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NEAR EAST UNIVERSITY
INSTITUTE OF HEALTH SCIENCES

The Inhibitory Activity of the Pesticide Abamectin Against the Enzyme Butyrylcholinesterase Purified from Horse Serum: Kinetic and Docking Studies

A THESIS SUBMITTED TO THE GRADUATE INSTITUTE OF HEALTH SCIENCES NEAR EAST UNIVERSITY

BY:

Qëndresa Hoti

In Partial Fulfillment of the Requirements For The Degree of
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APPROVAL

The Directorate of Graduate School of Health Sciences,
This study has been accepted by the thesis committee in Medical Biochemistry program as a Master of Science Thesis.

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According to the relevant article of the Near East University Postgraduate Study-Education and Examination Regulation, this thesis has been approved by the above-Mentioned members of the thesis committee and the decision of the board of Directors of the Institute.

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Director of Graduate School of Health Science

DECLARATION

I hereby declare that the work in this thesis entitled "**THE INHIBITORY ACTIVITY OF THE PESTICIDE ABAMECTIN AGAINST THE ENZYME BUTYRYLCHOLINESTERASE PURIFIED FROM HORSE SERUM: KINETIC AND DOCKING STUDIES."** is the product of my own research efforts undertaken under the supervision of Assistant Professor Kerem Teralı. No part of this thesis was previously presented for another degree or diploma in any University elsewhere, and all information in this document has been obtained and presented in accordance with academic ethical conduct and rules. All materials and results that are not original to this work have been duly acknowledged, fully cited and referenced.

Name, Last Name:

Signature:

Date:

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ABSTRACT

The Inhibitory Activity of the Pesticide Abamectin Against the Enzyme Butyrylcholinesterase Purified from Horse Serum: Kinetic and Docking Studies. Near East University, Institute OF Health Science, M.Sc. Thesis in Medical Biochemistry Program, Nicosia, 2017.

Butyrylcholinesterase (BChE) is a glycoprotein enzyme synthesized in the liver, BChE can be found in most tissues, the peripheral as well as the central nervous system. The plasma contains 5 mg l^{-1} of BChE. The Butyrylcholinesterase plays a very vital role in hydrolyzing acetylcholine and other choline esters, BChE is also able to functionally hydrolyze toxic esters such as cocaine or scavenge organophosphorus pesticides and nerve agents. In several clinical manifestations seen in Alzheimer patients, increase in the activity of BChE was observed, and as result of this, there is the need to investigate the inhibitor effect on the enzyme. Abamectin (ABM) a combination of avermectin B_{1a} and B_{1b} homologs, which are effective constituents, used in agricultural pesticides and antiparasitic agents and these are broadly employed in the agricultural, veterinary and medical fields. Avermectins (AVMs) are similar to the 16-membered macrocyclic lactone compounds, which have been derived from *Streptomyces avermitilis*, a soil microorganism. In this study, the interaction of abamectin with BChE (EC 3.1.1.8) extracted from horse serum was investigated. Activity measurements were carried out at 412 nm by using different concentration of BTC. Analysis of kinetic data indicated that the inhibition caused by Abamectin was competitive with IC_{50} value of 10.6 µM, the V_m and K_m were found as 252.59 ± 7.11 U mg^{-1} and 0.155 ± 0.020 µM, respectively. The K_i value was 2.26 ± 0.35 µM. Competitive inhibition shows that abamectin binds to the active site of the enzyme, the results demonstrate that even at very low concentrations, there are significant amounts of inhibition. The *in silico* analyses suggest that avermectin B_{1a} is placed in the enzyme active site with various noncovalent bonds including hydrogen and hydrophobic bonds, that may obstruct activity of BChE.

Key Words: Butyrylcholinesterase, Abamectin, Avermectin.

ÖZET

At Serumundan Arındırılmış Enzim Butirilkolinesteraza Karşı Pestisit Abamektinin Önleyici Aktivitesi: Kinetik ve Doklama Çalışmaları. Yakın Doğu Üniversitesi, Sağlık Bilimleri Enstitüsü, Yüksek Lisans Tıbbi Biyokimya Programında Tez, Lefkoşa, 2017.

Butirilkolinesteraz (BChE) karaciğerde sentezlenen bir glikoprotein enzimidir, BChE çoğu dokuda, periferik sinir sisteminde ve merkezi sinir sisteminde bulunur. Plazma 5 mg l^{-1} BChE içerir. Butirilkolinesteraz, asetilkolin ve diğer kolin esterlerini hidrolize etmek için hayati bir rol oynar; BChE, kokain gibi toksik esterleri veya organofosforlu pestisitleri ve sinir ajanlarını temizleyerek işlevsel olarak hidrolize edebilir. Alzheimer hastalarında görülen çeşitli klinik tablolarda, BChE aktivitesinde artış gözlemlendi ve bunun sonucu olarak, enzim üzerindeki inhibitör etkisini araştırmaya ihtiyaç duyuldu. Abamectin (ABM) tarımsal zirai ilaçlar ve antiparazitik ajanlarda kullanılan etkili bileşenler olan avermectin B_{1a} ve B_{1b} homologlarının bir kombinasyonu olup bunlar tarım, veterinerlik ve tıbbi alanlarda yaygın olarak kullanılmaktadır. Avermektinler (AVM), bir toprak mikroorganizması olan Streptomyces avermitilis'den türetilen 16 üyeli makrosiklik lakton bileşiklerine benzerdir. Bu çalışmada, atlık serumdan abamektin ile BChE (EC 3.1.1.8) arasındaki etkileşim araştırılmıştır. Aktivite ölçümleri farklı konsantrasyonda BTC kullanılarak 412 nm'de gerçekleştirildi. Kinetik verilerin analizi, Abamectin'in neden olduğu inhibisyonun 10.6 µM IC_{50} değeri ile rekabet ettiğini, V_m ve K_m'nin sırasıyla 252.59 ± 7.11 U mg^{-1} ve 0.155 ± 0.020 µM olarak bulunduğunu gösterdi. K_i değeri 2.26 ± 0.35 µM idi. Rekabetçi inhibisyon, abamektinin enzimin aktif bölgesine bağlandığını göstermektedir; sonuçlar, çok düşük konsantrasyonlarda bile önemli miktarda inhibisyon olduğunu ortaya koymaktadır. *In silico* analizleri, avermektin B_{1a}'nın BChE'nin aktivitesini engelleyebilecek hidrojen ve hidrofobik bağlar da dahil olmak üzere çeşitli kovalent olmayan bağlarla enzim aktif alanına yerleştirildiğini önermektedir.

Anahtar Kelimeler: Butirilkolinesteraz, Abamektin, Avermektin.

Table of Contents

SYMBOLS AND ABBREVIATIONS

Abbreviations Items	Scientific Name
6-MAM	6-Monoacetylmorphine
ABM	Abamectin
Ach	Acetylcholine
AChE	Acetylcholinesterase
AD	Alzheimer's disease
AVMs	Avermectins
BBB	Blood-Brain Barrier
bCA	Carbonic Anhydrase
BChE	Butyrylcholinesterase
BE	Benzoylecgonine
BTC	S-Butrythiocholine Iodide
CAD	Coronary Artery Disease
ChAT	Choline Acetyltransferase
CNS	Central Nervous System
CRP	C- reactive protein
DMSO	Dimethyl sulfoxide
DTNB	5, 5-Dithio-Bis-(2-Nitrobenzoic Acid)
EME	Ecgonine Methyl Ester

Abbreviations Items	Scientific Name
GABA	**γ-Aminobutyric Acid**
HDL	**High Density Lipoprotein**
IC_{50}	**Half maximum inhibitory concentration**
K_i	**Inhibitory constant**
K_m	**Michaelis constant**
mAChRs	**Muscarinic Acetylcholine Receptors**
nAChRs	**Nicotinic Acetylcholine Receptors**
PAS	**Peripheral Anionic Site**
PDB	**Protein Data Bank**
TcAChE	**Torpedo californica Acetylcholinesterase**
TNB	**5-thio-2-bis-nitrobenzoate**
V_{max}	**Maximum velocity**

LIST OF FIGURES

1. Introduction

The neurotransmission in the peripheral and central nervous systems is much controlled by cholinergic system. Choline acetyltransferase (ChAT) synthesizes the cholinergic neurotransmitter acetylcholine (Ach) is and acetylcholinesterase (AChE) and butyrylcholinesterase (BChE EC 3.1.1.8.) terminates its action (Reid, 2013).

A highly integrative process of chemical neurotransmission is mediated by the nicotinic and muscarinic receptors (n- and mAChRs) of acetylcholine (ACh) released by cholinergic neurons (Wessler *et al.*, 2008). In lymphocytes segregated from thymus, spleen, lymph node, and peripheral blood, both muscarinic and nicotinic acetylcholine (ACh) receptors have been recognized and their stimulation is reduced by muscarinic and nicotinic agonists on a diversity of biochemical and functional properties (Kawashima, 2000).

A group of enzymes that hydrolyze acetylcholine and other choline esters are called cholinesterase (Davis *et al.*, 1997). Cholinesterase of erythrocytes is different from that found in plasma (Kutty, 1980). The two main types of cholinesterase are present which have different biochemical properties, true or specific cholinesterase or acetylcholinesterase found in all excitable tissues of central and peripheral nervous system and muscles and also in red blood cells. It has great affinity for acetylcholine and it is a high-turnover enzyme. It is inhibited by the high concentrations of acetylcholine and has little affinity for noncholine esters (Davis *et al.*, 1997).

The other type of cholinesterase is the nonspecific, pseudocholinesterase or serum cholinesterase, also called butyrylcholinesterase which hydrolyses both benzylcholine (Kutty, 1980) and choline aliphatic esters (Santarpia, *et al*., 2013).

One of the most recognized components of central cholinergic pathways is acetylcholinesterase (AChE). Through hydrolysis it terminates the synaptic action of acetylcholine and produces the choline moiety which is required for the recycling of transmitter. In contrast to acetylcholinesterase which was generally of neuronal origin, butyrylcholinesterase is commonly of glial origin (Mesulam, 2002).

Butyrylcholinesterase (BChE) is α-glyco protein which is existent in most tissues of the central and peripheral nervous system and in liver. It has low affinity for acetylcholine and a high concentration of acetylcholine does not inhibit it (Santarpia *et al*., 2013). The half-life of BChE is approximately 12 days (Pan *et al*., 2009) and the normal value ranges from 5,900 and 13,200 IU l^{-1}. A raise in activity of BChE has been observed in obesity, uremia, diabetes, hyperlipidemic subjects and hyperthyroidism (PAES *et al*., 2006).

The BChE enzyme predominated than AChE enzyme in the human body. When enzyme activity level is multiplied by the mass of the tissue, the highest quantity of AChE enzyme is found in muscles whereas the highest level of BChE is found in the plasma and liver. Overall, the human body of an adult has ten times BChE protein (680 nmol) additional than AChE (62 nmol) (Manoharan *et al*., 2007).

The most important function of acetylcholinesterase (AChE) enzyme is catalyzing the hydrolysis for acetylcholine (ACh) in cholinergic synapses, while the important role of butyrylcholinesterase (BChE) is not as much obviously defined as it has been observed to hydrolyze ACh and other esters. Demonstrating of the three-dimensional structure of AChE of *Torpedo californica* (TcAChE) (Sussman *et al*., 1991)

BChE (Nicolet *et al*., 2003) displayed resemblance of this two enzymes, however, showing differences in the composition of the amino acids lining a deep, narrow gorge, which hosts a catalytic site at its bottom. In human's BChE, six of the fourteen aromatic amino acids that contour the gorge of AChE are substituted by aliphatic ones; therefore, it deficiencies the peripheral site found in AChEs, so the BChE acyl pocket is also greater (Groner, *et al*., 2007). While acetylcholinesterase enzyme function in hydrolyses of acetylcholine, this enzyme also affects differentiation and proliferation of cells, also responds to different insults such as, stress (Grisaru *et al*., 1999).

The studying for BChE rise dramatically after Clarence Broomfield, at the Institute of Chemical Defense, performed an important experiment in which monkeys were protected from the poisonousness of nerve agents after being pretreated with BChE (Broomfield *et al*., 1991). The butyrylcholinesterase (BChE) found in humans gained attention since it can hydrolyze toxic esters like cocaine and scavenge organophosphorus insecticides and nerve agents. Also since in Alzheimer disease the BChE activity rises progressively as the severity of dementia progresses, therefore researchers investigate for selective BChE inhibitors in the treatment of Alzheimer disease as well (Wszelaki & Kiss, 2010).

Abamectin (ABM) is a combination of avermectin B_{1a} and the B_{1b} homologs (Awasthi *et al*., 2013). It consists of 80% of avermectin B_{1a} and not more than 20% of B_{1b} avermectin (Campbell, 2012). It is widely used as an anthelmintic and antiparasitic agent for animals as well as humans. Avermectins (AVMs) are closely related 16-membered macrocyclic lactone compounds which are derived from *Streptomyces avermitilis* a soil microorganism, (Awasthi *et al*., 2013).

Avermectins are antiparasitic drugs and agricultural pesticides which belong to a class of neurotoxic macrocyclic lactone compounds (Valenzuela *et al*., 2000). They are primarily used in the protection of animals and harvests, such as peanut, corn, cattle, pigs and against insects and mites (Howells & Sauer, 2001). Edible oils extracted from plants and animals may contain avermectin residue, due to which public health is at risk. Avermectins are toxic to the nervous and growth systems and their harm to the environment and humans has raised increasing concerns on the topic residue analysis in food and agro-products (Kose *et al*., 2016).

In this study, the objective is to explore the inhibitory effects of abamectin on butyrylcholiesterase purified from horse serum.

2. General Information

2.1. Butyrylcholinesterase

BChE is an enzyme whose active site includes serine and belongs to serine hydrolase family. BChE requires the functioning catalytic triad: Ser198, Glu325, and His438 for its catalysis (Nicolet *et al*., 2003). Organophosphorus compounds irreversibly inhibit the activity of BChE, as an example the nerve agent sarin and the pesticide metabolite chlorpyrifos Oxon. The serine active site is covalently modified by reaction with organophosphorus esters that when join together destroys the organophosphorus poison and inactivate BChE (Fidder *et al*., 2002).

The presence of a single *BCHE* gene was confirmed to be found on human chromosome 3, between nucleotides 165,490,692–165,555,260. The mRNA is encoded by 4 exons. The 64.57 kb BCHE gene is located on the long arm of chromosome 3 at 3q26.1–q26.2. There are no other merged shapes of human BChE. The same *BCHE* mRNA encode the soluble, globular tetrameric BChE in plasma also in the membrane bound forms in muscles and brain (Massoulie, 2002).

