

However, other indices such as body weight and pathological/histological changes of organs were not evaluated in these studies. Therefore, to ensure the safety of consumers, it is advisable to examine the toxic effects of medicinal plants in addition to their therapeutic effects and active ingredients (51). The review of previous research on the protective effects of medicinal plants against ischemia indicated that the majority of these studies have been conducted on animal models, while so far, no research has been conducted on the protective effects of medicinal plants on cerebral ischemia in humans. Since the biochemical compounds of plants are metabolized during enzymatic processes of the body and liver (causing changes in the structure and influence of these plants), it is recommended that in addition to pre-clinical studies, clinical studies be conducted, as well. During ischemia and reperfusion, cellular and molecular processes damage neuronal and non-neuronal cells, as well as brain capillaries. Some of these processes include stimulus-induced cytotoxicity, oxidative and nitrative stress, lipid peroxidation, ion imbalance, release of inflammatory mediators, and mitochondrial dysfunction. Considering the complex pathological events in the process of ischemia-reperfusion, use of one single agent for treatment does not seem ideal. The review of studies on the protective effects of medicinal plants and their compounds indicated that these plants could reduce the damage caused by ischemia through several different pathways. Therefore, development of protective factors from medicinal plants could be a promising strategy for the treatment of ischemic brain damage and the associated neurological disorders.

It is suggested that future studies focus on the effects of medicinal herbs and their constituents in human models. In addition, the toxicity and leakage of these compounds through the blood-brain barrier should be determined.

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References