Research Article

Synthesis and the Inhibitory Effects of Amino Acid Derivatives of β-Boswellic Acid on Acetylcholinestrase

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ABSTRACT

Extract of gum resins of Boswellia species contains compounds such as β-boswellic acid (BA) that has been identified as the major bioactive ingredients involved in anti-cancer and anti-inflammatory activities as well as the treatment for a broad range of neurodegenerative disorders such as Alzheimer's disease (AD). The molecular mechanism of advantageous pharmacological effects of such extracts is still not recognized. In this study, we prepared BA coupled with leucine, isoleucine, valine and alanine in alkaline conditions of reaction. Moreover, we evaluated their pharmaceutical efficacy by measuring acetylcholinesterase (AChE) activity. We concluded that some amino acid complexes of BA may be used as potential drugs in the treatment of AD due to suppress AChE activity; however, more in vivo experiments would be needed.

Keywords: Alzheimer disease (AD), Acetylcholinesterase, β-Boswellic acid (BA), Synaptosome.

INTRODUCTION

Phytotherapeutic agents have been commonly used in the treatment of many human diseases, and their pharmaceutical potential is attributed to various chemical ingredients separated from their extract. Frankincense, also referred to olibanum or oleogum resin, is a resin extracted from the trees of the genus Boswellia of Burseraceae family and is obtained after an incision into the bark of the tree. Twenty five species in the Boswellia genus have been recognized totally.[1] In recent years, compounds derived from Boswellia have attracted more researchers because of its therapeutic capabilities. Gum resin from Boswellia genus contains various compounds of terpenoids and sugars including more than 200 different materials such as polysaccharides, oils, protein and inorganic substances.[2] The pentacyclic triterpenic acids available in oleogum resin, named Boswellic acids (BAs) are the major natural components for therapeutic applications. Many of these BAs are naturally hydrophobic (fat-soluble) as non-boswellic acid compound isolated from frankincense known as incensole acetate.[3]

It has been demonstrated that compounds obtained from Boswellia have therapeutic and pharmaceutical effects for the treatment of inflammatory diseases such as rheumatoid arthritis, bronchial asthma, chronic bowel diseases and others.[4] Based on in vitro and in vivo studies, boswellic acids inhibit the synthesis of 5-lipoxygenase (5-LO) that is a pro-inflammatory enzyme responsible for the catalysis of two steps in biosynthesis of leukotrienes (LTs), a group of lipid mediators of inflammation.[5] Besides the anti-inflammatory effects, many studies have been done on its potential role in the treatment of cancer[6,7], anti-fungal[8], anti-diarrheal[9], hypolipidemic[10], hepatoprotective[11] and hypoglycemic[12] activities. Moreover, in several studies, it has been observed that the Boswellic acids exhibited therapeutic effects on crohn's disease[13] and has improving effects on neurodegenerative disorders such as Alzheimer's disease (AD).[14]

AD is a progressive neurological disorder in which the increasing impairment of learning and memory leads to a conclusive diagnosis. It is one of the most common causes of dementia in elderly people. Dementia is associated with the decline of cognitive functioning and behavioral capabilities that disturbs an individual's routine daily life and activities. AD is a multi-factorial disorder which its pathogenesis and its cause are still mostly unknown. The disease influences regions of brain that are responsible for higher mental activities, such as the neocortex and hippocampus, are those most disrupted by the known pathology of AD. Extracellular beta-amyloid deposits in senile plaques might be the leading cause of the disorder.[15] On the other hand, tau protein abnormalities could create neurofibrillary tangles inside nerve cell bodies[16] which cause microtubules to disintegrate and disrupt the neuron transport system.[17] Finally, cholinergic hypothesis which most presently accessible medicines for treatment of AD are based on that, suggests that reduced synthesis of the neurotransmitter acetylcholine (ACh) leads to the pathology of AD.[18] Declined activity of ACh-producing enzyme called choline acetyl transferase (ChAT)[19], decreased choline uptake[20] and the release of

ACh are the key factors in the deterioration in cognitive function observed in patients with AD.[21] It seems that cholinomimetic agents might be effective in the treatment of abnormal behavioral symptoms of AD. Thus, it has been shown that AChE inhibitors are greatly beneficial in the improvement of many symptoms of behavioral disturbance such as delusions, agitation, apathy and psychotic symptoms.[22]

