Boswellic Acids as Promising Leads in Drug Development against Alzheimer’s Disease

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Abstract
Biological activity of Boswellia extract (BE) has been attributed to its main active ingredients; i.e. Boswellic acids (BAs). BE/BAs possess a promising therapeutic potential in neurodegenerative disorders; including Alzheimer’s disease (AD). The multifactorial nature of AD pathophysiology necessitates the development of the disease-modifying agents (DMA). Recent multi-targeting approaches for the DMAs development have brought more attention to the plant-derived compounds regarding their better human compatibility because of their biological origin. This review addresses the current knowledge on the anti-AD activity of BE/BAs based on the available in silico, in vitro, in vivo studies and clinical trials. The contribution of BE/BAs in inflammatory pathways, Tau and β-amyloid proteins, microtubule functions, oxidative stress, cholinesterase and diabetes/insulin pathways involved in AD have been discussed. BAs efficacy in different AD-related pathways has been confirmed in vitro and in vivo. They can be considered as valuable scaffold/lead compounds for multi-targeted DMAs in anti-AD drug discovery and development.

Introduction
Boswellia plant is native to India, Arabia and the northeastern coast of Africa.1,2 Boswellia extract (BE) or Frankincense gum resin has been used in traditional medicine in India and Iran.3,4 Different species of Boswellia produce BE. BE is known as Kundur in Iran. Persian physician, Avicenna, has recommended BE for the memory function improvement and prevention of amnesia in elderly persons, in his book (the Canon).4,5 In addition to medical applications, it is also used as incense in religious ceremonies and cosmetic ingredients (antiseptic agents in mouthwash, a fixative in perfumes, soaps, creams, lotions, and detergents).3 Chemical composition and biological activities of BE has been studied for years.1,2,6 BE contains essential oil (5–15%), mucus-like cluster (12–23%) and a lipophilic part (55–66%). The composition varies across different species (Serrata, Papyrifera, Carterii) and different grades.6,9 The lipophilic part includes terpenoid compounds. Tri-terpenoids that are known as Boswellic acids (BAs) are the biologically active entities of BE. BAs have a carboxylic group which connected to a pentacyclic triterpene containing at least one other functional group.2,10 Figure 1 shows the chemical structure of the most studied BA derivatives; i.e. β boswellic acid (βBA), Acetyl-11-keto-boswellic acid (AKBA), 11-keto-boswellic acid (KBA). These compounds are highly lipophilic and possess poor absorption characteristics.11 Acidic forms of these derivatives have ionization constant around 4 which could be a reason for their poor absorption in contrast to their lipophilic nature. More details about the BAs chemical characteristics along with their synthesis

Figure 1. Chemical structures of Boswellic acid derivatives.
and biosynthesis could be found in Al-Harrasi recent book about the BAs chemistry and bioactivity. AKBA and KBA are the most potent compounds and βBA is the most abundant derivative in extract. Some derivatives of BAs are available commercially. BAs are responsible for the majority of the medical properties of Boswellia. The therapeutic properties of for BE/BAs such as anti-inflammatory, antioxidant, immune-modulatory, chemotherapeutic, anti-hyperlipidemic and anti-obesity properties have been reported. BAs efficacy in asthma, brain tumor edema, rheumatic diseases, and ulcerative colitis and degeneration of specific neuronal populations are among the most studied factors that lead to cognitive failure. The etiology of AD is unknown and the multifactorial pathogenesis of it has been discussed. Some of the pathophysiologic mechanisms of AD such as cholinergic mechanisms, protein abnormalities, oxidative stress, mitochondrial dysfunction, and neuroinflammation have been studied for years. The available approved drugs against AD; i.e. acetylcholine esterase inhibitors (AChEIs: Donepezil, Galantamine) and N-methyl D-aspartate (NMDA) antagonists (Memantine), are symptomatic relief agents. The multifactorial nature of AD pathogenesis makes effective treatment development challenging. Recent approaches in AD treatment are suggesting multi-target agents with disease-modifying capabilities. Different synthetic and natural compounds with known multifunctional capabilities were studied as probable disease-modifying agents (DMA) in AD. Different studies suggested an effective role for BE/BAs in AD-related pathways. Tau and β Amyloid (Aβ) pathways, cholinergic pathways, inflammatory pathways, oxidative stress pathway have been studied in a relatively early phase. The inhibition of 5 lipoxigenases (5-LO) and nuclear factor κB (NFkB) have been reported recently. The inhibition of acetylcholinesterase (AChE) enzyme activity, and microtubule (MT) assembly dynamics for BAs have been reported. Also, possible beneficial effects of BAs on the experimental models of AD have been investigated. In vivo studies showed that oral administration of BAs has resulted in the inhibition of Aβ and Tau deposition in the brains of AD animal models. Also, improvements in behavioral impairment were observed in the same study. A number of review papers published in recent years which mentioned the anti-AD capabilities of BAs have reviewed the molecular targets attributed to BAs pharmacological uses and biological activities. Along with a diverse range of diseases, they also discussed the contribution of BAs to AD treatment briefly. Phytochemistry and potential therapeutic actions of BAs were reviewed in the year 2017 by Iram et al., in which they discussed BAs neuroprotective effects along with other medicinal effects. The pharmacological activities of BE derived compounds were reviewed by Sultana and co-authors. They discussed the anti AD activity of BAs and suggested that it might be related to their anti-inflammatory activity. Neuropharmacological effects of triterpenoids were reviewed by Parmar et al. in the year 2013. They listed BAs among the pentacyclic triterpenoids which possess behavioral and psychopharmacological effects that could be contributed to their anti-inflammatory activity. Hamidpour et al. reviewed the Boswellia application as the novel phytotherapy for the prevention and treatment of serious diseases including memory function. Yoo and Park in the year 2012 reviewed the role of terpenoids in AD disease. They discussed the anti-AD effect along with molecular targets of Ginkgolides Panax ginseng, Bilobalide from Ginkgo biloba, and Cannabis indica. Terpenoids originated from Boswellia species omitted in their paper. BAs biological efficacies were reviewed by Shah et al. in the year 2008 as a group of medicinally important compounds. The authors included the results of the publications from 1980-2008 in their study. They mentioned the anti-AD activity of BAs, and related to the BAs anti-inflammatory activity. An evidence-based systematic review of Boswellia, published in the year 2004 in which the authors, discussed Boswellia's role in inflammatory disease, while no evidence or study relating its effect in NDDs such as AD were included. BE/BAs as phytotherapy agents and lead compounds for AD treatment have not been reviewed and there is no publication including detailed mechanism-based discussion on their probable role in AD. The present study provides an overview of the BE/BAs role in AD treatment based on the pathophysiology of AD. The available studies, patents, clinical trials that provided information about the BE/BAs in AD pathways have been reviewed and discussed. The included publications have been discussed in 4 main categories; i.e. in vitro studies, animal studies, human studies, and clinical trials.