BChE structure is a tetramer of four undistinguishable subunits (each 85 kDa) which are related to each other through four-helix bundle at the C-termini. In the middle of the four-helix bundle a polyproline rich peptide exists. The four-helix package is the tetramerization area. The horizontal vision of the modeled BChE tetramer indicates four helix bundles outstanding from the globule can be seen in (figure 1.1B). İnside the midpoint of the 4 helix bundles the prolyproline rich peptide form hydrogen bond to Trp543, Trp550, Trp557 and with 3 extra hydrophobic residues, all at the identical side of the amphiphilic helix. Once BChE enzyme is diluted, the polyproline wealthy peptide isn't always released from the tetramer (Pan et al., 2009). The polyproline rich

peptides function is to pull collectively BChE identical subunits to the tetramer form (Larson et al., 2014).

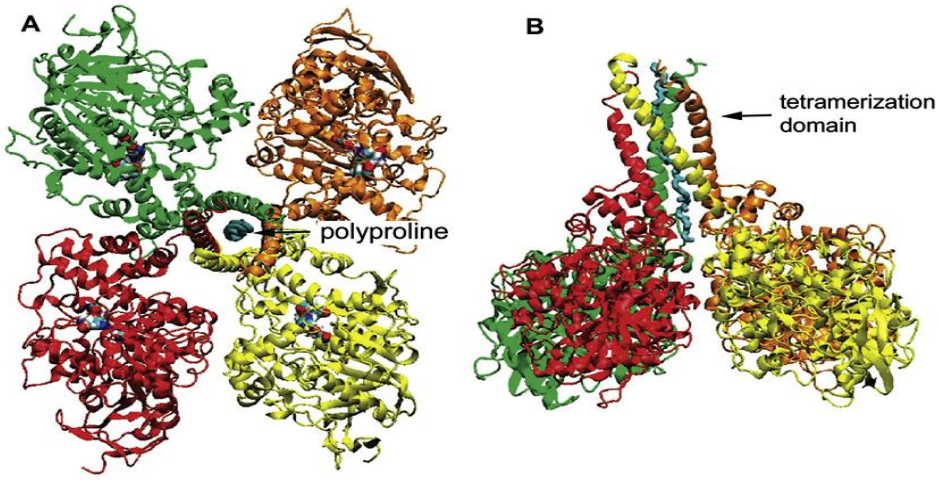

Figure 1.1 the Modeled Structure of Human BChE in Tetrameric Form

A) The view from top of the BChE tetramer with the center showing polyproline. **B)** The view from side of the BChE showing tetramer form. Collect into tetramer of the four undistinguishable subunits via the interaction of a polyproline peptide with the BChE tetramerization domain at the C-terminus (Pan *et al*., 2009).

Additional than BChE and AChE no proteins are identified to be use polyproline peptides as a mechanical concept to shape the subunits into tetrameric form. Different proteins might be recognized that involve noncovalently bound polyproline peptides for oligomerization in the future (Lockridge, 2015).

2.2. Difference between acetylcholinesterase and butrylcholinesterase

Acetylcholinesterase (AChE) enzyme hydrolyzes acetylcholine, while the larger molecules, such as butyrylcholine are hydrolyzed by butyrylcholinesterase (BChE). These two cholinesterases enzymes belong to a major protein family comprising the α/β hydrolase fold (Bajda *et al*, 2013). The AChE structure was first elaborated from the electrical organ of the electric ray, *Torpedo californica* (TcAChE) (Sussman *et al*. 1991).

(TcAChE) consists of 537 amino acids and carry 12 β-sheets encompassed by 14 α-helices. The structure of BChE is closely related to AChE. Therefore, based on homology model of electric ray acetylcholinesterase, all the structural analyses were performed for BChE (Nicolet *et al*., 2003).

The hydrolysis reaction are performed at the active site of the enzyme located at the end of a deep, and narrow gorge (20 Å deep and 5 Å wide) for AChE. The catalytic site of AChE is possessed of preserved aromatic amino acids also called the esteratic site that comprises the three fundamental amino acids, Ser200, His440 and Glu327 (TcAChE), which generate the catalytic triad of the active site.

They are implicated in the transference of the acetyl group from acetylcholine to Ser200. Human BChE catalytic triad is setting up of Ser198, His438 and Glu325. In the hydrolysis process an essential role is also played by aromatic amino acids, such as Trp84 and Phe330 (Harel *et al*., 1993).

The substrate quaternary ammonium group is bonded with cation-π interactions by the AChE anionic site which is composed of Trp84, Tyr130, Phe330 and Phe331. The appropriate location of acetylcholine in the gorge is provided due to the interactions through the anionic site, also enabling the inhibitor to bind with the enzyme. In BChE, the tryptophan key residue (Trp82) is preserved, on the other hand one phenylalanine (Phe330) is exchanged by Ala328.The absence of this phenylalanine impact the affinity of certain inhibitors (Nachon *et al*., 1998).

Serine hydrolase stabilize the transition state by amino acids of the oxyanion hole through hydrogen-bond formation. The transition complex is generated and become stable by Gly118, Gly119 and Ala201 from AChE throughout the enzymatic reaction. In hBChE, the oxyanion hole amino acids are derived from the main chain, that is, Gly116, Gly117 and Ala199, is alike and contains of highly preserved N–H dipoles. For the substrate specificity the acyl pocket is responsible.

The active gorge in *h*BChE is bigger than in TcAChE (500 Å3 versus 300 Å3). In TcAChE the active site is lined by 14 aromatic residues (Xu *et al*, 2008), in BChE, six of the aromatic residues are exchanged by smaller aliphatic residues, even polar ones. In TcAChE the two residues aromatic amino acids Phe288 and Phe290 regulate the shape of the acyl pocket, but in *h*BChE the aliphatic residues Leu286 and Val288. The access to the catalytic center by large molecule is preserved from Phe288 and Phe290.

In the case of BChE, the alteration of two phenylalanine residues with smaller amino acids, valine and leucine, creates a larger hollow in the acyl pocket and allows entrance of larger molecules in, causing lower enzyme specificity (Johnson, *et al*., 2006).

AChE contains of five amino acids in the peripheral anionic site (PAS), that is, Tyr70, Asp72, Tyr121, Trp279 and Tyr334. The PAS is positioned at the entrance of the active gorge and is responsible for the interaction with β-amyloid (Johnson, *et al*., 2006). At the peripheral site, BChE does not own counterparts of Tyr70, Tyr121 and Trp279. The PAS an essential structural component is have essential role for binding of numerous inhibitors. The differences present in the structure of the active sites of these two enzymes explain the alterations in their activities (Bajda *et al*., 2013).

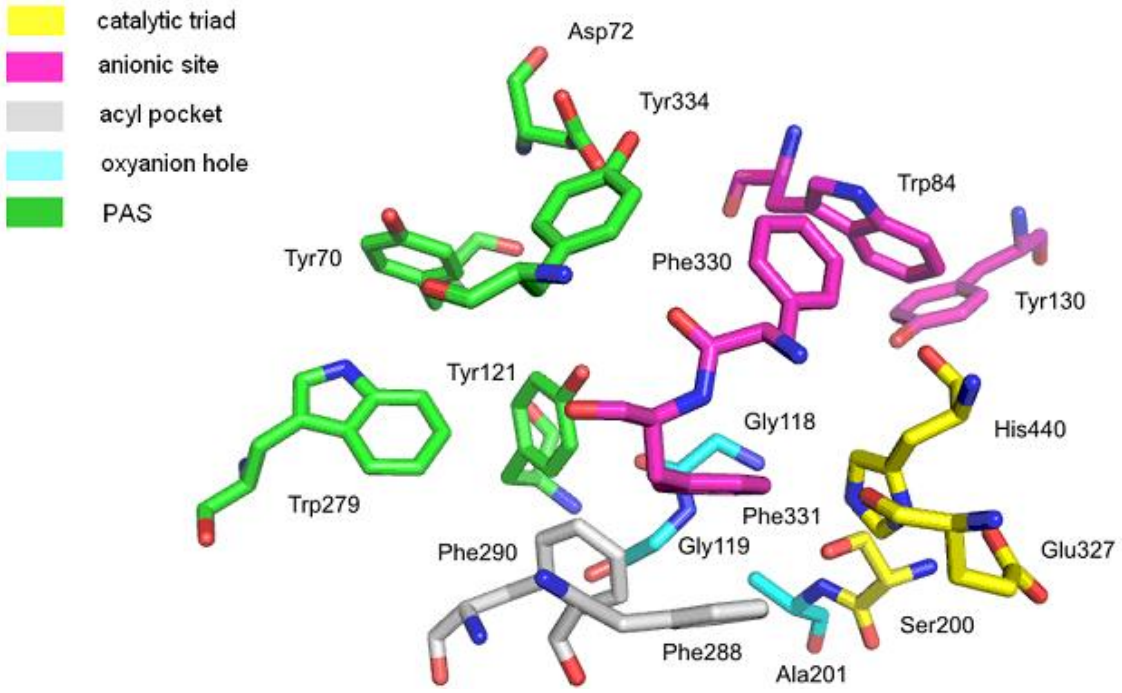

Figure 1.2 the active site of acetylcholinesterase (TcAChE)

(*Torpedo californica* acetylcholinesterase; from Protein Database Bank (PDB): 1EVE).

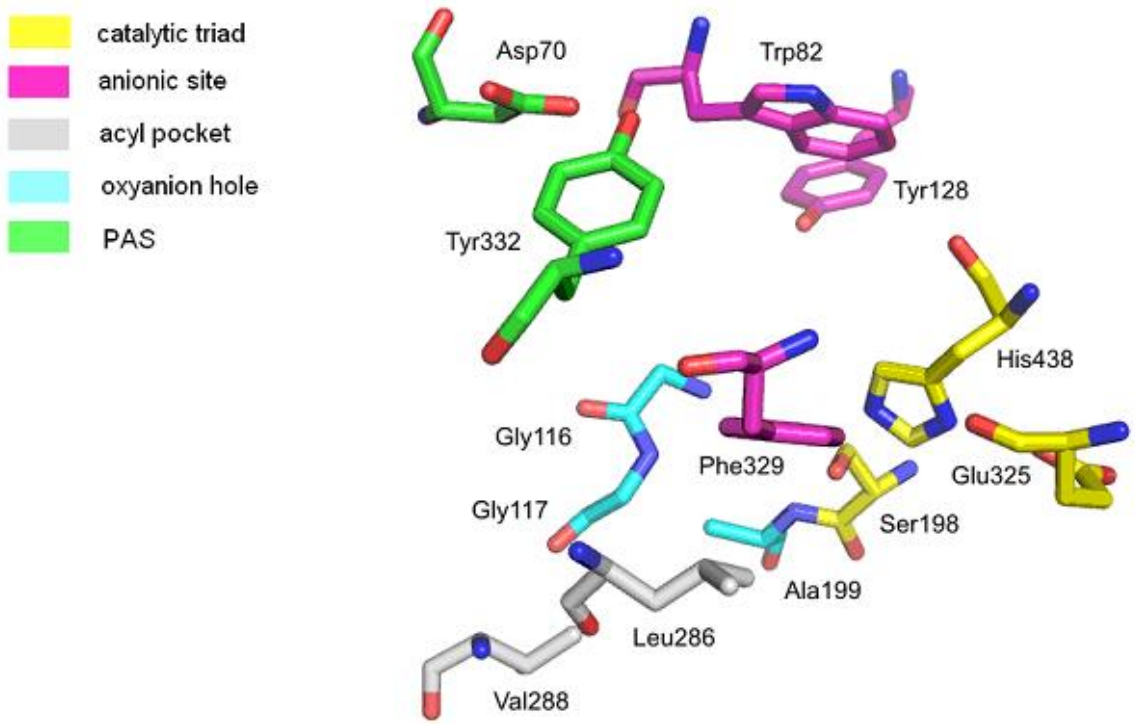

Figure 1.3 the active site of butyrylcholinesterase (hBChE)

(Human butyrylcholinesterase PDB: 1P0I) (Bajda et al., 2013).

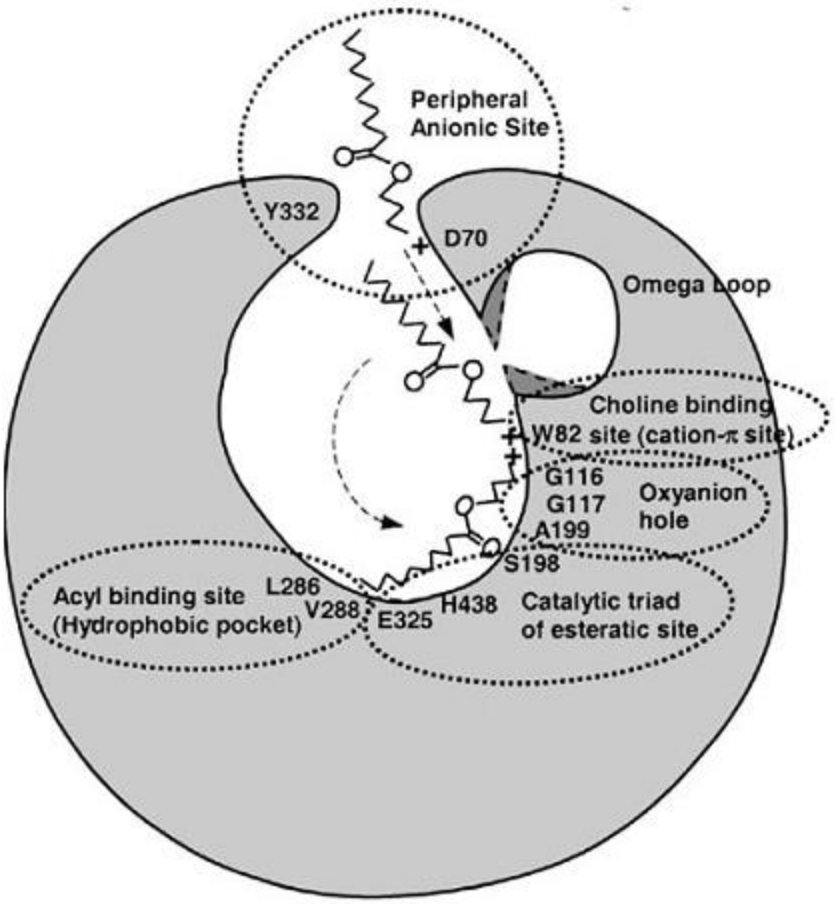

Figure 1.4 the active site of butyrylcholinesterase in monomer form (Çokuğraş, 2003).