The aim of this study was to combine BA with leucine, isoleucine, valine and alanine to synthesize new compounds in order to investigate their potential effect on the activity of AChE. We reported that some new compounds of pentacyclic triterpenes, including Boswellic acids, could block AChE activity in vitro. Leucine, isoleucine, valine and alanin were used to form amino acid salts of BA (I, II, III, IV) .The structure of new compounds was evaluated and confirmed by fourier transform infrared spectroscopy (FTIR), GC-mass spectroscopy (GC-MS) and CHN measurements and then, synaptosomal AChE activity was measured in the presence of these new compounds by Ellman method.[23] Maintaining ion homeostasis and membrane potentials by functional ion channels, carriers and receptors on synaptosome membranes, make them suitable elements as nerve terminal models to study synaptic cleft reactions very close to real state. According to these favorable features and the existence of critical components such as pre-synaptic, synaptic cleft and post-synaptic, synaptosomes were used as a perfect model for neural terminals in related researches.[21,24-26]

MATERIALS AND METHODS

Instruments

IR spectra were obtained with a JASCO FT-IR 410 spectrophotometer by KBr pellet method. MS spectra were determined with an HP-5973 network selective detector (electron impact, 70 eV) instrument. We used a PE 2400 Series II CHN Analyzer USA (Perkin Elmer) instrument to generate CHN spectra.

Materials

BA was provided by Sabinsa Corporation (Piscataway, NJ) and all other reagents in this study were produced by Merck (Darmstadt, Germany). All chemicals were reagent grade and were used without further purification.

Salt of the amino acid Leucine derivative of BA (BA-Leu) (I)

A solution containing 2g of leucine in 8 ml water was added to solution of BA (5g) in 95% aqueous methanol (125 mL) and stirred for 15 min. 2.6 mL aqueous solution of potassium hydroxide 20% (0.52 g KOH) was then added drop wise for 10 min and stirred continuously for 1 h. The solvent was evaporated under reduced pressure and dried to obtain compound (I) as yellow color powder.

Salt of the amino acid Isoleucine derivative of BA (BA-Ileu) (II)

Isoleucine (2g) was dissolved in water (8 mL) and obtained solution was added to a solution of BA (5g) in 95% aqueous methanol (125 mL). The mixture was stirred for 15 min and 2.6 mL aqueous solution of potassium hydroxide 20% (0.52 g KOH) was then added slowly for 10 min. The mixture was stirred for 1 h and the solvent was evaporated under reduced pressure and dried to acquire compound (II).

Salt of the amino acid Valine derivative of BA (BA-Val) (III)

0.2 g of BA powder dispersed in 5ml of 95% aqueous methanol and was added to a solution containing 0.2 g of valine in 1ml of water. The mixture stirred at room temperature for 15min and 2.6 mL aqueous solution of potassium hydroxide 20% (0.52 g KOH) was then added slowly to the reaction system, and stirred again at room temperature for 1h. Afterward, the mixture was evaporated under reduced pressure and dried in order to yield the bright-cream color powder as compound (III).

Salt of the amino acid Alanine derivative of BA (BA-Ala) (IV)

0.2 g of BA powder dispersed in 5ml of 95% aqueous methanol and was added to a solution containing 0.2 g of alanine in 1ml of water. The mixture stirred at room temperature for 15 min and then 2.6 ml of aqueous solution of potassium hydroxide 20% (0.52 g KOH) was gently added and stirred at room temperature for 1h. Finally, the mixture was evaporated under reduced pressure and dried in order to gain a high viscous brown matter as compound (IV).

Animal

Synaptosomes extracted from sheep brain were used to investigate the effects of new compounds on cholinergic synapses. The study was approved by the University of Tehran and Animal Sciences Research Institute of Iran. In this study, one adult male sheep (Afshari Persian) with 72.320 Kg body weight and appropriate body conditions was decapitated. The process of decapitation was carried out in the presence of Animal Sciences Research Institute representative and The Iranian Society for The Prevention of Cruelty to Animals agents in Ehsan slaughterhouse (Shahr-e-Ray, Iran). The carcass was delivered to Ehsan slaughterhouse. Sheep skull was cracked and intact brain was isolated. Then, cerebral cortex was separated and kept in sucrose 0.32 M to be used in synaptosome preparation step.