**Methods**

All published papers and patents from the year 1980 to 2020 were searched using different combinations of relevant keywords; i.e. Boswellia, boswellic acid, Alzheimer's disease, terpenoid, natural compounds, disease-modifying agents, multi-target. Google Scholar,
PubMed, Science Direct, ACS, Proquest, US patents, Clinical trials, were searched. Clinical trials were included in the review if they investigated the AD symptoms even in other diseases. Overall 165 papers were found that have investigated/reviewed the BA/BEs role in AD-related targets or pathways. Some of the included papers have not been investigated BE/BAs in AD but they have reported the relevant molecular mechanisms of BE/BAs.

Pathophysiology of AD and Related Treatment Strategies
AD is a progressive NDD, in which cognitive function impairment followed by behavioral changes happens. Although AD is known for about a century, effective medication to prevent, halt, or reverse of AD is not available yet. One reason for such a failure in AD treatment development is due to the complicated pathophysiology of AD. The pathophysiologic mechanisms involved in AD was shown schematically in Figure 2. According to the Figure 2, different pathways and mechanisms have been identified for AD in which Cholinergic, Aβ/Tau and, inflammation pathways are among the most studied pathways. Due to various types of targets, different approaches have been utilized for AD drug development. Table 1 shows a summary of the available strategies for AD drug development. Most of the strategies are in the research phase. The most studied AD development mechanisms and the related drug development strategies are reviewed in the following sections.

Cholinergic pathway
Cholinergic neurons depend on choline for returning to resting state after activation. Choline is provided to them mainly by acetylcholine (ACh) hydrolysis with AChE. The deficiency of choline, in AD patients, ended up with the cholinergic hypothesis. Cholinergic neurons as the main players in AD neurotransmission are responsible for maintaining the cortical function, cerebral blood flow, cognitive/memory function, learning a task, cerebral cortex development, and sleep-wake cycle regulation. A part of the complexity of the symptoms in AD is due to the multifunctional nature of the cholinergic neurons. The dysfunction of the cholinergic system in AD occurs at various levels including a decreased AChE activity, reduced choline uptake, decrease in ACh synthesis and altered levels of ACh receptors (AChR). AChEIs (e.g. Tacrine, Donepezil, Rivastigmine and Galantamine enhance cholinergic neurotransmission, and are the major class of FDA approved drugs for AD. AChE possesses some choline independent roles in AD. Its contribution to inflammation-related mechanisms has been studied and considered as possible targets for AD drugs. The other mechanisms such as complex formation with Aβ and its contribution to cell toxicity have been studied.

Protein abnormalities
Aβ and Tau proteins are among the most studied proteins in AD. Their aggregation is known as one of the main hallmarks in the AD brain. Tau is an unfolded cytosolic protein located in the brain, specifically in the axon of neurons. Human Tau which is a microtubule-associated protein (MAP) encoded on chromosome 17q21. It plays an important role in axonal

Figure 2. Probable Alzheimer’s disease pathways affected by Boswellic acids
Table 1. AD treatment drug development strategies

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Involved mechanisms</th>
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<tbody>
<tr>
<td>Modulating neurotransmission</td>
<td>Acetylcholinesterase inhibitor (AChEI)*</td>
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<tr>
<td></td>
<td>N-methyl D-aspartate (NMDA) antagonism*</td>
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<td></td>
<td>GABAergic modulation</td>
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<td>Serotonin receptor modulation</td>
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<td>Histaminergic modulation</td>
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<td>Adenosine receptor modulation</td>
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<td></td>
<td>Phosphorylation inhibition</td>
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<td></td>
<td>Microtubule stabilization</td>
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<tr>
<td>Tau-based therapy</td>
<td>Oligomerization blocking</td>
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<td></td>
<td>Enhancing Tau degradation</td>
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<td></td>
<td>Immunotherapy</td>
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<td></td>
<td>Secretase enzyme modulation</td>
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<tr>
<td>Amyloid based therapy</td>
<td>Aggregation preventing</td>
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<tr>
<td></td>
<td>Cleavage promoting</td>
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<tr>
<td></td>
<td>Immunotherapy</td>
</tr>
<tr>
<td>Intracellular signaling cascade</td>
<td>Inhibition of the NF-kB system</td>
</tr>
<tr>
<td>Oxidative stress reduction</td>
<td>Inhibition of the production of ROS</td>
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<tr>
<td>Anti-inflammatory therapy</td>
<td>Inhibition of COX2, 5 lipoxygenase enzymes, interfering with complement pathway and reduction of inflammatory mediators</td>
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<tr>
<td>Mitochondrial targeting therapy</td>
<td>Microtubule stabilization</td>
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<td></td>
<td>Intranasal insulin</td>
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<tr>
<td>Others</td>
<td>Cell replacement</td>
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<tr>
<td></td>
<td>Autophagy activators</td>
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<td></td>
<td>Metal chelators</td>
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* Approved by FDA