2.3. Genetic variants of human cholinesterases

Acetylcholinesterase (AChE) is a physiologically essential objective for organophosphorus toxicants (OP), nerve agents and insecticides. Butyrylcholinesterase (BChE) serves as a bioscavenger in blood that protects AChE enzyme in nerve synapses from inhibition thru OP. Exposure to OP can be traced by mass spectrometry methods measuring adducts on the active site serine of plasma BChE.

In the both enzymes AChE also BChE genetic variants occur but mutations with loss of function only in the BChE gene were observed. His353Asn is the most common AChE variant (Lockridge *et al*., 2016). This mutation defines the Yt blood group antigen without changing AChE activity (Masson, *et al*., 1994).

The BChE gene was observed to have 34 losses of function mutations with none present in the homozygous state (Lek *et al.*, 2015). Humans homozygous for silent BChE are healthful, fertile and remain active till old age (Manoharan *et al*., 2007).

Missense mutations were found to be in the heterozygous state. They were reported in the residues of the catalytic triad of BChE. Five alleles out of 121,110 had Ser226Gly in the active site serine, a mutation that results in complete loss of BChE activity. One allele out of 121,174 had a mutation in the catalytic triad: His466Arg. No mutations were seen in the catalytic triad residue Glu353. One BChE allele out of 121,142 had the mutation Trp110Arg at the choline binding site, this mutation is expected to severely impair BChE activity. The mutation that is responsible for prolonged apnea in response to the muscle relaxants succinylcholine and mivacurium, is the missense mutation Asp98Gly (McGuire *et al*., 1989).

Individuals who are homozygous for atypical BChE are incapable to breathe for around two hours from a dose of succinylcholine that can paralyzes most people in three minutes. Other, less recurrent mutations including those that produce silent BChE with no activity (Lushchekina *et al*., 2016) are also correlated with prolonged apnea in response to a muscle relaxant.

The popular BChE variant, Ala567Thr or the K-variant has 33% reduced plasma BChE activity. The K-variant is known by a single amino acid substitution that can turn alanine (539) in to threonine (Lockridge *et al*., 2016).

The genetic variant accompanying with extended reaction to muscle relaxants, that is, Asp98Gly or atypical BChE, has decrease enzyme concentration and activity (Lockridge *et al*., 2016).

Nerve agents inhibit AChE more readily than they inhibit BChE. Humans have moderate signs of toxicity after a single oral dose of 0.028 mg kg^{-1} sarin or an intravenous dosage of 0.0015 mg kg^{-1}. The 50 nM BChE in plasma easily captures low doses of nerve agent, preventing the inhibition of AChE. Genetic variants with reduced levels or with inactive BChE are expected to be at more risk of toxicity from the nerve agents (Lockridge *et al*., 2016).

2.3.1. *BCHE* Expression in Human Tissues Gene

Human *ACHE* and *BCHE* gene expression profiles are markedly different. AChE levels are highest in the brain, followed by skeletal muscle and nerve impulse transmission. BChE levels are highest in the liver, adipose-visceral, esophagus, colon, fallopian tube, uterus, cervix, and lung consisting with the function of BChE in detoxification of poisons that may are eaten or inhaled.

Whole body autoradiograms of mice injected intravenously with 3H-soman, a potent irreversible cholinesterase inhibitor, showed that five minutes after its administration, elevated concentration of radioactivity was found in the blood, heart, kidney, lung, nasal cavities, lacrymal glands, salivary glands, skin, and some striated muscles (Kadar *et al*., 1985). No radioactivity in the central nervous system was seen. The tissue distribution of soman resembles that of BChE (Lockridge *et al*., 2016).

In human body there are more BChE than AChE. When the level of enzymatic activity are multiplied be the mass of tissue, the highest quantity of AChE enzymes are found in muscles, whereas the highest level of BChE enzymes are found in plasma and liver. Overall, the adult human body has approximately 10 times more BChE (680 nmol) protein than AChE protein (62 nmol) (Manoharan *et al*., 2007).

2.4. Butyrylcholinesterase as an Effective Therapeutic Scavenger than Acetylcholinesterase

An enzyme to be an effective scavenger for organophosphorus toxicans, must have the following:

1. Rapid reaction of the enzyme with organophosphorus toxicants.
2. Availability of sufficient quantities of the enzyme.
3. Stable for elongated storage.
4. The enzyme in vivo must have an elongated half-life.
5. The enzyme should be immunoreactive.
6. The toxic agent and the scavenger enzyme stoichiometry should approach 1:1.
7. It shouldn't cause any side effect in large quantities of the enzyme and shouldn't perform decrements. (Lockridge, *et al*., 2011).

Human acetylcholinesterase does not meet all the criteria, as compared to the BChE enzyme. A liter of blood contains 0.5 mg AChE, a quantity that is 20% of the amount of BChE in the whole blood. Liberation of AChE from erythrocytes is not as simple as withdrawal of soluble BChE from plasma (Lockridge *et al*., 2005). The monomeric and dimeric AChE is filtered from blood circulation of the animals in few minutes (Chitlaru *et al*., 2001). AChE is sticky on plastic and glasses which results in loss of large amount of the enzyme unless amount of albumin is added or the protein concentration is high.

The criteria for butyrylcholineserase to be an effective scavenger for organophosphorus toxican are:

1. It acts immediately with organophosphorus toxicans.

2. The human plasma contains soluble concentration of BChE of 5 mg l^{-1}. (Lockridge *et al*., 2011).

3. The purified enzyme is stabile for years and the sugarcoated of butyrylcholinesterase tetramer protect it from proteolysis (Saxena *et al*., 2006).

4. Purified butyrylcholiesterase tetramer has a half-life of eight to twelve days after injection into human body.

5. Various transfusion of blood comprise human butyrylcholinesterase have cause no reverse effect in humans although existence of naturally occurring mutation in BChE (Souza *et al*., 2005). Injected BChE to human doesn't predict to cause any immune reaction (Rosenberg *et al*., 2002).

6. The Butyrylcholiestarse covalently bind to one molecule of toxicant per molecule of enzyme.

7. By following the direction of quantities of BChE up to 800 times higher than the endogenous blood levels in animals, no performance reduction or reverse side effects have been observed (Lockridge, *et al*., 2011).

8. The wide substrate specificity of Butyrylcholinesterase is another advantage. It has the capacity to hydrolyze other esters than Acetylcholinesterase, and it can be used for protection from toxicity of nerve agents and also pesticides, furthermore to protect from Succinylcholine apnea, cocaine toxicity and mivacurium apnea (Ashani *et al*., 2000).

2.5. Examples of Hydrolytic Reactions of Butyrylcholinesterase

2.5.1. Succinylcholine (SuCh)

In general succinylcholine which is used clinically today is the only ultrashort-acting depolarizing neuromuscular blocking agent (Kaufman *et al*., 2011). It is still the drug of choice to enable rapid-sequence endotracheal intubation for the period of stimulation of anesthesia or emergency tactics related to gastro esophageal regurgitation (Mohamed & Lien, 2010).

Succinylcholine structurally contains of 2 acetylcholine molecules joined through the acetate methyl groups which mimic the neurotransmitter acetylcholine. The short action interval of succinylcholine (estimated extraction half-life 47 seconds) is due to its speedy hydrolysis by butyrylcholinesterase (Torda *et al*., 1997).

Infrequently, patients with deficiencies in butyrylcholinesterase activity show remarkable signals of sensitivity to succinylcholine, exhibited clinically by extended neuromuscular paralysis and consequent apnea (Kaufman *et al*., 2011).

In patients with genotypically ordinary butyrylcholinesterase, 90% recovery from succinylcholine-induced paralysis is dose-dependent, well generally 9–13 min are required after a norm dose of 1 mg kg^{-1} (Mohamed & Lien, 2010). Hence, an extended "wake-up" period with continuous muscular paralysis may give the attention to the medical caring staff of the probability for unrecognized hereditary butyrylcholinesterase deficiency (Maiorana & Roach, 2003). This may also be complemented as sedation wears off with unexplained tachycardia and patient anxiety happens in the face of continued paralysis, signifying the need for reassurance and supplementary sedation.

BChE genetic variations provided rise to extended succinylcholine effects (Kaufman *et al*., 2011). Many non-genetic factors such as advanced age, renal disease, malnutrition, liver disease, collagen vascular disease, pregnancy, malignancy, burns, hypothyroidism, acute infection, myocardial infarction and leprosy can also cause reduction in butyrylcholinesterase activity (Kaufman *et al*., 2011). Furthermore, certain medications and chemicals, including oral contraceptives, clindamycin, echothiophate eyedrops, insecticides (organophosphates, carbamates), pancuronium, metoclopramide, carbon disulfide, organic mercury have been involved to the elongate effects of succinylcholine-induced muscle paralysis (Soliday & Conley, 2010).

2.5.2. Cocaine

Cocaine abuse one of the medical problems all over the world. Symptoms of the cocaine toxicity ensure grand-mal seizure, cardiac arrest, stroke, and increase body temperature. Deaths due to cocaine abuse are not dose-related and toxicity cannot be predicted by blood levels (Duysen *et al*., 2008).

The deteriorating of cocaine addiction, both medically and socially, like aggressive crime, decrease in individual constructivism, sickness and death, giving high priority to effective pharmacological treatment. The main cocaine breakdown pathway is the enzymatic hydrolysis, at the benzoyl ester group or methyl ester group (Gorelick, 1997).

Benzoyl ester hydrolysis produces ecgonine methyl ester (EME), while methyl ester hydrolysis produces benzoylecgonine (BE). Butyrylcholinesterase (BChE) is the major cocaine-metabolizing enzymes in humans, which catalyzes benzoyl ester hydrolysis and two liver carboxylesterases (denoted by hCE-1 and hCE-2) that are able to catalyze hydrolysis at the methyl ester and at the benzoyl ester, correspondingly.

In human BChE is the principle cocaine hydrolase, around 95% of cocaine in humans is hydrolyze by BChE enzyme. The left behind part of 5% is deactivated by the liver microsomal cytochrome P450 system through amine oxidation, yielding nor cocaine (Poet *et al*., 1996).

Ecgonine methyl ester (EME) shows to be the lower pharmacologically active of the cocaine metabolites and may even cause to vasodilation, while both benzoylecgonine (BE) and norcocaine seem to cause vasoconstriction and lesser the seizure threshold, alike to cocaine itself. Norcocaine can be hepatotoxic and a local insensitivity (Pan & Hedaya, 1999). Therefore, hydrolysis at the benzoyl ester of cocaine by BChE is probably most appropriate pathway for amplification.

Studies in animals and humans have shown that the enhanced activity of BChE by administration with the exogenous enzyme basically reduce cocaine half-life. In case of the addition of human BChE (taken from donated blood) to human plasma containing cocaine 2 μg ml^{-1} reduce the in vitro half-life of cocaine at a BChE concentration of 3.02 μg ml^{-1} from 116 min to 10 min at a BChE concentration of 37.6 μg ml^{-1}.

In vivo studies in animals have also exposed significant improvement of BChE activity on cocaine's effects. A single injection of enzyme also increases BChE activity in plasma for numerous days (Zhan *et al*., 2003).

2.5.3. Heroin

The heroin (3, 6-diacetylmorphine) is a highly addictive prohibited opiate drug (Chou *et al*., 2013). Heroin makes euphoria or pleasurable feelings followed by drowsy feeling for more than a few hours due to depression of the central nervous system (CNS). The pills may damage ruling, memory and cloud mental operative since of the sleepy feeling and depression of the CNS (Chou *et al*., 2013).

The disastrous consequences of heroin addiction have increased the need to device effective pharmacological treatment of heroin abuse. Heroin indeed has a very low binding affinity to μ-opioid receptor, due to the low potency of heroin in activating the G-protein thence to produce its effects on neurotransmitter systems (Selley *et al*., 2001).

Even so, injected heroin is fastly metabolized through hydrolysis to 6-monoacetylmorphine (6-MAM), it can readily cross the blood-brain barrier (BBB) and then quickly concentrated in the brain (Qiao, & Zhan, 2013). Heroin can be hydrolyze by human serum BChE to 6-MAM with a reaction average of 4.5 min^{-1} per μmol l^{-1}, that is nine times of the hydrolysis rate (0.5 min^{-1} per μmol l^{-1}) by human erythrocyte AChE (Salmon *et al*., 1999). Thus, BChE can be consider as the major responsible enzyme for the activation process of hydrolyzing heroin into 6- monoacetylmorphine (MAM) in human body. Developing a new therapeutic treatment that may target the activation process will make a progression. One of the alternatives is designing a small molecule as an allosteric inhibitor which can significantly elevate the free energy barrier for BChE-catalyzed hydrolysis of heroin to 6-monoacetylmorphine (MAM) with maintaining the other enzyme functions.

The desirable allosteric inhibitor shouldn't block the enzyme active site possibly bind to a binding site (another than the enzyme active site), like the peripheral anionic site of BChE (which could be nearby Asp70), (Qiao & Zhan, 2013).

2.6. BChE Role like a Biomarker of Organophosphorus Exposure

BChE is considering being a prime biomarker of exposure to nerve agents and organophosphorus pesticides due to following:

A) Presenting the BChE in human plasma a tissue that is very easy to obtain.

B) BChE even at too low doses of organophosphorus esters (OP) it reacts rapidly to intoxicate.

C) Reaction of OP with BChE makes an irreversible covalent bond that stay stable in the circulation also in stored plasma.

D) BChE half-life in the circulation is for 12 days, meaning that even after several days from an incident blood drawn may still show detectable amounts of OP-BChE adduct.

E) Techniques to educe BChE from plasma have been developed in simple steps by binding to immobilized monoclonal (Sporty *et al*., 2010).

F) Digestion of BChE with pepsin yields the active site peptide FGES198AGAAS modified on Ser198 by OP. The small peptides ionize more readily than large peptides size is an advantage for mass spectral analysis.

G) Laboratories such as TNO laboratory in Rijswijk, Netherlands developed mass spectrometric methods and adopted worldwide to identify exposure to OP by analyzing OP adducts on BChE (Fidder *et al*., 2002).

The United Nations reported that the chemical weapons used in Damascus contained the nerve agent sarin have been used in that incident. Without describe any methods used to recognize sarin. Nevertheless, the mass spectrometry analyses may have been used to identify the sarin-BChE adduct (Lockridge, 2015).