Preparation of synaptosomes

The approach we used to prepare Synaptosomes was based on the method of Dodd et al[27-28] using sucrose gradient centrifugation. Hence, the synaptosomes were prepared from the cerebral cortex of sheep brain. Extracted cerebral cortex was minced and homogenized

with Motor-Driven Potter Teflon-Glass Homogenizer at 800 rpm. The obtained homogenate was centrifuged at 3000 g for 30 min. Supernatant was loaded on top of sucrose 1.2 M. The sucrose gradients were centrifuged at 113000 g for 35 min. The soft middle white layer between the sucrose layers of 0.32 M and 1.2 M was then acquired and loaded on top of sucrose 0.8 M which was centrifuged at 113000 g for 35 min. The resulting pellet, containing synaptosomes, was dissolved in sucrose 0.32 M solutions. Finally, synaptosomes were stored at -20°C.

Transmission electron microscopy (TEM)

TEM micrographs were taken to assess and verify morphology of synaptosomes. The samples were prepared and stained with uranyl acetate and Pb citrate and finally observed with a HU-12A electron microscope (Hitachi, Japan).

Measurement of AChE activity

Specific activity of AChE was measured by Ellman method after incubation of 1 mg/ml of synaptosome suspension with 0.1 mg of each synthesized compounds.[23] This method is based on NTB^{2-} (2-nitro, 5-thiobenzoic acid) production and its absorption at 412 nm. The samples consisting of synaptosomal suspension (200 µg protein), acetylthiocholine 1.2 mM and 5'-dithiobis-2-nitrobenzoic acid (DTNB 5) 1mM were prepared in phosphate buffer 50 mM, pH 7.2 and the enzyme activity was assayed at 37ºC. The protein concentrations were determined for enzyme specific activity using Bradford method.

RESULTS

FT-IR, GC-mass spectra and CHN analysis

All of the synthesized compounds 1-4 were characterized by FT-IR and mass spectroscopy and CHN analyzer. Infrared spectroscopy is an effective technique in order to identify the presence of certain functional groups in a molecule. An infrared spectrum represents a fingerprint of a sample with absorption peaks which corresponds to frequencies of vibrations between the bonds of atoms making up the material. Therefore, infrared spectroscopy can lead to qualitative analysis of every different kinds of material. Mass spectrometry is used to produce spectra of the masses of the atoms or molecules comprising a material. In this method, chemical structure of molecules is elucidated. CHN analysis is a form of elemental analysis that is based on combustion analysis where the sample is first fully combusted and then its elements (C, H and N) are analyzed. Hence, GC-mass and FT-IR spectroscopy and CHN analyzer were utilized to evaluate the structure of synthesized compounds (I-IV) that were indicated below.

Leucine derivative of BA (BA-Leu) (I)

Yield, 31.8%; FT-IR: $\bar{\nu}$ 1027.87 (-C-O-), 1356.68 ($-CH_3$), 1402 ($-CH_2$-), 1517 (-C=C-), 1633.41, 1590.02 (-NH,$-COO^-K^+$,$-CO_2^-$ -ring), 2936.04 (-CH-), 3423.03 ($-NH_2$),cm^{-1}; MS $(M+1)^+$, m/z: 607 ($C_{36}H_{58}NO_4K^+$), 579 ($C_{34}H_{54}NO_4K^+$), 563 ($C_{33}H_{50}NO_4K^+$), 520 ($C_{31}H_{46}O_4K^+$), 466 ($C_{31}H_{47}O_3^+$), 439 ($C_{30}H_{48}O_2^+$), 424 ($C_{29}H_{45}O_2^+$), 406 ($C_{27}H_{35}O_3^+$), 392 ($C_{26}H_{36}O_3^+$) (Fig. 1); Anal. Calcd for $C_{36}H_{58}NO_4K^+$ (%): C, 85.72%; H, 11.5%; N, 2.77%. Found (%): C, 81.58%; H, 12%; N, 6.4%.