microtubule (MT) stabilization, neuronal development, neuronal polarity\(^6\) and maintenance of DNA.\(^6\) Tau aggregation is promoted by charge compensation of the basic middle part of Tau which is triggered by polyanions.\(^6\) Aggregates of hyperphosphorylated Tau makes intracellular neurofibrillary tangles (NFTs). Distribution of Tau oligomers correlates with the duration, progression and clinical stages of AD (Braak stages).\(^5,6\) In addition, impairment of axonal transport in the mature neurons is a common factor in many of the major NDDs, including AD.\(^6\) MTs are cytoskeletal structures that are critical for stable neuronal morphology and physiologic functions of neurons. Impairment of axonal transport in mature neurons (that depends on normal functions of MTs), is a common factor in many of the NDDs, including AD.\(^6\) Defects in MT assembly and organization have been reported in AD. Brandt and Bacota\(^6\) described the involvement of structure and dynamics change (Tau dependent/independent) of MTs in the neurodegenerative triad of AD. They argued that MT dynamics modulation could be regarded as a potential target for AD drugs.

Aβ is responsible for extracellular senile plaques.\(^6\) At physiological concentrations Aβ peptide (picomolar), improves memory function. The overproduction of Aβ in the brain of AD patients and individuals with Down’s syndrome is known as a promoter of oxidative damage which produces a chain of damage in neurons and synapses and starting point for the AD development.\(^6\)\(^7\)\(^8\) The AD initiation is connected to Aβ accumulation. Aβ-induced neurotoxicity is dependent on NFTs. The levels of Aβ plaques is not a discriminative factor between normal aging and AD.\(^6\)\(^7\) Recent studies results are supportive of the idea of targeting Aβ and Tau, as a promising approach, for the development of disease-modifying therapies in AD.\(^7\)\(^7\) Inhibition of Aβ and Tau aggregation in the brain is one of the most studied strategies for the development of AD therapies. During the past decade, various phytochemical compounds (e.g. Curcumin, Cinnamon) have been reported to be able to interfere with both Aβ and Tau aggregation and evidence provided using in vitro studies.\(^4\)\(^8\)\(^7\)\(^2\)\(^7\)\(^3\) Inflammation

Neuroinflammation exacerbates AD pathogenesis. Aβ, NFTs and neurodegeneration are the most likely
sources for inflammation in the AD brain. They induce inflammation via inflammatory cytokines and chemokines (e.g. IL-1β, IL-6, IL-8, TNF-α, TGF-β, MIP-1α, IFN-γ, NF-kB) as well as the complement system activation mechanisms which are unique to the central nervous system (CNS).4,25 Joshi and Pratico reviewed the role of lipoxygenases (LOXs) in the pathogenesis of AD and tried to discuss LOX contribution in both oxidative and inflammatory pathways.6, 7 5-LOX is playing a role in the metabolism of Tau and Aβ through FLAP (5-lipoxygenase activating protein),29 γ-secretase,37 protein kinases A and C (PKA, PKC) and MAP kinases (ERK1/ERK2, p38).78 Different studies showed that microsomal prostaglandin E synthase-1 (mPGES-1) is upregulated in the AD brain of rats29 and humans.80 The results of the study of mPGES-1 upregulation in the human AD brain suggested a potential role for the functional replacement of cytosolic PGES or inactive mPGES-1 in later stages of AD.40 The role of mPGES-1 in inflammatory brain diseases including AD was reviewed by Ikeda-Matsu.41 They discussed the promising capability of mPGES-1 as a target for the new therapeutics in this area. Different studies suggested that treatment with anti-inflammatory drugs, such as NSAIDs, reduces the incidence and progression of AD.28,82

**Oxidative stress**

Oxidative stress has an important role in the pathogenesis of AD through damage to vital cellular elements such as nucleic acids, lipids, and proteins.83 It is well known that oxidative stress and antioxidant imbalance is one of the hallmarks of AD. Oxidative stress pathway is one of the most interesting pathways in the multi-targeting anti-AD agents’ development.44-46 Wojsiat et al.84 reviewed the impact of oxidative stress and oxidant/antioxidant imbalances in AD. They concluded that both the reactive oxygen species (ROS) generation and the cellular oxidative stress defense mechanisms are compromised in the brain as well as in the peripheral tissues in AD.44 Mitochondria dysfunction is known as an important factor involved in the pathogenesis of AD, through the production of ROS,29,30,79 and modulation of Tau phosphorylation.63,89 Aβ and Tau-induced oxidative stress in neuronal cells is a cause of AD pathology and antioxidant therapy is one of the pharmacological approaches for AD treatment.63