2.7. Protective function of BChE

Organophosphorus compounds (OP) are widely used as chemical compounds that kill pests, like plasticizers, pharmaceuticals, also used in hydraulic fluid, and in jet engine fuel. Some are chemical weapon or fear. Exposure to organophosphorus agents causes acute toxicity result from inhibition of acetylcholinesterase (AChE) in contrast of inhibition of BChE that is thought to have a protective role, by scavenging OP molecules.

Plasma BChE whom prevent inhibition of neuromuscular and neuronal AChEs by scavenging of OP compounds. Pretreating monkeys with human BChE (*h*BChE) can be completely protected against OP toxicity, such us up to5 LD_{50} of soman (Nachon *et al*., 2005).

Studies elucidated that BChE have a protective role from the toxicity that occur by Organophosphorus compounds. (Saxena *et al*., 2011a) BChE enzyme has specific characteristics making it the gold standard for curative use by rapidly binding it protects from nerve agent toxicity and inactivating the nerve agents.

The enzyme BChE is a soluble protein in human plasma and the concentration is around 4 mg l^{-1}. Possibilities to produce great amounts of pure BChE are available due to the methods of enzyme purification from human plasma and Cohn fraction IV-4 BChE (Saxena *et al*., 2010). In the purified form the enzyme stay unchanging for years (Saxena *et al*., 2005; Doctor *et al*., 2010). BChE has a half-life of twelve days in the human circulation (Ostergaard *et al*., 1988).

2.8. The Relation between the Butyrylcholinesterase and Several Diseases

2.8.1. Role of Cholinesterases in Alzheimer's disease

Alzheimer's disease is a progressive neurodegenerative disorder the characteristic of this disease is the irreversible loss of memory and cognitive decline, and is associated with personality changes (Rakonczay *et al*., 2005). One of the major risk factor is aging (Saido *et al*., 2006).

The prevalence is reported to be nearly 1.5% at age 65 years and doubles each four years at age 80 years to reach around 30% (Foroughan *et al*., 2008). Alzheimer disease has grown to be a problem in high rate with the increase worldwide life expectancy (Bermejo *et al*., 2010). It became a critical challenge in the public health due to aging global population (Cummings, 2011). For millions of people the quality of life is affected by Alzheimer disease and the disastrous situations cause for the patients also for their families and the society (Rakonczay *et al*., 2005).

A combination of clinical assessment, psychological tests, imaging is need for its diagnosis to eradicate the possibility of other neurological disorders (Bermejo *et al*., 2010). AD certainly can be diagnosed through autopsy (Bakhtiari *et al*., 2017). The absence of tests for early diagnosis of AD is one of the main obstacles to its effective treatments (Lansbury *et al*., 2004).

The cholinergic defects in the CNS are the main neurochemical disorder in AD (Saido *et al*., 2006). The main groups of neurons that are destroyed in the early stages are cholinergic neurons, causing reduction in the levels of Acetylcholine (ACh) (Sayer *et al*., 2004). The significant reduction in cholinergic activity is a main phenomenon in neurochemistry of Alzheimer's (Urbanelli *et al*., 2009).

The Butyrylcholinesterase hydrolyze acetylcholine and other choline esters. It was notice that in AD patients the BChE levels elevate. Although the enzyme full role is not completely understood there are some suggestion in studies that BChE promote amyloid

plaque formation, therefore the study researches in cholinesterase inhibition has been begin (Darvesh *et al*., 2012).

In Alzheimer disease patients as the cholinergic nerve terminals was lost the membrane bound from of AChE deny, while the BChE stays unchanged or almost elevate (Lane *et al*., 2006). In rats with lesions of cortical cholinergic neurons the level of AChE are reduce but its level can be preserved by selective inhibitors of BChE (Giacobini, 2000). Thence, recently it was recognized drugs that effect and inhibit both of the enzymes may be favorable for patients with Alzheimer disease than the selective inhibitors drugs like donepezil for AChE (Lane *et al*., 2006).

2.8.2. Role of Butyrylcholinesterase in Regulation of Ghrelin

Purified BChE releases ghrelin's (a peptide hormone involved in hunger, feeding, and stress) octanoyl group in vitro, converting it into a putatively inactive "desacyl" form (De Vriese *et al*., 2004).

In vitro study after gene transfer to male BALB/c mice with high plasma BChE investigate that mouse aggression decreases with the increase level of BChE, which hydrolyze ghrelin and moderate emotional states that predispose to fighting. In contrast, the loss of BChE activity generates an increase in ghrelin hormone and the tendency to fight between young adult males also increase (Chen *et al*., 2015).

2.8.3. Relation between Butyrylcholinesterase and Coronary Artery Disease (CAD)

The predominant manifestation in cardiovascular disease patients is the atherosclerosis, is demonstrated with long term mortality also with a high morbidity (Goliasch *et al*., 2012). Large assortments of cardiovascular risk factors have been marked as the risk factors to contribute to coronary artery disease (Graham *et al*., 2007).

SV erum butyrylcholinesterase has been involved in the evolution of coronary artery disease (Alcantara *et al*., 2002). In a previews study, it was elucidated that individuals with lowest quintile of butyrylcholinesterase activity significantly had elevated rates for cardiovascular mortality (Calderon-Margalit *et al*., 2006). In another recent study, a strong and independent association between low butyrylcholinesterase activity and long-term outcome in patients with known CAD has been found (Goliasch *et al*., 2012).

2.8.4. Butyrylcholinesterase Activity and Low-grade Ssystemic Inflammation

The cholinergic anti-inflammatory pathway exerts directly inhibitor effect on the pro inflammatory cytokine production mediated by the neurotransmitter enzyme acetylcholinesterase (Rosas, Ballina, & Tracey, 2009). An increase in AChE and BChE enzyme activities cause a greater hydrolytic destruction and reduce acetylcholine concentrations, and this could trigger and continue the systemic inflammation (Das, 2007). Several studies elucidate the association of chronic low-grade inflammation, as defined by level of serum C-reactive protein (D'Amore, 2005) and BChE activity (Stojanov *et al*., 2011) with metabolic syndrome, obesity, and cardiovascular risk and insulin resistance. Therefore, increased BChE activity in plasma and tissues in different clinical issues suggest that it could be a marker for low grade systemic inflammation (Das, 2007).

Recently, marked increases of plasma BChE activity correlated with different inflammatory markers, such as fibrinogen, interleukin-6, and CRP, during the acute phase in stroke patients have been reported (Assayag *et al*., 2010). In number of pathophysiological states an elevate in BChE enzyme level have been characterize such as in obesity, hyperlipidemia, hypertension, insulin resistance, metabolic syndrome, hepatic steatosis, diabetes mellitus also Alzheimer disease, in cases that the low-grade systemic inflammation have a role in its development. Therefore, elevated levels of BChE enzyme in plasma and tissues is suggested being as a marker in low-grads systemic inflammation (Lampón *et al*., 2012).

2.8.5. Butyrylcholinesterase Correlation with Metabolic Syndrome

The characterizations of metabolic syndrome are associated with visceral fat, abnormal lipid profile (low level of high density lipoprotein and high levels of triglyceride), hypertension, and glucose intolerance (known as insulin resistance). Individuals that manifest with abnormality of metabolic syndrome are in increased risk for coronary artery disease (CAD) as well in risk developing diabetes mellitus type two. The correlation of these metabolic syndromes including lipid abnormalities with obesity and the diabetes may also implicate involvement of BChE enzyme level. Therefore, BChE enzyme may be used as a marker for the pathological processes intermediating in the metabolic syndrome (Randell *et al*., 2005).

Serum BChE activity demonstrate a strongly positive relation with total cholesterol levels and the serum triglyceride, also a negative relation with high density lipoprotein (HDL) (Alcantara *et al*., 2002). In patient's serum with hyperlipidemias, diabetes mellitus also obesity shown high levels of enzyme activity by means of compared to individuals with healthy and lower body weight. On the other hand, stimulation of animal models with diabetes or obesity is associated with a parallel elevation in both levels of serum triglyceride and BChE activity (Kutty & Payne, 1994).

Serum BChE activity was elevated in equivalent with most patients that shows marked elevation in the very low-density lipoprotein (Nassar *et al*., 2002). As an explanation for the relation of serum BChE activity with increased lipid levels it was guessed that the enzyme changes in structure of tertiary and quaternary, more than increased enzyme synthesis. Additionally, a positive correlation of serum BChE activity with midriff perimeter, more precisely with trunk fats may propose an undeviating relationship among enzyme activity, insulin resistance syndromes and obesity, (Randell *et al*., 2005).

2.9. Abamectin

Human being is exposed to pesticides directly, as employees in green-houses and in the agriculture, or indirectly, via foodstuff ingesting. In addition, a considerable amount of pesticides and their metabolites reach rivers and estuaries through run-off from farmland that are toxic to environment also (El-Shenawy, 2010).

Abamectin (Figure 2.1) is the widely used name indicated to the avermectins, a combination that contain 80% avermectin B_{1a} and around 20% B_{1b}, homologs that have biological activity almost equivalent. Miticide/Pesticide used is composed of Clinch(r) which is fire ant bait, and Avid(r). Abamectin are known to have numerous local systemic properties. When only the upper surface is treated it can lead to the death of mites on contact with the underside of the leaf. The most favorable usages of these constituents are to control spider bugs, leaf miners and the interior parasites in animal household's other difficult monitoring glasshouse pests, (Ware & Whitacre, 2004).

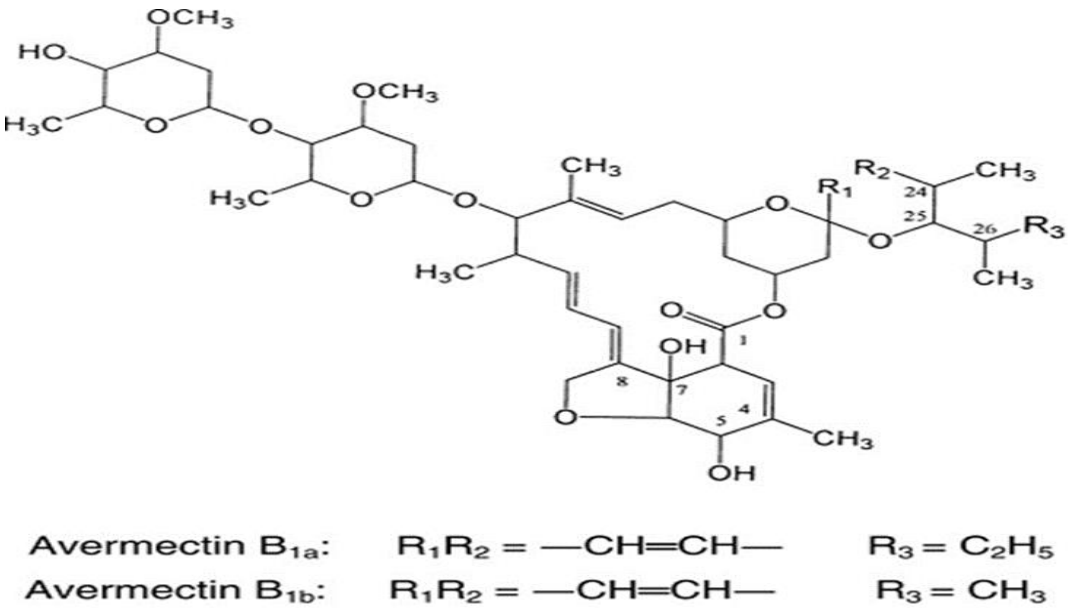

Figure 2.1 Chemical structure of abamectin composed of avermectin B_{1a} and B_{1b} (Zanoli et al., 2012).

The macrocyclic lactone components are composed of two subgroups. The first group is the avermectins, that include abamectin, emamectin, doramectin and some others. Avermectins, as antiparasitic drugs and agricultural pesticides, are a kind of neurotoxic macrocyclic lactone compounds (Valenzuela *et al*., 2000) They are exceedingly used in the protection of animals and crops, such as corn, peanut, cattle and pigs, against insects and mites (Howells & Sauer, 2001). The other subgroup has saccharide substituents (Durden, 2007).

2.10. Avermectins

Avermectins are 16-membered macrocyclic lactones figure out from soil-dwelling actinomycetes of the kind Streptomyces. They are highly vigorous against nematode and arthropod species as well as acarine and insect plant pathogens. Due to their use in the treatment of endoparasitic infections and ectoparasitic infestations they award the name as endocetocide. The avermectins have a disaccharide moiety attached to the 13-position of the macrocyclic ring as a primary structure (Schenck *et al*., 1999).

From fermentation of Streptomyces auermitilis, the avermectins are yield as a combination variant of eight constituents. These natural compounds indicate A_{1a}, A_{1b}, A_{2a}, A_{2b}, B_{1a}, B_{1b}, B_{2a}, also B_{2b}. A-compounds consist a group of methoxy at the 5-placement, while the B-compounds consist a group of hydroxy, the 1-compound between the 22- and 23-placement contain a double bond, while the 2-compound at the 23-placement contain a hydroxy group with single bond; and the A-compounds at the 25-placement consist a secondary butyl side chain, while B-compounds at the 25- placement consist an isopropyl substituent. Split-up of A-compounds from the B-compounds in huge fermentation scale is unpractical and needless considering that the two homologs ensure practically indistinguishable actions (Shoop *et al*., 1995).

Avermectins are toxic to the nervous and growth systems, and their harm to the environment and humans has raised increasing concerns on the topic of residue analysis in food and agro-products (Huang *et al*., 2014).

With high attraction they bind to various insects species from site of muscle head of neuronal membrane (Rohrer *et al*., 1995) performing in that way as agonists for the GABA- gated chloride channel (Lasota & Dybas, 1991).

The rapidly degradation of avermectins residues, forms a diversity of products. The mainly residues with toxicological importance are avermectins as the existence in the fruits of these residues can cause effect to the consumer healthiness (Valenzuela *et al*., 2000). *S. avermitilis* produce multiple avermectins drugs against nematode and arthropod invasions which are one of the widely most employed. The ivermectin, in addition to abamectin, are commonly used pesticide and anthelmintic (Burg *et al*., 1979).

Abamectin is a type of large-ring lactone disaccharide compound *(*Luo *et al*., 2013) is the avermectin isomers main pair that was commercialized, and the cis-hydrogenated product of abamectin is ivermectin (Tolan *et al*., 1980).