Isoleucine derivative of BA (BA-Ileu) (II)

Yield, 33.3%; FT-IR: $\bar{\nu}$; 1033.66 (-C-O-), 1394.28 ($-CH_3$), 1402 ($-CH_2$-), 1517.70 (-C=C-), 1624.73 (-NH, $-COO^-K^+$,$-CO_2^-$ -ring), 2936.09 (-C-H-), 3390.24 ($-NH_2$), cm^{-1}; MS $(M+1)^+$: m/z, 607 ($C_{36}H_{58}NO_4K^+$), 578 ($C_{34}H_{53}NO_4K^+$), 566 ($C_{36}H_{56}NO_4^+$), 552 ($C_{36}H_{58}NO_3^+$), 538 ($C_{35}H_{56}NO_3^+$), 524 ($C_{35}H_{58}NO_2^+$), 510 ($C_{34}H_{56}NO_2^+$), 496 ($C_{33}H_{54}NO_2^+$), 481 ($C_{32}H_{51}NO_2^+$), 467 ($C_{31}H_{49}NO_2^+$), 451 ($C_{31}H_{47}O_2^+$) (Fig. 2); Anal. Calcd for $C_{36}H_{58}NO_4K^+$ (%): C, 85.72%; H, 11.5%; N, 2.77%. Found (%): C, 81.79%; H, 12.3%; N, 5.89%.

Valine derivative of BA (BA-Val) (III)

Yield, 32.3%; FT-IR (KBr, cm^{-1}): $\bar{\nu}$1027.87 (-C-O-), 1398.14 ($-CH_3$, bending), 1423.21 ($-CH_2$-, bending), 1509.03 (-C=C-), 1590.02-1724.05 (-OCO-, ring), ($-COO^-$), 2937.06 (-CH-), 3423.03 ($-NH_2$); MS $(M+1)^+$, m/z: 593 ($C_{35}H_{56}NO_4K^+$), 578 ($C_{34}H_{53}NO_4K^+$), 538 ($C_{35}H_{56}NO_3^+$), 524 ($C_{34}H_{54}NO_3^+$), 510 ($C_{33}H_{52}NO_3^+$), 496 ($C_{32}H_{50}NO_3^+$), 482 ($C_{32}H_{52}NO_2^+$), 468 ($C_{31}H_{50}NO_2^+$), 452 ($C_{31}H_{50}NO^+$), 438 ($C_{30}H_{48}NO^+$), 411 ($C_{28}H_{45}NO^+$), 395 ($C_{27}H_{41}NO^+$), 368 ($C_{25}H_{38}NO^+$) (Fig. 3); Anal. Calcd for $C_{35}H_{56}NO_4K^+$ (%): C, 85.8%; H, 11.3%; N, 2.9%. Found (%): C, 84.5%; H, 10.4%; N, 5.1%.

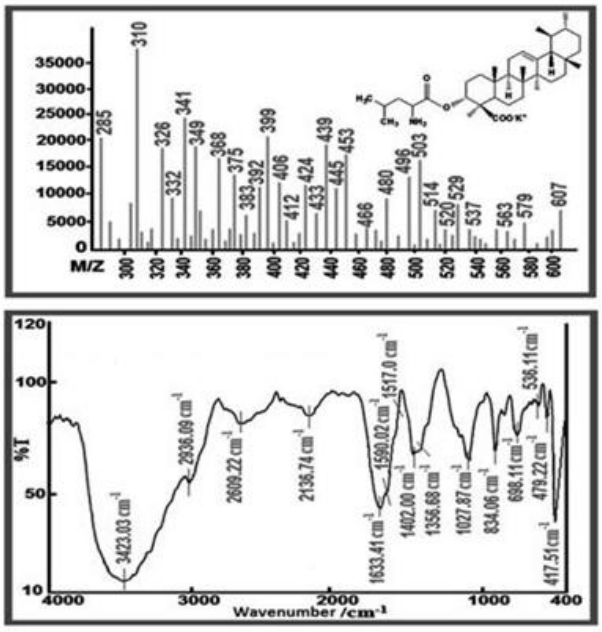

Figure 1: GC-MS and FT-IR spectra of compound (I). *The appearance of absorption 3423.03 cm^{-1} and 1633.41 cm^{-1} is attributed to presence of amine and ester group, respectively and the presence of peak at m/z 607 is due to fragment $\{K[C_{36}H_{58}NO_4]\}^+$, which affirmed the production of BA-Leu.*

Alanine derivative of BA (BA-Ala) (IV)