**Diabetes and Insulin**

Evidence for the prevalence of diabetes and AD is getting higher.90 There are a lot of studies which confirm the hyperglycemia as a potential risk factor in the development of AD. A lot of studies showed that diabetes-induced impairment of memory is associated with an increase in oxidative stress.91 Hyperglycemia induces brain insulin resistance, increases Aβ accumulation on brain lesions, neuroinflammation and mitochondrial dysfunction. It also impairs neuronal integrity and causes neurodegeneration.92,93 In both cell culture and transgenic mice studies, high glucose condition increases Aβ production by inhibiting Aβ precursor protein (APP) degradation, not by increasing APP synthesis and abnormal insulin signaling in the brain. Insulin resistance increases Aβ accumulation and Tau phosphorylation in diabetic rodent models.94 Systemic administration of insulin is associated with reduced penetration in the brain and a higher risk of hypoglycemia.95,96 In several clinical studies, intranasal administration of insulin has been tested.97 After intranasal administration, insulin bypasses the Blood-Brain Barrier (BBB) and reaches significant biologic concentrations in the brain.96,97 Intranasal insulin administration improves memory and enhances the mood in AD patients in which improves cerebral glucose metabolism and preserves the volume of brain regions affected by AD pathology.96,97 In vitro studies showed that insulin inhibits neuronal apoptosis via activation of protein kinase B.96,98 In vivo studies revealed that insulin regulates Tau phosphorylation, APP metabolism and Aβ clearance.99 Prevent or ameliorate cognitive dysfunction through Type 2 diabetes-specific treatment is one of the AD treatment approaches which has been interested in researches in recent years.96 One of the main characteristics of this approach is the availability of a large number of common hallmarks between AD and diabetes in which oxidative stress and inflammation could be highlighted.

**BEs’ and BAs’ Efficacy in AD**

**In Vitro studies**

The probable molecular mechanisms of BE/BAs efficacy as anti-AD treatments were investigated in vitro and the results are discussed in different studies. Table 2 contains the AD-related in vitro studies of BE/BAs. The details of the related studies are discussed in the following sections.

**Inhibition of AChE activity**

Riazi and coworkers reported the effect of amino acid derivatives of βBA and AKBA on the inhibition of AChE in the in vitro studies.91,100 They showed that the coupling of AKBA and βBA with valine and leucine amino acids can successfully strengthen the effect of BAs on the decline of the AChE activity. Other studies suggested high AChEI activity (46-71% inhibition) for BE,98 and the Boswellia oil (96% inhibition and IC_{50} value 0.043±0.02 mg/ml).101 Ota and Houghton reported high AChEI activity (80%) for 11-hydroxy–βBA.102

**Microtubule stabilization**

In an in vitro study, Karima et al.40 reported the effect of βBA on hippocampal neurite outgrowth and branching. They showed that βBA has the capability of the enhancement of neurite outgrowth, branching, and tubulin polymerization dynamics. Following that study, they examined the effect of βBA on the assembly dynamics behavior of tubulin to address the mechanism of neurites outgrowth and branching enhancement by βBA. They reported that βBA could significantly enhance the MAP polymerization dynamic. No references are needed for this section.
and MT length, which may consequently prevent axonal degeneration and MT disruption. They concluded that βBA can be a useful agent against NDDs and memory loss.

**Tau and Aβ proteins**

Different studies reported the effect of terpenoids on Aβ/Tau aggregation, and Tau hyperphosphorylation.\(^{45,91}\) Investigation of the effect of βBA on human astrocytes revealed its capability to reduce hyperphosphorylated Tau.\(^{103}\)

Our investigation on the probable direct interaction between Tau (hTau34) protein and βBA using Surface Plasmon Resonance (SPR) technique, suggested the formation of Tau-βBA complex under physiologic pH.\(^{104}\) Molecular docking investigation in two different studies revealed that terpenoids are the most potent and safe inhibitor of both AChE and Aβ aggregation.\(^{105,106}\)

We investigated the Tau interaction with βBA and the results showed the capability of βBA binding to Tau via PGGG loops by hydrophobic interactions as the main driving force for the binding.\(^{104}\) Additional studies are required to investigate the precise activity of BAs on Aβ and Tau related AD treatment.

**Antioxidant activity**

Antioxidant activity of BEs was studied using methanol extract of *Salvia macrochlamys* and the results confirmed their antioxidant potency and capability for use in pharmaceutical as antioxidant agents.\(^{16}\) Antioxidant activity (free radical scavenger capacity) of *B. serrata* aqueous extracts were studied and the results revealed a dose-dependent antioxidant activity.\(^{107}\) The similar results were obtained for *Boswellia* oil\(^{108}\), and methanolic leaf extract.\(^{109}\) A recent *in vitro* study on the antioxidant power of BEs revealed the higher antioxidant potency for the extracts with higher concentrations of polyphenols and AKBA.\(^{110}\)

**Anti-inflammatory activity**

BE/BAs anti-inflammatory activity has been reported in different studies.\(^{8,19,111}\) Different studies showed that AKBA reduces chronic inflammation through the inhibition of the NFkB system\(^{14}\), while other inflammatory targets; e.g. LL-37 (cathelicidin related peptides) and Human leukocyte elastase (HLE) have been reported\(^{112}\) as well. Also, BAs inhibit the 5-LOX enzyme and consequent inhibition of 5-hydroxyeicosatetraenoic (5-HETE) and leukotriene B4 (LTB4) production\(^{86,113}\), HLE.\(^{1,112}\) They also reversibly suppress the transformation of prostaglandin (PG) H2 to E2 mediated by mPGES-1 (IC\(_{50}\) \(\approx 3–10\) mM).\(^{114}\)

As discussed earlier in this paper 5-LOX plays a significant role in Tau and Aβ metabolism and its inhibition with BAs could be considered as one of the main AD treatment mechanisms of BAs.