It is highly lipophilic and used both as a biocide and as an anthelminthic drug. The toxicological mechanism of abamectin is believed to affect the γ-aminobutyric acid (GABA) system and the Cl^- channels of animal cells (Maioli *et al*., 2013) in which the GABA receptor is in charge for fixing the neural basal tone (Turner *et al*., 1989).

Abamectin compounds are toxic especially to phytophagous mites and to a choice panel of insect species, but it is obviously lower in potent against certain lepidopterin and homopterin types (Lasota & Dybas, 1991). Abamectin is consider to be fewer poisonous to the benefited insects like the honey bee, parasitoids and predators (Zhang & Sanderson, 1990) so it may be considered as selective insecticide.

Ivermectin is a product gained from avermectin a semi-synthetic, synthesized naturally by the microorganism *S. avermitilis*. Ivermectin with wide range for medical applications in it is used for treatment of rashes, worms and lice, by acting on the muscles functions, in the nervous system, causing in paralysis and demise of the parasites (Rolim *et al* ., 2015).

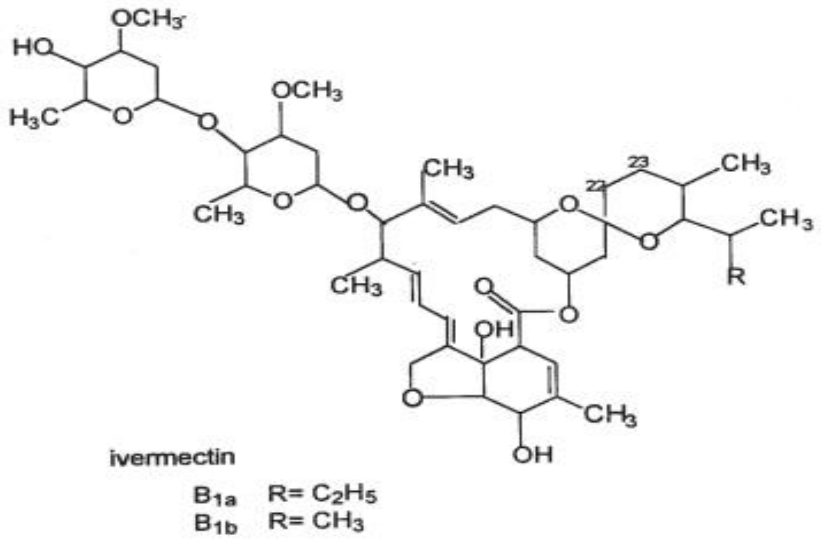

Figure 2.2 Structure of ivermectin (Schenck et al., 1999).

Numerous medicines are also enzyme inhibitors which are been used as herbicides and insecticides. The enzyme inhibitor is a fragment that impasses to enzymes and result to reduces activity of the enzyme. By means of blocking an enzyme's activity this can destroy a pathogen or recover an imbalance in the metabolism.

The substrate can be prevented from binding to enzyme active site by binding of an inhibitor to the enzyme and prohibiting the enzyme from catalyzing its reaction. Numerous medications works like enzyme inhibitors, therefore discovery and progression is an important research area in pharmacology and biochemistry (Shapiro & Vallee, 1991).

It is important to estimate the potential impact of these antibiotics on ecological and living systems after it has been reported that wide use of avermectins can outcome in environmental pollution. These antibiotics have been demonstrated to raise the rate of apoptosis and the expression levels of caspase and mRNA in the liver of pigeons. By increased exposure dose some ultra-structural alterations, inclusive mitochondrial damage and chromatin aggregation, become intense.

Exposition to avermectins stimulate significant changes in antioxidant enzyme including superoxide dismutase activities and glutathione peroxidase and malondialdehyde levels, and enhance protein carbonyl content and DNA-protein crosslinking, in a concentration-dependent methods in the liver of pigeons (Zhu *et al*., 2013).

In a research concerning the effect of avermectins on the cytosolic bovine erythrocyte carbonic anhydrase (bCA) enzyme was proved that bCA enzyme was effectively inhibited with inhibition constants in the nanomolar range by avermectins including abamectin. Concerning the structure of carbonic anhydrase enzyme that has a Zn^{2+} ion in the active site and Avermectins include electronegative atoms that interact with the active site and inhibit the bCA enzyme (Kose *et al*., 2016).

By ongoing contamination of the ambient all organisms are exposed to the effects of these pollutants. In many countries the waste materials of factories are buried or released into the air. Naturally, this pollution may then be transmitting to resources and to living creatures in nature, which is a considerable threat to future generations. Thence, regarding the use of pesticides, enzyme activity studies are of great importance (Akbaba *et al*., 2014).

2.11. Bioinformatics and Docking

The bioinformatics present a new, processing area in the computational access to answer biological questions, answering those questions requires the investigators to use large complex date in order to reach adequate biological conclusions. This approach helps to more efficiently guide the experimental projection in laboratory (Baxevanis & Ouellette, 2004). The investigation in bioinformatics, and drug discovery also biology furthermore the characterization of interaction in protein-ligand complex is needed. There are nearly 100,000 deposited protein structure in Protein Data Bank (PDB) more than 75% where settle in small molecule ligands complex.

Binding of a protein to a ligand require specific configurations of attractive, characteristically non-covalent links among both molecules. We can get deep insight into how a protein interacts with a ligand with such rich data (Salentin *et al*., 2015). Detailed individual characterizations are important to elucidate the molecular recognition also the protein function or to enhance and optimize lead compounds. The relative studies can enhance protein–ligand deducting or virtual screening (Liu *et al*., 2013).

In this study, the molecular docking calculations were performed in order to explain the interaction of avermectins with the butyrylcholiesterase enzyme.

3. Materials and Methods

3.1. Chemicals Used To Perform the Experiment

5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB), S-Butrythiocholine Iodide (BTC), Butyrylcholinesterase (BChE) extracted from horse serum, Dimethyl sulfoxide (DMSO), were obtained from sigma Aldrich (St. Louis, MO, USA). Abamectin (avermectin b_{1a} 95% and avermectin B_{1b} 5%) from Agrikem (Izmir, Turkey), MOPS, KOH,

3.2. Measurement of the BChE Enzyme Activity

According to the colorimetric method of Ellman (*et al*., 1961), kinetic studies were carried out for the determination of K_m and V_m values, all the experiment was carried out by using a Perkin Elmer Lambda 25 UV/VIS Spectrophotometer.

For measuring the butyrylcholinesterase activity the butyrylthiocholine (BTC) was employed as reaction substrates with 5,5-dithio-bis-(2-nitrobenzoic acid) the (DTNB)ion was used as chromogenic substrate, in the reaction thiocholine was formed by enzyme hydrolysis activity, leads to the formation of yellow 5-thio-2-bis-nitrobenzoate (TNB) ion. Therefore BChE activity was measured by following the increase of absorbance at 412nm. Over all reaction is catalyzed by the enzyme butyrylcholinesterase which is demonstrated below:

$$\text{Butyrylcholine} + H_2O \longrightarrow \text{Butyrate} + \text{Thiocholine} \qquad (3.1)$$

$$\text{Thiocholine} + 5,5\text{-dithio-bis-2-nitrobenzoic acid} \longrightarrow \text{TNB} \qquad (3.2)$$

The enzyme activity was measured in present of six different concentrations of BTC (0.05 mM, 0.1 mM, 0.25 mM, 0.50 mM, 1 mM, 2 mM). The reaction mixture were: 250 µl of 200 mM MOPS at pH 7.5, 165 µl of distilled water, 50 µl of 2.5 mM DTNB, 10 µl of BTC in varied concentrations and 25 µl of BChE. Constantly the reaction was started by adding the enzyme to the mixture. For each BTC concentration activity measurement was performed in triplicate for 20 s at 37 °C.

Average activity (U ml^{-1}) values was calculated and converted to Specific Activity (U mg^{-1} protein) in order to draw Michealis–Menten, Lineweaver–Burke, Dixon and other plots (Segel, 1975).

Specific Activity (Unit mg^{-1} protein) = $\frac{\boldsymbol{Abs412 \times Vt \times 1000}}{\boldsymbol{13.6 \times Vs \times [Protein]}}$

ΔAbs412 min^{-1}: Change in absorbance min at 412 nm

V_t : The total volume of activity measurement mixture (500 µl)

V_s : Enzyme volume used in the measurement of enzyme activity (25 µl)

13.6: Extinction coefficient of 5-thio-2-nitrobenzoic acid (mM)

3.3. The Inhibitory Effect of Abamectin on BChE Activity

Abamectin was dissolved in dimethyl sulphoxide (DMSO). From the experiment its confirmed that DMSO had no effect on the assays by measuring the enzyme activity in present of DMSO without abamectin. Reaction mixture was consisted of 250 µl of 200 mM MOPS pH at 7.5, 160 µl of distilled water, 50 µl of 2.5 mM DTNB, 10 µl of 1 mM BTC, 5 µl of Abamectin and 25 µl of BChE. Constantly the reaction was started by adding the enzyme to the mixture, abamectin was prepared in different concentrations with final concentrations ranged from (2.5 µM, 5.0 µM, 7.5 µM, 10 µM, 12.5 µM, 15 µM, 20 µM, 5 µM, 30 µM). Enzyme activity for each Abamectin concentration was measured at 412 nm for 20 s at 37 °C. Each measurement for the activity was performed in triplicates. Average specific activity was calculated and used to draw related graphics. The inhibition type shown (Figure 4.9), by using constant concentration of abamectin 10 µM, with different volume of BChE. The reaction mixture was consisted of 250 µl of 200 mM MOPS pH at 7.5, 1 mM BTC, 2.5 mM DTNB, the distilled water volume was various, used for adjustment the final volume, by increasing the enzyme volume the distilled water volume decreased in the reaction mixture.

3.4. Inhibitory Kinetic Studies with Abamectin

Inhibitory kinetic experiments were performed to define K_m and V_m values of BChE in the present of variable concentrations of abamectin. Five different abamectin concentrations (1 µM, 2 µM, 4 µM, 6 µM, 8 µM) and six various concentrations of BTC (0.05 mM, 0.1 mM, 0.25 mM, 0.50 mM, 1 mM, 2 mM) were tested. Reaction mixture consisted of 250 µl of 200 mM MOPS at pH 7.5, 160 µl of distilled water, 50 µl of 2.5 mM DTNB, 10 µl of 1 mM BTC, 5 µl of abamectin and 25 µl of BChE. Constantly the reaction was started by adding the enzyme to the reaction mixture, increase in the absorbance at 412 nm was monitored for 20 s at 37 °C. For every measurement three reading were taken. The average specific activity was calculated and used to related graphics.

3.5. Statistical Analysis

Statistica (Statsoft Inc., Tulsa, OK, USA) edition `99, non-linear estimation module was used in calculation of the kinetic parameters and the valuation of the inhibition sort.

3.6. Homology Modeling

3.6.1. Selection of Template for Protein

The three-dimensional crystal structure of human butyrylcholinesterase (*h*BChE) was downloaded from RCBS PDB database (Protein Data Bank) (PDB ID: 3O9M; resolution 2.98 Å) consisting of the protein crystal structure complexed with benzoic acid (the hydrolyzing product of cocaine). From the tetrameric crystal structure of *h*BChE, three of the subunits were deleted. Only one monomeric subunit in complex with benzoic acid was used. The *h*BChE was used in the molecular studies because the 3D structure of the horse BChE was not available on protein data bank and is closely related to horse BChE than other BChE with 90% identity (Alpi *et al.*, 2015).

3.6.2. Modeler

CPHmodels-3.0) was the web-server used to predict the protein three dimension structure by use of single template homology modeling based on crystal structure of hBChE and generate the 3D structure of horse BChE (Nielsen et al., 2010). The residue profiles of the three-dimensional models were further checked using VERIFY3D which elucidates the compatibility of an atomic model (3D) with its own amino acid sequence (Tiwari et al., 2012).

3.6.3. Ligand Molecule

In the present study, avermectin B_{1a} was used as the ligand. The 3D structure was retrieved from the NCBI PubChem database with compound ID: 6434889, by using canonical smile code to generate the 3D structure using a 3D Structure Predictor, COSMOS.

3.6.4. Docking

Molecular docking was carried out to elucidate the protein–ligand interaction. This was performed between the horse BChE and avermectin b_{1a} as a ligand using a docking algorithm, PatchDock (Schneidman *et al*., 2005). Both the horse BChE homology model and avermectin B_{1a} were inputted into the PatchDock request form in PDB file format. The horse BChE was the receptor while the avermectin B_{1a} was the ligand. The solution with the highest geometric score was selected.

3.6.5. Validation of Docking Results

To analyze the interatomic association between the horse BChE and avermectin B_{1a}, a web server for the visualization and comprehensive detection of protein–ligand interaction, Protein Ligand Interaction Profiler (PLIP), was used (Salentin *et al.,* 2015). Then a molecular visualizing system, PyMOL, was used to obtain desired images from the outcome file.

4. Results

4.1. Substrate Kinetics

Kinetic parameters for the butyrylcholinesterse enzyme were determined by using different concentration of butyrylthiocholine (BTC) while keeping the DTNB in constant concentration. In the reaction mixture, final concentration of BTC was (0.05 mM, 0.1 mM, 0.25 mM, 0.50 mM, 1 mM, 2 mM) and DTNB was (2.5 mM). The whole activity measurements were achieved by repeating three times and the specific activity (U mg^{-1} of protein) was calculated. The specific activity was plotted versus BTC concentration and Michaelis–Menten graph was obtained (Figure 4.1). The Lineweaver–Burk plot was drawn by using the same data (figure 4.2).