Yield, 33.9%; FT-IR (KBr, cm^{-1}): $\bar{\nu}$1019.19 (-C-O-), 1363.43 ($-CH_3$), 1410.67 ($-CH_2$-), 1558.20 (-C=C-), 1602.56 (-OCO-), 1686.44 ($-COO^-$, -NH-), 2881.13 (-CH-), 3484.74 (-NH-) cm^-

[1]; MS $(M+1)^+$, m/z: 565 ($C_{33}H_{52}NO_4K^+$), 547 ($C_{33}H_{48}O_4K^+$), 509 ($C_{33}H_{51}NO_3^+$), 481 ($C_{32}H_{51}NO_2^+$), 453 ($C_{30}H_{47}NO_2^+$), 439 ($C_{29}H_{45}NO_2^+$), 425 ($C_{28}H_{43}NO_2^+$), 411 ($C_{27}H_{41}NO_2^+$), 395 ($C_{29}H_{47}^+$), 368 ($C_{27}H_{44}^+$) (Fig. 4); Anal. Calcd for $C_{33}H_{52}NO_4K^+$ (%): C, 85.8%; H, 11.3%; N, 2.9%. Found (%): C, 77.4%; H, 13.7%; N, 8.9%.

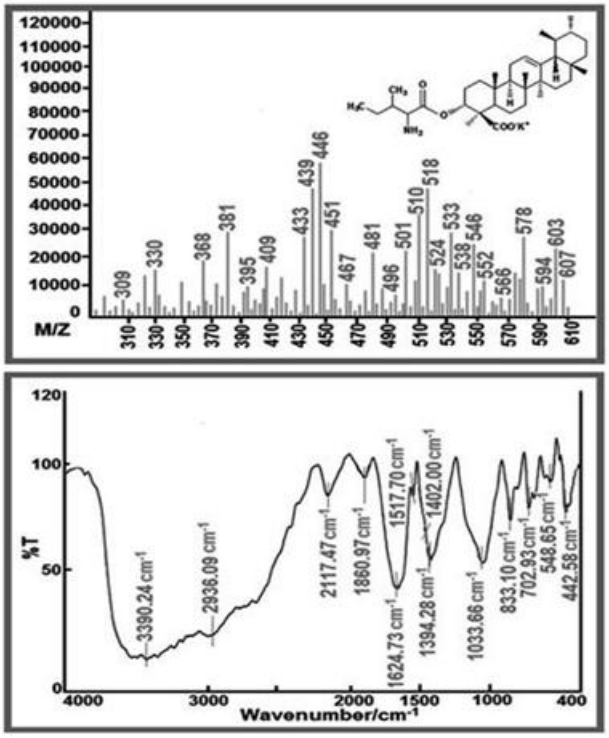

Figure 2: GC-MS and FT-IR spectra of compound (II). The appearance of absorption 3390.24 and 1624.73 cm^{-1} is attributed to presence of NH and COO group, respectively that indicates isoleucine attached to BA. The presence of peak at *m/z* 607 is due to fragment $\{K[C_{36}H_{58}NO_4]\}^+$, which affirmed the production of BA-Ileu.

Chemistry

Production of compounds I-IV were initiated when the OH group located on C3 of BA loses H^+ in basic medium and gets negative charge which makes it strong nucleophile. Consequently, leucine, isoleucine, valine and alanine amino acids can attach to BA through nucleophilic attack of O^- to carboxylic group of mentioned amino acids in order to form compounds I-IV, respectively via esterification reactions (Figure 5). The yields of the reactions were confirmed by CHN analyzer, FT-IR and GC-mass spectroscopy (Fig 1-4 related to compounds I-IV, respectively).

Inhibitory effects on AChE activity

AChE activity was measured to verify the integrity of synaptosomes.[29-30] AChE specific activity was 25.89 µmol /h 100 mg protein ± 1.07 as control enzyme activity. In addition, normal morphology of synaptosomes was observed by TEM (Figure 6). The impact of new compounds (I-IV) on the AChE activity was investigated in vitro in order to evaluate whether such agents can be exploited in the treatment of AD or not. As expected, a whit inhibitory effect of Boswellic acid was displayed as decreased AChE activity by 23% (Figure 7). On the other hand, conjugation of BA with four amino acids increased its inhibitory efficacy further. We showed that the inhibitory performance of BA was significantly improved when combined with leucine compared to BA as the standard inhibitor (53.90% for leucine conjugate of BA and 77.50% for BA) (Figure 7). Moreover, the activity of AChE treated with isoleucine conjugate of BA was lower than that of all other synthetic compounds in the study (31.97% for isoleucine conjugate compared to 53.90%, 66.55% and 70.82% for leucine, valine and alanine conjugates, respectively) while compound IV (BA-Ala) was the least effective form which decreased AChE activity about 7% compared to BA alone (Figure 7). Finally, no significant difference was observed between the valine and alanine conjugates of BA related to inhibitory effect on AChE activity and they had similar efficiency.