The results of some BAs and their semisynthetic derivatives 5-LOX inhibitory activity showed that AKBA and KBA are more potent inhibitors than βBA and 3-O-acetyl-BA.\(^{8,111,115,116}\)

Suppression of PGE2 formation by BAs (AKBA, KBA, and βBA) via interference with mPGES-1, was studied using a protein fishing approach and the results showed that BAs could reduce PGE2 formation by inhibiting mPGES-1.\(^{114}\) Also, they reported concentration-dependent blocking of PGE2 biosynthesis in intact A549 cells.\(^{114}\) The results of a cell-based assay in the same study suggested a functional role for BAs in PGE2 formation reduction, in which βBA

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Assay method</th>
<th>Studied ingredient</th>
<th>Results</th>
<th>Ref.</th>
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<tbody>
<tr>
<td><strong>Microtubule stabilization effect</strong></td>
<td>Embryonic hippocampal cells</td>
<td>10,20,30 nM βBA</td>
<td>βBA could increase both MT length and axonal outgrowth and branching</td>
<td>4,40</td>
</tr>
<tr>
<td><strong>Inhibition of AChE activity</strong></td>
<td>Spectroscopy</td>
<td>βBA/ AKBA</td>
<td>Inhibited AChE activity</td>
<td>34,102</td>
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<tr>
<td></td>
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<td>BE, AChE-I</td>
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<tr>
<td><strong>Anti-oxidative activity</strong></td>
<td>Free radical scavenging assay</td>
<td><em>B. serrata</em> 500 µg/mL</td>
<td>Antioxidant activity in a concentration dependent manner</td>
<td>107</td>
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<tr>
<td></td>
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<td><em>Boswellia</em> oil</td>
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<tr>
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<td>15,114</td>
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<tr>
<td></td>
<td>HLE, 5-HETE, LTB4 production by spectroscopy</td>
<td>AKBA, BE</td>
<td>Inhibition of 5-LOX</td>
<td>8,11,36,112,113</td>
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<td></td>
<td>NF-κB-dependent cytokine expression</td>
<td>BE</td>
<td>Inhibiting NF-κB activity, decrease NO production</td>
<td>19,42,111,117</td>
</tr>
<tr>
<td></td>
<td>Human whole blood, A549 cells</td>
<td>βBA</td>
<td>Suppressed levels of PGE2 mediated by mPGES1</td>
<td>114</td>
</tr>
</tbody>
</table>

Table 2. The *in vitro* studies of BE/BAs on the AD related targets
selectively reduced the PGE2 formation. This evidence suggests the contribution of the anti-inflammatory activity of BEs/BAs, as a part of their promising effect in AD treatment.

**Anti-diabetic activity**

Both type 1 and type 2 diabetes activity of BE and KBA have been studied. Ammon reviewed the present evidence of the therapeutic effects and the underlying molecular mechanisms of BE and/or KBA in the prevention/ treatment of diabetes mellitus recently. The author concluded that the BE and/or KBA may prevent insulin resistance in type 1 and type 2 diabetes by inhibiting the expression of proinflammatory cytokines from immunocompetent cells. There is no in vitro study which specifically studies the effect of BE/BAs on shared mechanisms of AD and Diabetes. We studied the effect of βBA-Tau interaction in the presence and absence of the glucose in which the results showed that the presence of glucose interferes with the βBA-Tau complex formation in a way that affinity decreased significantly by the enhancement of glucose concentration.

**Preclinical studies of BEs/BAs role in AD**

BE/BAs effect in AD has been studied extensively in animal models. Some of the reported preclinical studies are summarized in Table 3. According to the table, most of the conducted studies confirmed the capability of BE/BAs in the AD treatment. BBB permeability of BAs studied using a rat model and the results revealed the highest brain/plasma ratio for βBA compared to KBA and AKBA which indicates facilitated BBB permeability for βBA. The crossing of the BAs from the BBB and improvement of Aβ metabolism studied using a rat model and NFTs formation studied in AD induced rats. Oral administration of BE (90 mg/kg) to the adult male Sprague Dawley rats had protective effects against oxidative damage. Also returning to the healthy neurons in the affected brain regions and NFTs level reduction was reported in their study. The different probable underlying mechanisms for the contribution of BE in AD treatment were studied on adult male Wistar rats with AD (induced using AICl). Oral administration of BE resulted in a significant reduction in brain ACh along with significant elevation in serum and brain AChE. Also, a significant decrease in brain CRP, NFkB, MCP-1, and LTβ4 levels was observed. They concluded that the potent effect of B.serrata against AD stems from its ability to ameliorate cholinergic dysfunction, inhibit the inflammatory mediators and promote neuronal survival. A group of researchers reported the increased level of ACh and decreased level of AChE enzyme in AD rats (induced using AlCl3) after oral treatment with BE. The reported variation in the cholinesterase pathway showed a dose-dependent activity.

In a recent study, the effect of BE on the cognitive impairment of diabetic rats was investigated. The results showed that hippocampal elevated levels of AChE were significantly decreased due to BE consumption. Different studies showed that oral BE reduces inflammatory mediators (IL-1β, IL-6, TNF-α, IFN-γ, cathepsin G (Cat G) and PGE2 level significantly, and increases the level of IL-10. The effect of B.carterii on memory loss in rats (induced by lipopolysaccharide (LPS)) was studied and the results showed a significant reduction of TNF-α in the hippocampus of rats as well as a significant increase in the step-through latency. The authors concluded that the hydroalcoholic extract of the Boswellia was able to retrieve the memory of studied rats probably due to anti neuro inflammatory property.