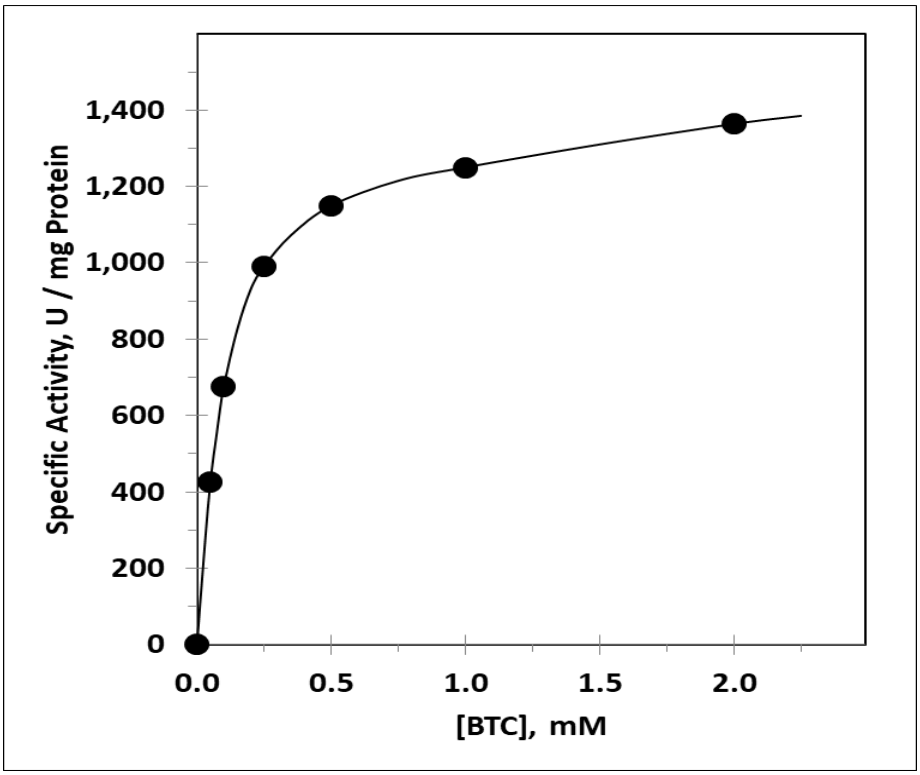

Figure 4.1 Michaelis–Menten plot of butyrylcholinesterase enzyme.

BTC concentration was (0.05 mM, 0.1 mM, 0.25 mM, 0.50 mM, 1 mM, 2 mM).

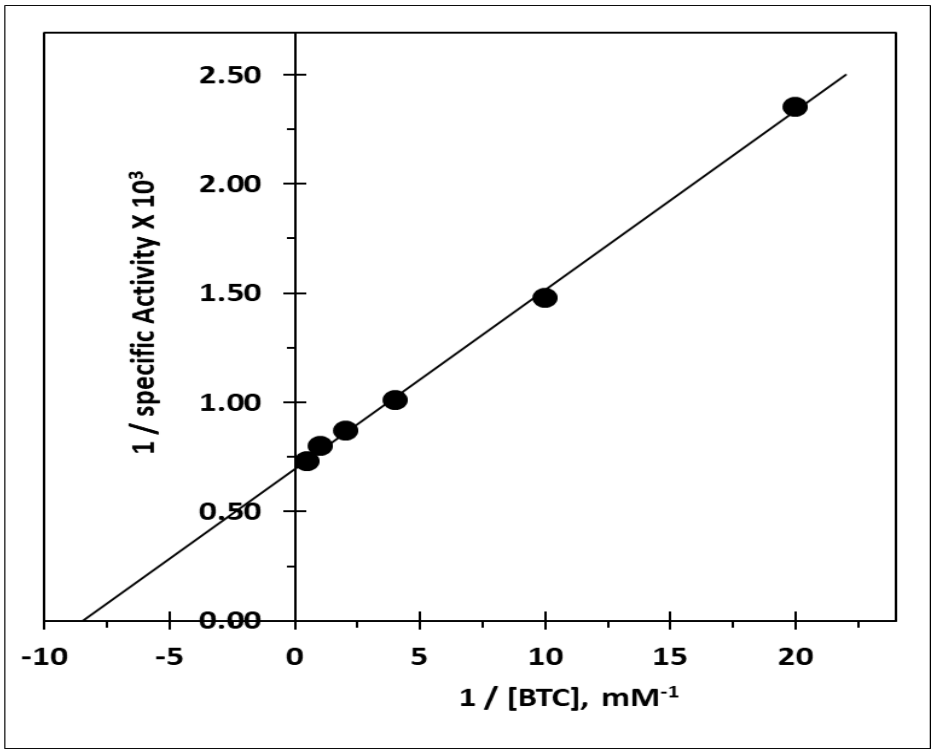

Figure 4.2 Lineweaver–Burk plot of butyrylcholinesterase enzyme.
BTC concentration was (0.05 mM, 0.1 mM, 0.25 mM, 0.50 mM, 1 mM, 2 mM).

4.2. Inhibitory kinetic behavior of butyrylcholinesterse enzyme with abamectin

The butyrylcholinesterase activity was measured by using nine different abamectin concentrations (2.5 µM, 5.0 µM, 7.5 µM, 10 µM, 12.5 µM, 15 µM, 20 µM, 5 µM, 30 µM). Final concentrations of DTNB and BTC in the reaction mixture were 2.5 mM and 1 mM, respectively. For each abamectin concentration the measurement was performed in triplicate and average specific activities were calculated. The (Figure 4.3) demonstrates that the inhibition of butyrylcholinesterase enzyme varies with different concentrations of abamectin. Even though, the enzyme activity gradually was reduced in the concentration range of the study the inhibition did not reach zero. The value of IC_{50} was calculated as 10.6 µM. The four various concentrations of abamectin (1 µM, 2 µM, 4 µM, 8 µM) were used for the inhibitory kinetic experiment these concentrations were carefully chosen from the range were the inhibition was linear.

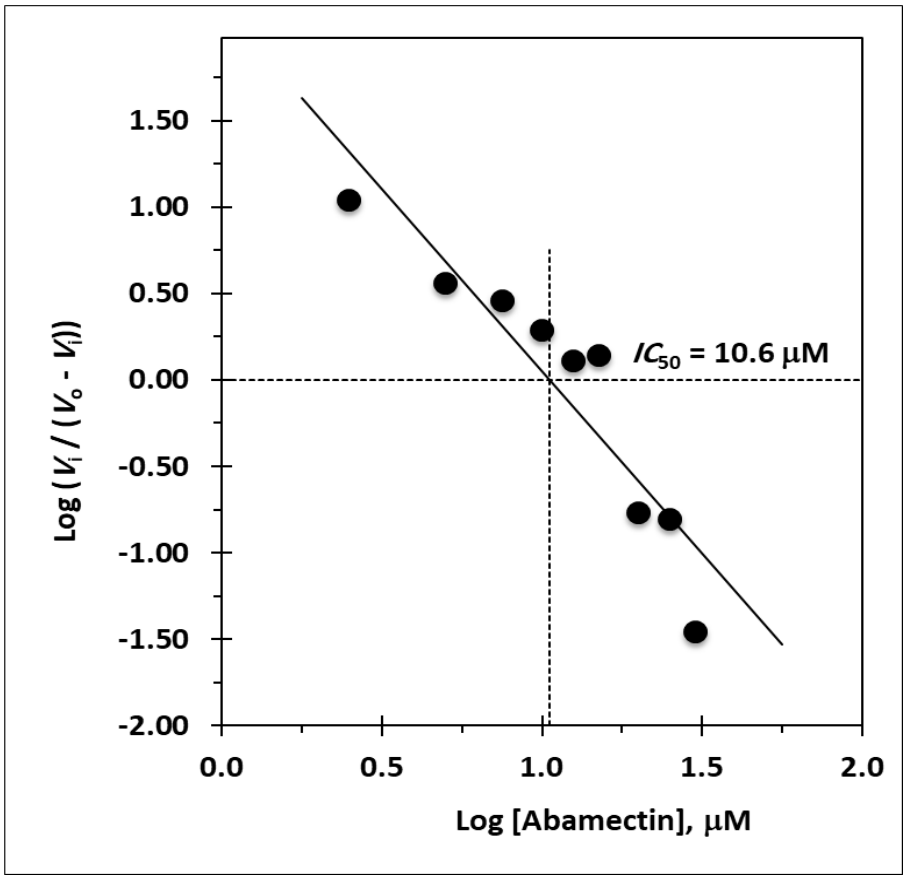

Figure 4.3 **Inhibitory hill plot for abamectin inhibition of BChE.**

Log (v1/(v0-v1) vs. Log[abamectin]. [BTC] = 1 mM; [Abamectin] = (2.5 µM, 5.0 µM, 7.5 µM, 10 µM, 12.5 µM, 15 µM, 20 µM, 5 µM, 30 µM).

The inhibition of butyrylcholinesterase in the present of various concentrations of abamectin (1 µM, 2 µM, 4 µM, 6 µM, 8 µM) was obtained. The activity of BChE enzyme was measured at different concentrations of BTC (0.05 mM, 0.1 mM, 0.25 mM, 0.50 mM, 1 mM, and 2 mM). Every activity measurements were repeated in three times specific activities calculations were used for drawing the Michaelis–Menten (shown in figure 4.4), Lineweaver–Burk (Figure 4.5) and Dixon plots (Figure 4.6). At variable concentration of BTC and abamecin the butyrylcholinesterase was inhibited competitively (figure 4.5). From the intercept points on Lineweaver–Burk the kinetic parameter K_m and K_i were calculated and found to be as 0.155 ± 0.020 µM and 2.26 ± 0.35 µM, correspondingly. The V_m was calculated as 252.6 ± 7.11 (U mg^{-1} protein) (Figure 4.5, figure 4.6).

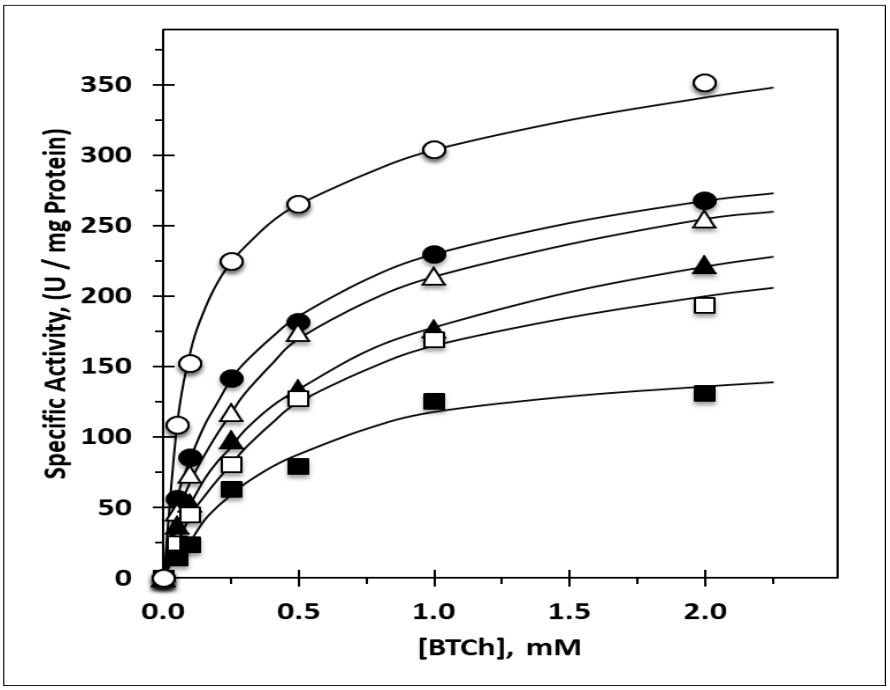

Figure 5 Michaelis–Menten plot of butyrylcholinesterase in the present of abamectin.

[BTC] concentration were varied (0.05 mM, 0.1 mM, 0.25 mM, 0.50 mM, 1 mM, and 2 mM). [Abamectin] = (○) 0 µM, (●) 1 µM, (Δ) 2 µM, (▲) 4 µM, (□) 6 µM, (■) 8 µM.

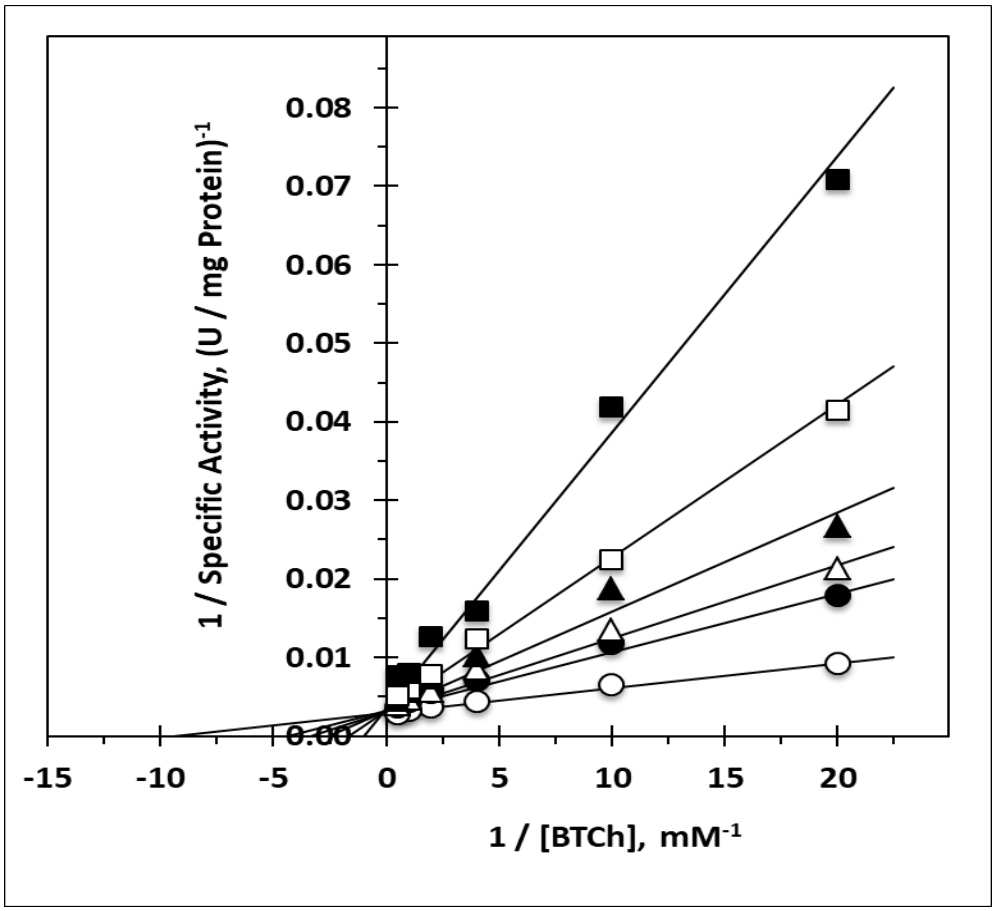

Figure 4.5 Lineweaver–Burk plot butyrycholinesterase enzyme in the present of abamectin.

[BTC] concentration were varied (0.05 mM, 0.1 mM, 0.25 mM, 0.50 mM, 1 mM, and 2 mM). [Abamectin] = (○) 0 μM , (●) 1 μM, (Δ) 2 μM, (▲) 4 μM, (□) 6 μM, (■) 8 μM.