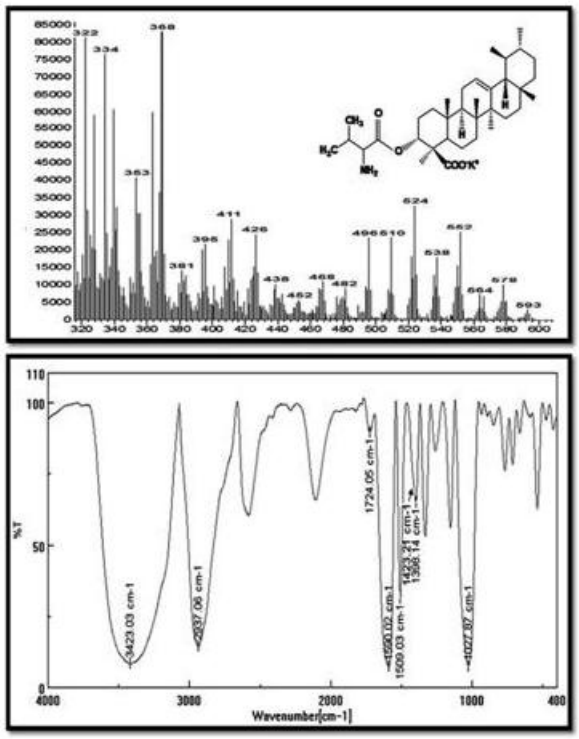

Figure 3: GC-MS and FT-IR spectra of compound (III). The absorption at 1590.2-1724.05 and 3423.02 cm^{-1} indicates the presence of ester (-COO-) and amine (NH_2, stretching), respectively. Also, observation of fragment $\{K[C_{35}H_{56}NO_4]\}^+$ at *m/z* 593 certified the production of BA-Val.

DISCUSSION

The active site of AChE is found at the bottom of a deep and narrow gorge.[31] AChE inhibitors have lately been used as cognition promoting agent and as a treatment for patients with AD. A few numbers of such drugs have been approved by U. S. food and drug administration (FDA) and the others are under study as potential marketable medications. Documents have shown that they improve behavioral disturbances and cognition and are primarily

different from each other based on the type of bond they create with AChE.[32-33]

Oleogum resin largely contains substances such as β-boswellic acid, acetyl-β-boswellic acid, acetyl-11-keto-β-boswellic acid and 11-keto-β-boswellic acid. These compounds have been reported to have therapeutic effects on neurodegenerative diseases through enhancing ACh levels in the groups of AD-induced rats treated with Boswellia serrata.[34] It was established that triterpene acids also possess antioxidant and anti-inflammatory activity which could be attributed to their positive effects on AD. Several investigations have been reported in the literature aiming to define the mechanism of action of BAs. The structure and biological activities of BAs have been well investigated, and formulations and various preparations in diverse forms including Boswellia serrata extract have been consumed for many years. Nevertheless, it is surprising that not much has been addressed regarding synthesis of new compounds by chemical modifications or preparation of various analogues of the BAs. Therefore, we have prepared the salts of leucine, isoleucine, valine and alanine amino acids derivatives of BA and then we assessed their AChE inhibitory activity in comparison with boswellic acid alone.