Werts et al. investigated the BAs' contribution to the reduction of PGE2 formation. They studied carrageenan-induced rat pleurisy and mouse paw edema after intraperitoneal or oral administration of BAs. They found that βBA selectively suppresses the production of PGE2, while cyclooxygenase (COX) pathway metabolites' levels were not altered. According to their results βBA clearly exhibited anti-inflammatory activity. They concluded that interference with mPGES-1 might represent a reasonable molecular mechanism contributing to some of the anti-inflammatory properties of BE. Increased or decreased level of cathepsin as a lysosomal protein, can lead to functional impairment and gradual death of neurons. A systematic review published recently provided an overview of the role of cathepsins in AD. According to their review, the main role of cathepsins is related to their contribution to Aβ degradation. Another review paper discussed the positive and negative contribution of cathepsin B in AD which mostly is related to the Aβ degradation/metabolism. Other researchers reported the upregulation of cathepsin D in the AD human brain neocortex and inducing of AD-like phenotype in LPS treated mice, by cathepsin B. Most of the other similar studies investigated the role of cathepsins B and D (cysteine cathepsins) in AD and another aging-related disease. The BAs effect on cathepsin G was investigated using cell based and computational methods, and the results showed that BAs interact directly and functionally with Cat G.

There are few reports about the contribution of cathepsin G in AD which are against the probable role for it in AD. There is no report on the probable interaction of BAs with other cathepsins.

The effect of the combination therapy of AKBA, as a 5-LOX inhibitor and Celecoxib as a selective COX-2 inhibitor on LPS cognitive dysfunction mice model, was studied and the results showed improved cognitive-behavioral functions for dual enzyme inhibitors compared to monotherapies. A histochemical study of the mice brain showed a reduction of the Aβ deposition in the cerebral cortical region in the same study. According to the results, the effect of AKBA on cognitive behavior improvement was comparable with Celecoxib.

The effect of BE on the cognitive impairment reduction of diabetic rats was investigated recently, and the results showed the probable contribution of the antioxidant...
effect of the active compounds on the preventive effect of BE on cognitive impairment.\textsuperscript{130} According to this study BE treatment produced a significant reduction in an oxidative damage marker (Malondialdehyde (MDA)), in the hippocampus of diabetic rats and enhanced the antioxidant enzyme activities of glutathione peroxidase (GSH) and Superoxide dismutase (SOD). Neuroprotective effect of BE due to the antioxidant effect was studied in a rat model and the results showed reduced GSH content and increased MDA level in the cerebral cortex that suggests the antioxidant activity as a mechanism of anti-AD efficacy.\textsuperscript{131}

Some studies reported that BEs can improve behavioral and anatomical deficits.\textsuperscript{132,133} Beheshti and Aghaei investigated the effect of BE in streptozotocin-induced AD models of adult male Wistar rats.\textsuperscript{55} Their results showed that chronic administration of BE improves dementia type AD in a time-dependent manner.\textsuperscript{55} In another study,\textsuperscript{132} the results of the administration of BE before LPS injection to the male Wistar rats indicated that BE reduces the LPS induced memory. Also, the reduction of TNF-\(\alpha\) levels in the hippocampus of rats was reported in the same study. They suggested that the anti neuroinflammatory activity of BEs could be involved in their Anti AD effect.

Protective effects of BE against AD in male Albino rats (\(\text{AlCl}_3\), induced AD) were studied, and the results were in agreement with previous findings of the probable anti AD activity of BE.\textsuperscript{134} The results showed that in addition to the memory and learning improvement, \textit{B. carteri} extract can possess a neuroprotective effect on rats with AD. Prevention of lipid peroxidation (LPO) rising, increased levels of GSH, Glutathione disulfide (GSSG) and SOD,

| Table 3. The \textit{in vivo} studies of BE/BAs anti-AD effects in animal models |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Animal model                    | Treatment                        | Neuroopathological and biochemical investigation                                                                 | Behavioral investigation                                | Ref. |
| Wistar rats                     | BSE (68.75 , 137.5 mg/ kg/d) orally for 3 months | A significant increase in brain ACh, a significant decrease in brain and serum AChE activity, CRP, NF\(\kappa\)B, MCP-1, LTB4, Bc-2 and A\(\beta\) plaque burden in hippocampus and striatum | ND                                                                                                    | 42   |
| Sprague Dawley rats             | BSE (90 mg/kg /day) orally for two weeks       | A significant increase in Brain ACh, a significant decrease in brain AChE activity and decrease A\(\beta\), NFT plaque burden | Retrieval of memory in the behavioral tests                                             | 21   |
| Wistar rats                     | BSE(50 mg/kg; orally for 6 weeks)              | Significant reduction in hippocampal TNF-\(\alpha\) level                                                                                           | Significant facilitation of spatial learning and memory                                      | 55,115 |
| CD1 mice, Wistar rats           | \(\beta\)BA (1 mg/kg; I.P. or orally 30 min before carrageenan) mouse paw edema and pleurisy  | Markedly lower PGE2 levels by inhibition of mPGES1, inhibition of cathepsin G and 5-LOX activity                                                           | ND                                                                                                    | 114  |
| Albino diabetic rats            | BE(500 mg /kg/day) orally for 5 weeks         | Ameliorate the NDDs characteristics of AD. Significant increase GSH content                                                                     | Improve memory and learning                                                                          | 130  |
| Wistar rats                     | \textit{B. Carterii} (40, 80 and 160 mg/kg/d, I.P for 3 weeks)  | Significant inhibitory effect against AChE increased the GSH content and reduced the MDA level                                                                 | Significant improvement in learning and memory                                                      | 131  |
| Wistar rats                     | BSE(100 mg/kg/d; orally for 8 weeks)           | Neuro-anatomical basis for memory improvement, attenuate dendritic regression in CA1 of Hippocampus.                                                   | ND                                                                                                    | 132,135 |
| Wistar diabetic rats            | BAs (200-400 mg/kg/d; orally for 8 weeks)      | Reduced significantly the hippocampal elevated levels of caspase-3, AChE, GSK-3\(\beta\), TNF-\(\alpha\), IL-1\(\beta\), IL-6, MDA and GSH, SOD, glutamate receptor expression also inhibited extracellular deposits of amyloid plaques and the intracellular NFT | Significantly ameliorates cognitive decline                                                              | 91   |
| NMRI mice, Wistar rats          | \textit{B. Papyrifera} (50-150 mg/kg, orally)  | ND                                                                                                                                                | Facilitation of spatial learning and memory                                                            | 133,136 |
| Albino Wistar rats              | \textit{B. Carteri} (500 mg /kg/day) orally for 8 weeks | Significant decrease brain neurotransmitters as DA, NE, GABA, GSH, GSSD, SOD and AChE activity. Improve hippocampus histopathological changes | Improve the learning and memory                                                                      | 134  |