Intercept point on ordinate elucidate that abamectin inhibits BChE enzyme competitively. The V_m value was not affected and the K_m increased. Competitive inhibition type was confirmed by Dixon plot (figure 4.7). In slopes versus 1/(BTC) plot, line passes through the origin demonstrating that the inhibition type is competitive (figure 4.8).

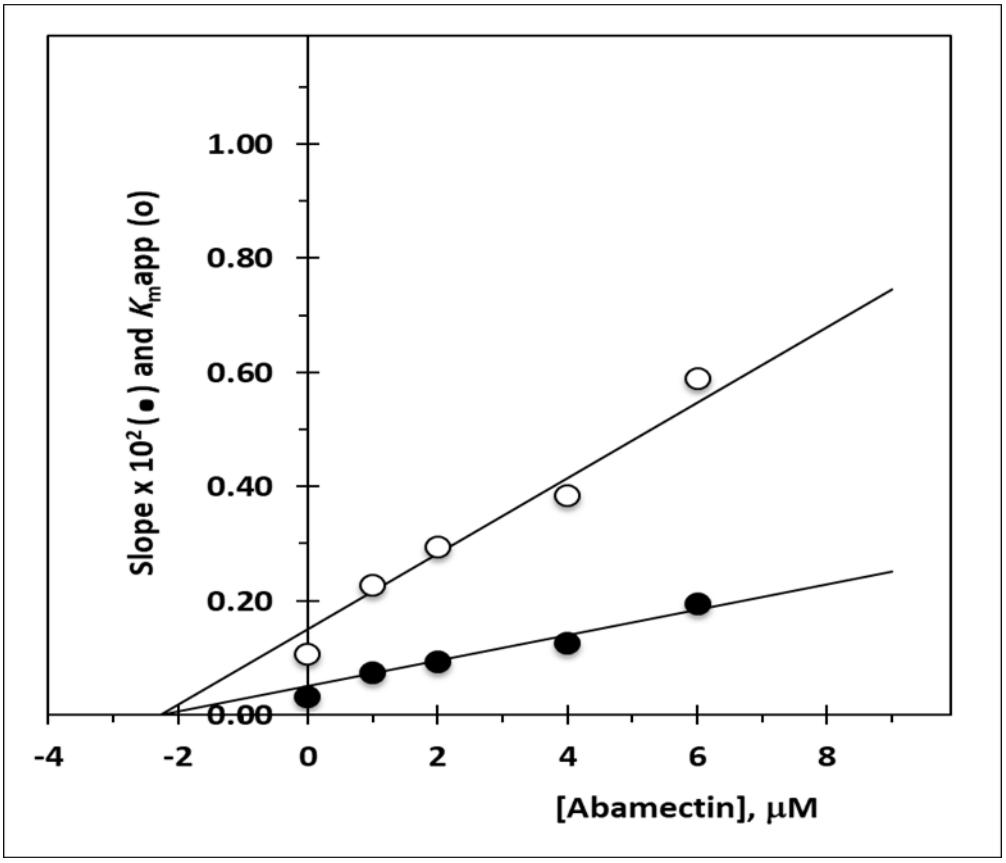

Figure 4.6 Kmapp vs. abamectin and slope of the reciprocal vs. abamectin plots.

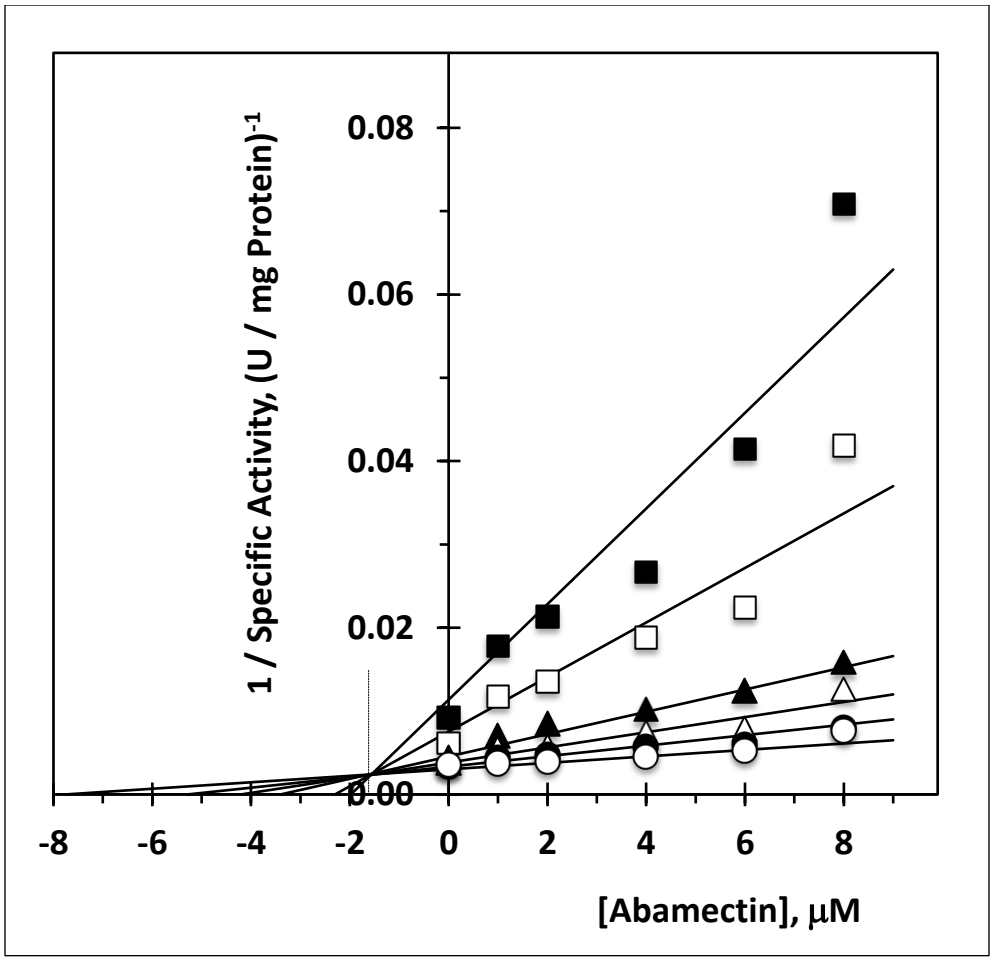

Figure 4.7 Dixon plot of butyrylcholinesterase in the presence of abamectin.

[BTC] concentration were varied (0.05 mM, 0.1 mM, 0.25 mM, 0.50 mM, 1 mM, 2 mM).

[Abamectin] = (○) 0 µM, (●) 1 µM, (Δ) 2 µM, (▲) 4 µM, (□), 6 µM, (■) 8 µM.

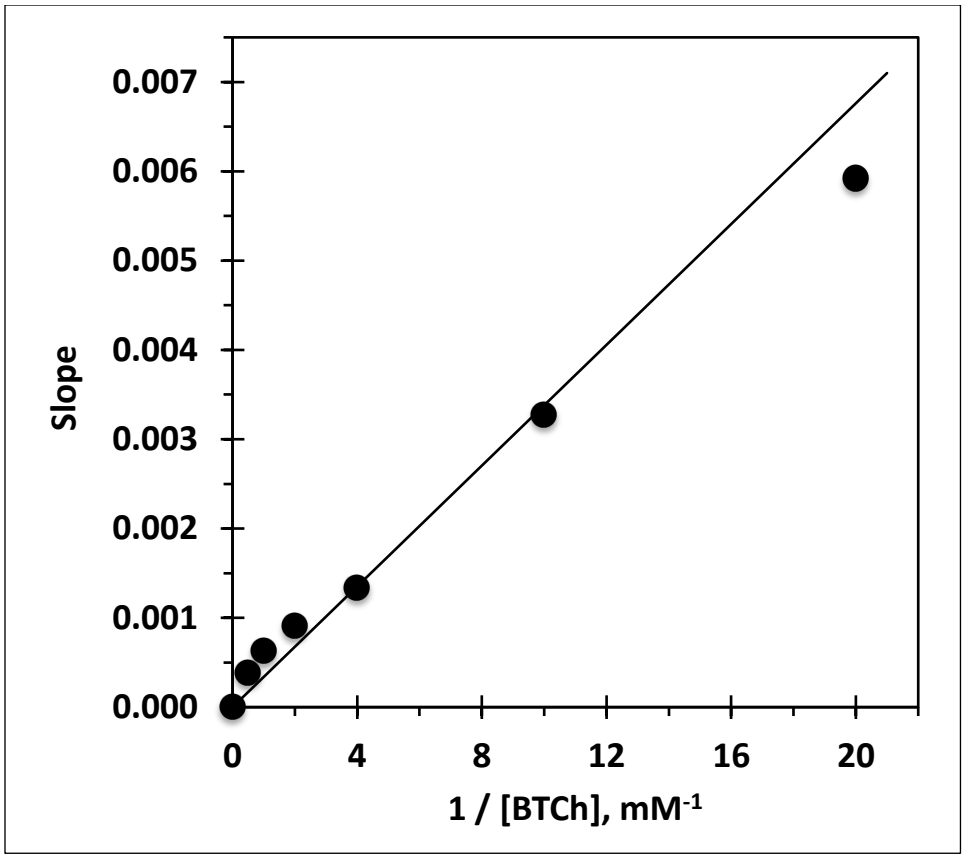

Figure 4.8 Slope versus 1/(BTC) plot

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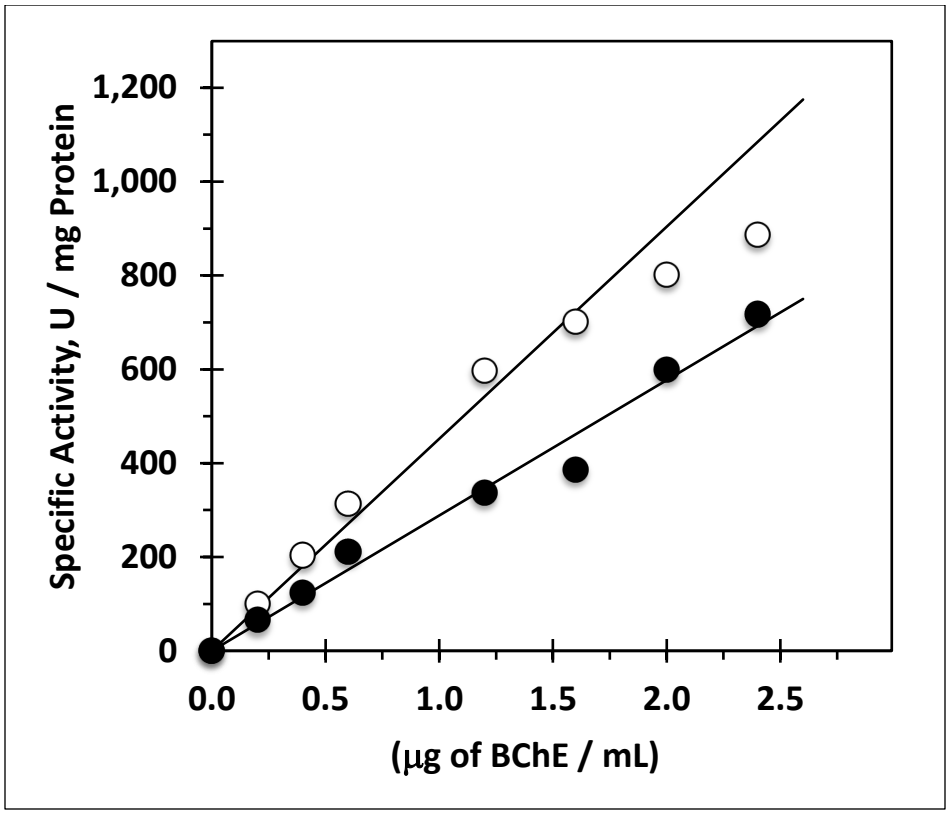

Figure 4.9 Reversible Inhibition of abamectin

Specific activity (U mg^{-1} protein) versus μg ml^{-1} of BChE.

This figure elucidate that abamectin inhibits BChE enzyme reversible without causing permanent effect on the enzyme. The line pass through the origin show that the inhibition is reversible.

4.3. *In silico* Analyses results

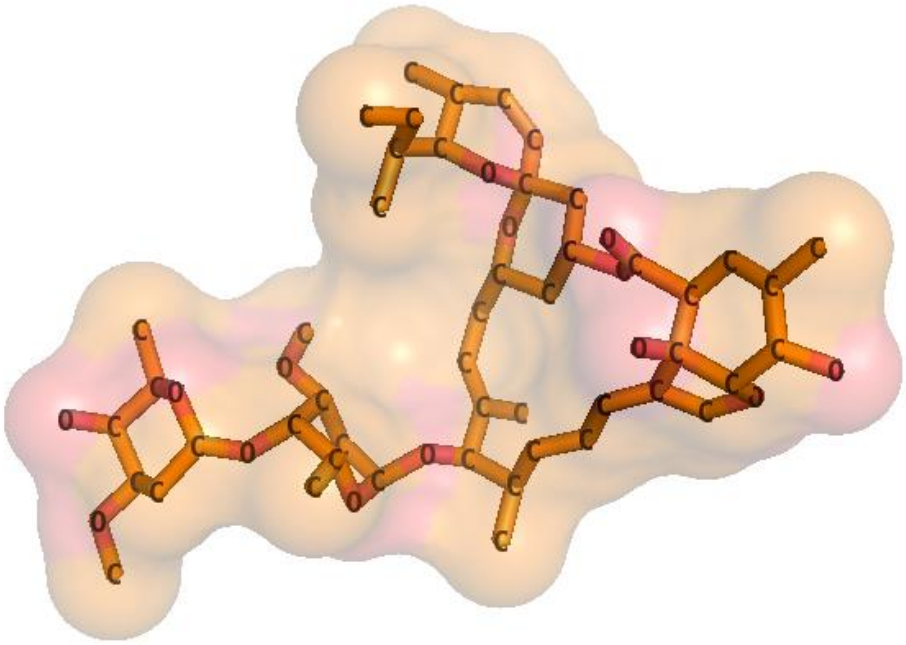

Figure 4.3.10 The chemical structure of avermectin B1a in three dimensions.

The figure shows avermectin B_{1a} structure as sticks appearance within transparent surface presented by Pymol.

Acermectin B_{1a} was used as ligand that bond to the enzyme, while it's the predominant compound of abamectin, it's a large molecule and form noncovalent bonds with BChE enzyme.