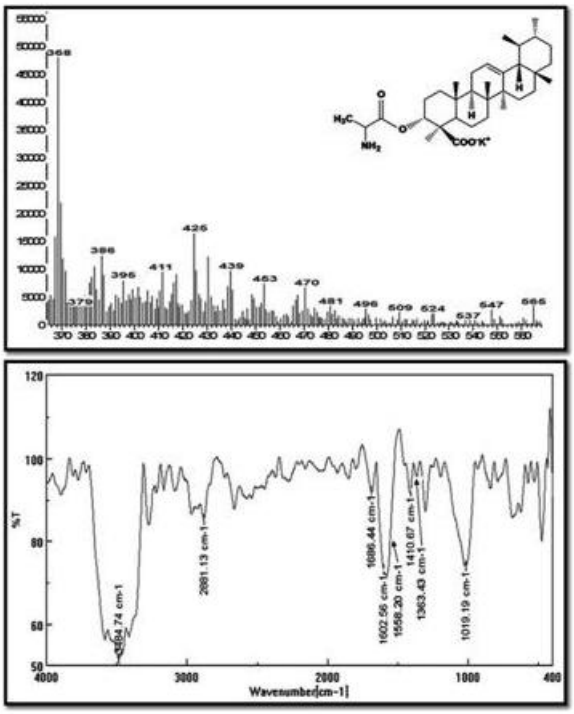

Figure 4: GC-MS and FT-IR spectra of compound (IV). The appearance of absorption 3484.74 and 1602.56 cm^{-1} is attributed to presence of amine and (-OCO-) group, respectively and the presence of peak at *m/z* 565 is due to fragment $\{K[C_{33}H_{52}NO_4]\}^+$, which affirmed the production of BA-Ala.

A group of researchers conducted a study to evaluate the effects of aqueous administrations of Boswellia Serrata on the rats with AD induced by $AlCl_3$. They found out that the activity of AD rats increased significantly when treated with Boswellia and histopathological findings of the brain tissue confirmed that amyloid plaques had disappeared compared to AD group. Moreover, an increased level of ACh and decreased level of AChE enzyme in a dose dependent manner after treatment with Boswellia extract were reported. In addition, regarding the anti-oxidant and anti-inflammatory activities of triterpene acids such as BA and their role in enhancing of ACh levels, they concluded that compounds available in Boswellia Serrata were beneficial and helpful in the treatment of rats with AD.[34]

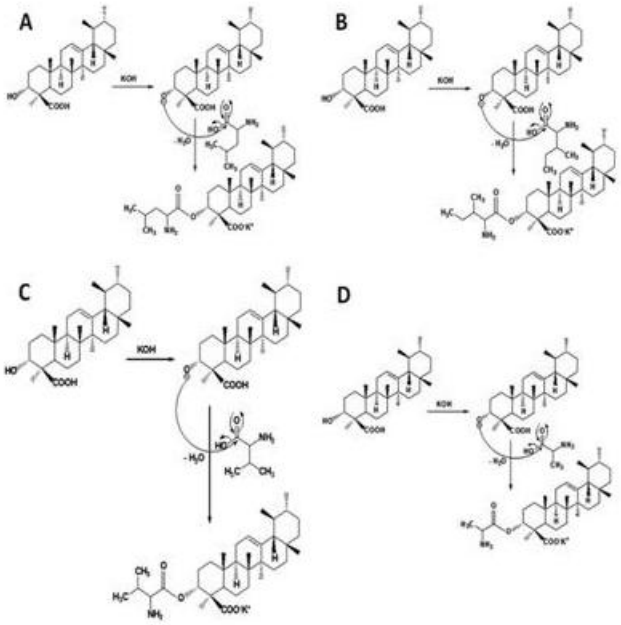

Figure 5: Mechanism of coupling reactions of leucine (a), isoleucine (b), valine (c) and alanine (d) with BA. The reactions were initiated by nucleophilic attack of O^- of BA that is yielded in basic medium.

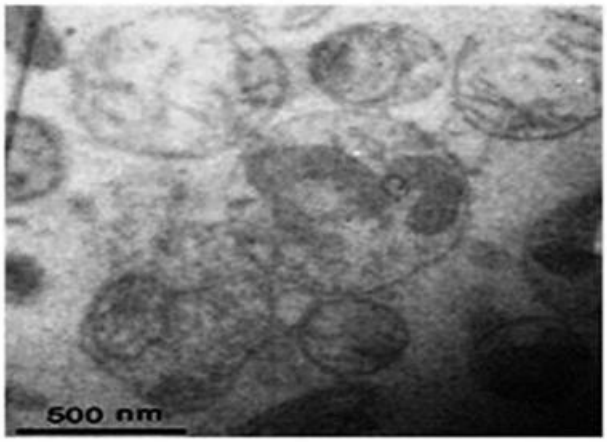

Figure 6: Electron microscopy image of sheep hemispheres synaptosomes displaying the normal morphology of synaptosomes