N.D (not described) - \textit{Boswellia Serrata} extracts (BSE) - Intraperitoneal( I.P)
enhancement in the neurotransmitter (Norepinephrine, Dopamine and gamma-Aminobutyric acid (GABA)) formation in the brain and decreased AChE activity was detected in BE treated AD rats in this study. According to these results, the authors suggested the contribution of antioxidative stress activity of the BE in its potential protective and therapeutic role in AD.

The effect of $B$. *papyrifera* extract on learning and memory of male Wistar rats and male NMRI mice was studied and the results showed that its oral administration significantly decreased the number of days required to make the mice learned as per set criteria and time took to find the food by the learned mice in the radial arm maze model. In addition, $B$. *papyrifera* extract leads to a decrease in escape latency as well as an increase in the animal swimming speed in the morris water maze model. Spatial memory retention in male rats was studied after administration of total extract of $B$. *papyrifera* and the results were compared with BAs fraction administration in the same study. According to the results, both total extract and BAs fraction reduced escape latency and distance traveled in studied rats significantly. In addition, their findings indicated a dose-dependent manner for the BAs fraction in spatial memory retention enhancement. Recently a group of researchers investigated the effects of chronic administration of BAs on the learning performance and the morphology of hippocampal granule cells in the aged rats. Their results showed that BE improved learning capability in the aged male Wistar rats. Also, their data showed the enhancement of dendritic arbors and dendritic spines in hippocampal granule cells.

According to the results of a recent study, *B*. *serrata* treated aged rats, had greater stratum pyramidal volumes and stratum radium lacunosum molecular. The results also indicated more numerical branching density in the apical dendrites of CA1 pyramidal neurons for the rats *B*. *serrata* extract treated rats. A recent study investigated the effect of BE on the cognitive impairment of diabetic rats, and the results showed that BE prevents cognitive impairment. The authors reported a significant decrease in Aβ deposits and p-Tau positive cells after BE consumption.

### Clinical studies

Few number of clinical trials have been reported the results of BE/BAs efficacy study in patients (Table 4). Most of the available trials are related to the inflammatory diseases such as bronchial asthma, osteoarthitis of the knee, joint discom fort, Crohn disease, collagenous colitis, chronic colitis, moderate plaque-induced gingivitis, erythematous eczema and psoriasis, osteo muscular pain, while other diseases such as heavy menstrual bleeding, diabetes, prevention of adju vant radiotherapy skin damage, multiple sclerosis (MS), osteoarthritis (as pain reliever) are studied too. The safety profile of BEs/BAs was investigated by monitoring the vital signs or measuring the hematological and biochemical parameter and no safety perturbation was observed. Overall good tolerability was reported in different trials. Only a few adverse effects (diarrhea, abdominal pain, and nausea) were reported. A prospective, placebo-controlled, and a double-blind pilot clinical trial was conducted on 27 patients with brain edema. 14 patients received a high dose of BEs (4.2 g per day) orally, while 13 patients received placebo. The results showed that BE was well tolerated after oral administration. Phase II studies for anti-inflammatory/anti-arthritic effects of BEs provide proof of the efficacy. The effect of a tablet containing *B*. *serrata* and *M*. *officinalis* extract, on older adults (70 years and older) memory, was studied through a randomized controlled trial and the results suggested beneficial effects for the administered tablets.

In a randomized controlled trial, a study in type 2 diabetic patients, administration of 400 mg *Boswellia* gum resin capsules two times a day after meal, leads to the reduced fasting blood sugar (FBS), glycosylated hemoglobin (HbA1c) and insulin. Also, cholesterol (Chol), LDL and triglyceride (TG) levels significantly decreased without any significant effects on the other blood lipid levels and liver/kidney function tests compared with the placebo at the endpoint.

A recent study of *B*. *serrata* extract effect on the blood glucose and lipid profile of diabetic patients showed considerable reduction after the intervention in the field of FBS, HbA1c, and TG in the BE administered group, while no significant difference was observed in all outcome measures between the two groups at the end of study. The effect of *B*. *papyrifera* extract on cognitive impairment in MS patients was investigated on 80 patients. The results showed that *B*. *papyrifera* significantly improved visuospatial memory, but it had no effect on verbal memory and information processing speed.

The efficacy of a mixture of *B*. *carterii*, *Z*. *officinale*, and *A*. *millefolium* was investigated on the severity of symptoms, anxiety, and depression in irritable bowel syndrome (IBS) patients. The results suggested a reduction of pain, anxiety, and depression in studied patients. Analgesic activity of *B*. *serrata* was investigated in healthy volunteers and the results suggested a significant enhancement in the pain threshold, pain tolerance force and time compared to placebo.