There are several studies regarding to preparation novel salts or ion pair complexes of BA with other chemical substances. In a study conducted by Shah et al (2007),

they synthesized novel 4-amino analogues of BA, 11-Keto-β-boswellic acid (KBA) and their epimers through isocyanat intermediates. In more details, they replaced carboxyl group in ursane nucleus with an amino group. The resulted compounds showed improved cytotoxicity compared to the natural molecules. They also induced DNA fragmentation leading to apoptotic activity.[35] Investigations also showed that acetylation or mild oxidation of BAs leads to their enhanced biological activity.[36] In another study, 2-cyano analogues of BA and KBA were assessed for their in vitro cytotoxicity in HL-60 and HeLa cells. They found that novel compounds show 50-100 fold higher cytotoxicity than their primary molecules.[37] Furthermore, Honda et al. prepared oleanan and ursane triterpenoids via modifying ring A and C finding that the combined modification of both rings enhances the efficacy about 10,000 times compared with the primary compound. The final preferred oleanan complex was found to be a promising multifunctional agent.[38]

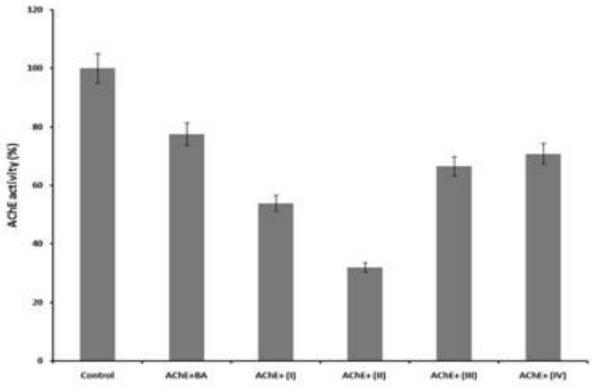

Figure 7: Effects of compounds (I-IV) on AChE activity. Specific activity of AChE was measured after incubation of 1 mg/ml of synaptosome suspension with 0.1 mg of each synthesized compounds. The enzyme activity was assayed at 37ºC and the absorbance was detected at 412 nm.

Kumar et al. (2012) prepared 28 various alkyl-acyl derivatives of BAs and assessed them for cytotoxicity against a range of human cancer cell lines in vitro. They aimed to evaluate the effect of alkyl-acyl groups with different lengths on carbon 3-hydroxyl functionality and also on its stoichiometry in association with the biological activity of BAs. In general, chemical modified BAs displayed better cytotoxic activity than the parent BAs. Moreover, evaluating of BA derivatives resulted in the identification of 3-O-n-butyryl-11-keto-β-boswellic acid as a potent compound agent that inhibited NF-kB (protein involved in the regulation of several anti-apoptotic genes expression) and could be developed as a novel potential anti-cancer agent.[39]

The results of the above-mentioned studies can support our whole idea behind the preparation of amino acid derivatives of BA. Our results showed that some amino acid compounds of BA might have more potent AChE inhibiting features and these new complexes resemble in the mode of action of drugs administered in the treatment of AD. We assume that the salt of amino acid leucine complexes with BA forms specific bonds with active site of the enzyme and inhibit its reaction. Nevertheless, the exact mechanism by which these compounds interact with AChE remains unexplored. Undoubtedly, molecular docking and simulation studies are required as a part of documentation process to improve the reliability and accuracy of our results and to investigate possible interactions between molecules.

CONCLUSION

Triterpenoids had shown an excellent capacity for the treatment of cancer, inflammatory diseases and neurodegenerative diseases. Novel compounds with more potent pharmaceutical effects can be synthesized via appropriate chemical reactions. The present work showed that the proposed techniques and chemical reactions are appropriate to synthesize complexes between BA with amino acids, which could exhibit more efficient pharmaceutical properties. Furthermore, we used spectroscopic techniques such as GC-MS and FT-IR in order to analyze and confirm the coupling process in this study. Regarding the type of bonds between novel compounds and AChE active site, they could affect the enzyme activity. Inhibited AChE activity was observed and it could be used as a potential agent for the treatment of AD.

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