Researchers investigated the effect of herbal medicine, D.Loban (DL: a mixture of *C*. *rotundus* L., *Z*. *officinale*, *A*. *calamus* L., *P*. *nigrum* L., and *B*. *carterii*) on 24 patients with mild to moderate AD. The results showed the improvement of memory in DL treated patients compared with the placebo group. But they didn't report any data on the application of *B*. *carterii* alone. There is no published or submitted clinical trial to investigate the effect of BAs independently on AD.

### Patents

There is one patent in the USA that mentioned the application of incense and different forms of BAs derivatives in the treatment of AD.
BAs as Disease-Modifying Scaffolds

Today, drug discovery for AD is one of the most challenging and difficult tasks in medicinal chemistry. AD has multifactorial etiology and complex pathophysiology. Understanding the pathogenesis of AD, the critical elements underlying the mechanism of it and the disease process are crucial bottlenecks of AD drug discovery. Accordingly, over the last decade, a growing number of researches has focused on DMAs in which more than 80% of phase II and III DMAs are belonging to the anti-Aβ and anti Tau drugs. Anti-inflammatory agents and cell-protecting drugs are also studied frequently. The drug-target space in AD still is not completely understood and more information is needed about the underlying mechanisms of AD beginning, progression and ameliorating. Interdisciplinary and collaborative approaches along with a correlation between clinical outcomes and biomarkers are the main facilitators of DMAs development. Researchers are using a combination of diverse drug discovery tools such as phenotypic based drug discovery (better tools are developing by the application of advanced assay tools, organoids, and artificial intelligence-based data translation), poly-pharmacology based screening and multi-target drug design methods to develop DMAs for AD. They are hopeful about the potential of these strategies to develop new promising AD treatment drugs.

At the same time, the enterprise of drug discovery for AD is still challenging with respect to addressing potency, toxicity, and pharmacokinetic problems during the hit optimization. The availability of the relevant cellular and animal models that closely mimic human clinical conditions in AD is another challenging issue. The absence of effective tests to monitor the course of treatment and certify the cure of patients is also a very serious problem that must be faced in parallel with efforts to develop new drugs.

To address one of the main challenges of multi-target drug development, researchers are implementing a combination of different approaches including the use of plant-derived compounds such as Boswellia serrata extract.
drug discovery; i.e. small drug discovery, researchers have been using natural products as a valuable source of excellent small molecule scaffolds. Chemical entities originated from nature are among the most valuable lead compounds in drug design and discovery especially for complex diseases like AD. Natural compounds could be regarded as biologically pre-validated platforms for the design of compound libraries in the search for new drug candidates.\(^{159}\) In addition, their incredible diversity provides a higher possibility of candidate compound selection in the current paradigm of drug discovery i.e. multi-targeting approach rather than one molecule one target approach),\(^{160}\) especially for the complex disease.\(^{161,162}\)

A large number of studies have used natural compounds and related databases in the design and discovery of multi-targeted anti AD agents.\(^{104,161,163-165}\)

The current review provided a comprehensive summary of the available evidence on the effectiveness of BEs and BAs in AD. The biologic activities of BAs have been studied extensively confirming their multi-targeting nature. Al Harrasi et al. discussed Boswellia\(s\) diversity and biological activity within a recently published book.\(^6\)

Our review showed that although there are a vast number of \textit{in silico}, \textit{in vitro} and \textit{in vivo} preclinical studies on the probable contribution of BE/BAs in AD treatment. Clinical trials that are investigating the effect of BE/BAs in AD patients are rare. We believe that translation of the results obtained from preclinical studies to the clinical applications needs rigorous pharmacokinetic/ pharmacodynamic analysis and more molecular and mechanistic studies. Fortunately, the druggability of the BAs has been examined and described in different diseases which facilitates and accelerates AD drug designing from BAs. In addition, animal studies have confirmed the effectiveness of BAs in AD treatment.

Due to acceptable potency and diversity in targets and mechanisms of action, BAs are regarded as a valuable scaffold for multi-target based DMAs development for AD. Both additive (act on different targets in one pathway) and synergistic (act on different targets on different pathways) approaches in DMAs development for AD can be covered by BAs.

**Conclusion**

To the best of our knowledge, most of the available studies related to the BE/BAs efficacy in AD were reviewed and included in this paper. According to the current comprehensive review, BEs along with their active ingredients; i.e. BAs, are promising agents for AD treatment. Their anti-oxidative stress, anti-inflammatory, and anti-diabetic efficacies along with their AChE inhibitory and anti-Aβ activities together with their effects on the modulation of Tau deposition and hyperphosphorylation in the brain have introduced them as powerful multi-targeting lead compounds. These wonderful natural compounds could be considered as successful scaffolds in DMA discovery and development, for AD treatment. Although no clinical trial has been reported to approve the actual benefits of BE/BAs in AD patients, according to the traditional medicine of Iran and India, BE/BAs are efficient in the reinforcement of memory, especially in elderly people. Also, clinical trials approved BE/BAs efficacy in some diseases with shared hallmarks with AD (e.g. inflammation, oxidative stress, diabetic symptoms, memory function, etc). In conclusion, further \textit{in vitro} and \textit{in vivo} studies are needed to clarify the detailed mechanistic contribution of BAs to AD treatment. In addition, detailed \textit{in silico} studies are needed to discuss BAs structure-activity relationships and apply the results for rational DMA development against AD.

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**Conflict of Interest**

The authors claim that there is no conflict of interest.

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