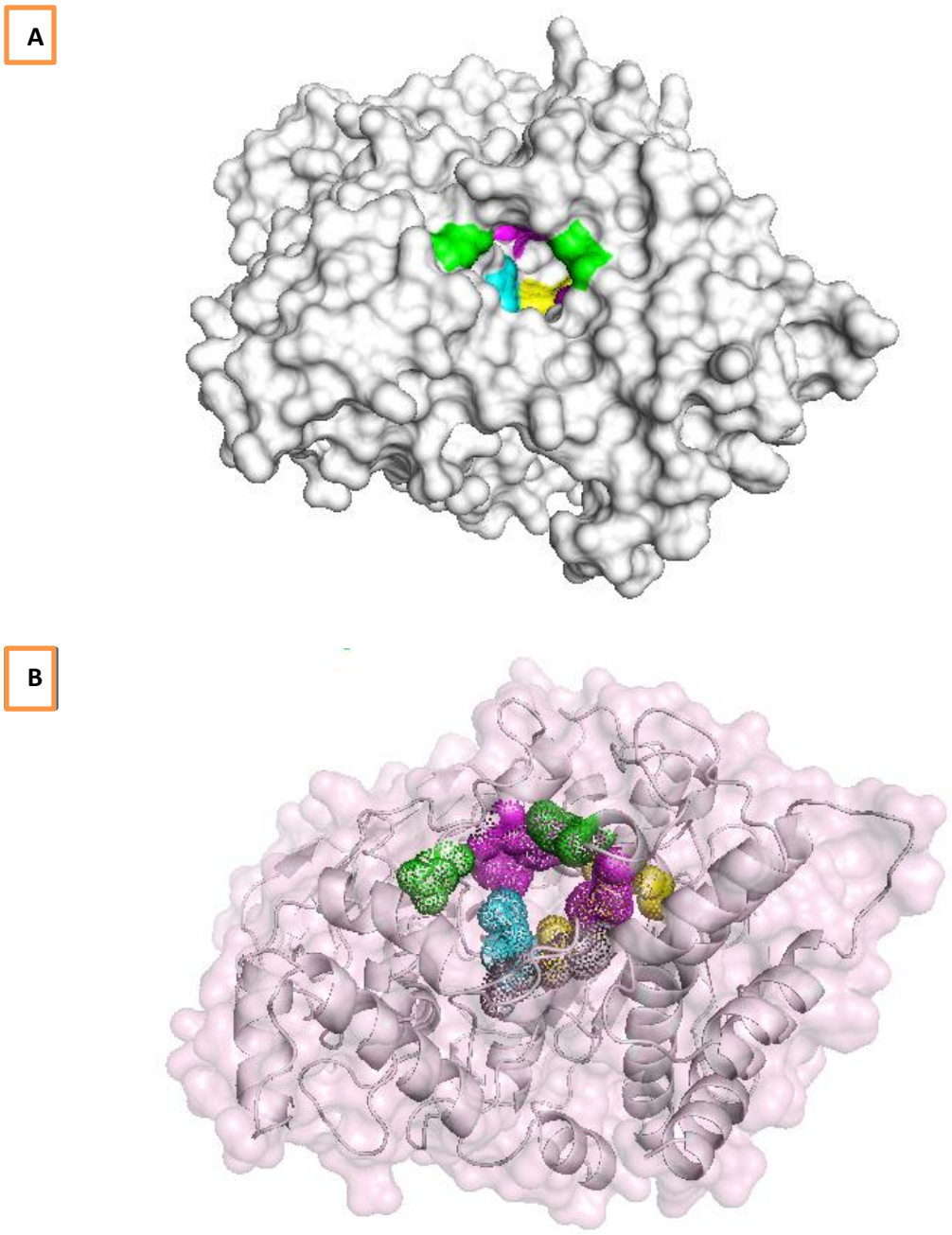

Figure 4.3.11 Three-dimensional structure of monomeric subunit of horse BChE homology model

A: Visualizing the deep gorge from the surface of the enzyme, composed of amino acids that construct catalytic tried, anionic site, acyl pocket, oxyanion hole and the peripheral anionic site (PAS),the green dots are the peripheral anionic site of the enzyme appearing from the external site.

B: Showing through the partially transparent surface, the secondary structure of the protein in helix sheet and loops. The yellow dots present the catalytic tried His434, Glu325, Ser198 of amino acids in active site, the magenta dots shows the anionic site Tyr128, Trp82, Phe329, gray dots for the acyl pocket Val288, Leu286, the cyan dots the oxyanion hole Gly116, Gly117, Ala199, and the green dots for the peripheral anionic site Tyr332, Aps71, the both figure poses were presented by PyMOL.

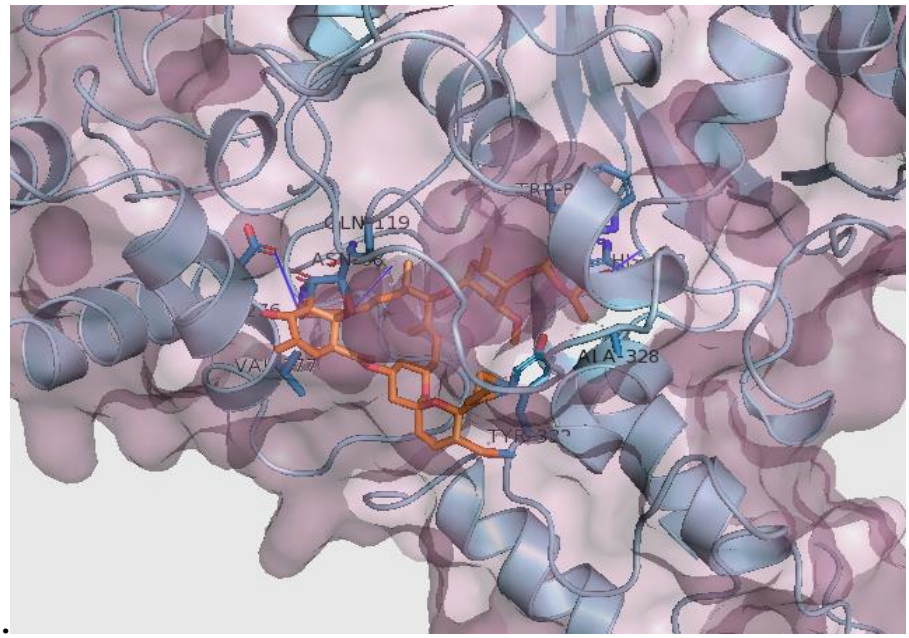

Figure 4.3.12 Structural view the docked complex between BChE and avermectin B1a, visualized with PyMOL.

Showing the secondary structure of a subunit of BChE through a transparent surface, the orange sticks presenting the ligand molecule interact with noncovalent bonds with the amino acids in the enzyme active site.

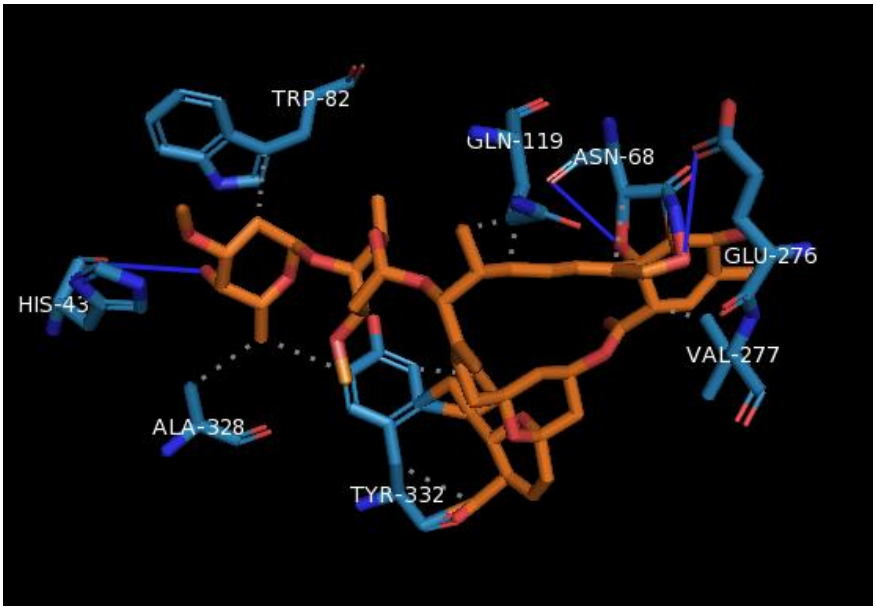

Figure 4.3.13 Noncovalent interaction between Avermectin b1a and hBCHE.

The avermectin B_{1a} is represented in orange sticks, *h*BChE interacting residues in blue sticks, hydrogen bonds in blue solid line and hydrophobic interactions in gray dashed lines. To elucidate the amino acids of the active side to BChE, indicating the noncovalent interaction of the avermectin b_{1a} with the amino acids of the enzyme active site. Docking outcome it shows that three hydrogen bonds were formed with these amino acids Asn68 (distance = 2.69 Å), Glu276 (3.08 Å) and His438 (2.91 Å). Followed by hydrophobic bonds, associates corresponding amino acids, Asn68 with a distance (3.14 Å), Trp82 (3.79 Å), two bonds with Gln119 (3.44 Å and 2.33 Å), Val277 (2.26 Å), Aln328 (3.15 Å), and four bonds through Try332 in different distances (3.23 Å, 2.62 Å, 1.83 Å, 3.53 Å). Although the avermectin b_{1a} is a large molecule it interact with His438 one of the catalytic triad of enzyme active site with a hydrogen bond, and with Trp82 amino acid of anionic site with hydrophobic bond, this may show that the ligand compete the substrate in the enzyme active site.

5. Discussion

The Butyrylcholinesterase (EC 3.1.1.8) (BChE) is a glycosylated protein which have a tetrameric molecule of four identical subunits, 574-amino acid found in each subunit, the evident molecular weight for a subunit is 84,551 Da (Moorad, *et al*., 1999). On each subunit nine Asn-linked carbohydrate chains are found (Blong, *et al*., 1997) the evaluated carbohydrate was 26%. Ser198, Glu325, and His438 are the active side burden triad.

Glu-Glu-Asp-Ile are the N-terminal aminoacids harmonious creating the mature form of enzyme. Comparison of horse BChE enzyme and other sorts demonstrate highly similarity, with human serum BChE its 90.1% homology, although one less N-glycosylation site and Cys residue are in horse serum BChE enzyme, if the preserved amino acid are included then it will reach 93.4% similarity (Moorad *et al*., 1999). The molecule is protected from proteolysis by a heavy sugar coating from nine N-linked carbohydrate chains. The combined molecular mass is 340,000 Da (Li *et al*., 2008).

Butyrylcholinesterase enzyme the clear physiological role is still indistinct, while the acylytlcholinesterase role is well defined in cholinergic neurotransmition. The BChE in its structure it's implicated in three various enzymatic activities, in esterase, aryl acylamidase also protease. (Massouliè J. *et al.,* 1992). The BChE and AChE are comparable in the molecular forms while it divers in distribution. The BChE in the absent of AChE it regulate cholinergic transmission, also act like scavenging of organophosphate and carbamate, and in hydrolyzing of some drugs like the cocaine amitriptyline, aspirin. (Chatonnet A, Lockridge O., 1989).

In this study, horse serum butyrylcholinesterase enzyme was obtained commercially. By using different concentration of butyrylthiocholine (BTC) starting from (0.05 mM, 0.1 mM, 0.25 mM, 0.50 mM, 1 mM, to 2 mM), the V_m and K_m were found to be 252.59 ± 7.11 U mg^{-1} protein and 0.155 ± 0.020 μM, respectively as showed in the (figure 4.5).

Elevation of BChE enzyme activity in various pathologies of diseases reported in previous studies such as in obesity, diabetes, uremia, hyperthyroidism, hyperlipidemic subjects (PAES *et al*., 2006) and in Alzheimer disease (Darvesh *et al*., 2012) attracted the investigation on potential inhibitors on butyrylcholinesterase enzyme. Therefore, we investigate the kinetic behavior of butyrylcholinesterase enzyme extracted from horse in the present of pesticide abamectin.

Abamectin is kind of big rings lactone disaccharide components and a natural fermentation output from soil dwelling *actinomycete S. avermitilis*. Avermectins isomer major type commercialized is abamectin. It is used as biocide and anthelminthic drug and it is highly lipophilic. The mechanism of action effect g-aminobutyric acid (GABA) system and Cl channels (Kose *et al*., 2016). Using abamectin as a pesticide it increase the opportunity to reach the body, therefore the effect of this pesticide at low concentration on the physiology is quiet important.

Activity measurements were performed at varied abamectin concentrations (2.5 µM, 5.0 µM, 7.5 µM, 10 µM, 12.5 µM, 15 µM, 20 µM, 5 µM, 30 µM) and BTC 1 mM. The enzyme activity was decreasing gradually as abamectin concentration increased elucidating that abamectin has an inhibitory effect on BChE with a low half maximal inhibitory concentration (IC_{50}) and it was calculated as 10.6 µM. Although, abamectin inhibit BChE in a dose dependent manner the inhibition didn't go to zero in the studied concentration range (Figure 4.3). The inhibitory kinetic experiments of the enzyme was additional tested in which (1 µM, 2 µM, 4 µM, 6 µM, 8 µM) of abamectin concentrations were chosen to be used since the inhibition pattern was linear at this range. Activity of the enzyme was measured on these abamectin concentrations and BTC concentration was in the range of (0.05 mM, 0.1 mM, 0.25 mM, 0.50 mM, 1 mM, 2 mM). Competitive type of inhibition was obtained**.** The inhibitory constant (K_i) was calculated as 2.26 ± 0.35 µM by using both the interception point on graph (figure 4.7) and statistical analyses. Competitive inhibition shows that abamectin bind to the enzyme

active site, the results demonstrate that even at very low concentrations; there are significant amounts of inhibition.

The inhibitory effect of pesticide avarmectin including abamectin was investigated on the carbonic anhydrase enzyme purified from fresh bovine erythrocyte. The inhibition results obtained from this study showed K_i values of 9.74 ± 1.91 nM and IC_{50} 14.39 nM for abamectin (Kose *et al* 2016). In another study the effect of abamectin on bioenergetics of mitochondria was demonstrated elucidating that abamectin disorganize through different mechanisms of mitochondria by inhibiting adenine nucleotide translocator (ANT) more than F_oF_1-ATPase, but with no effect on NADP dehydrogenase and succinate dehydrogenase, this effects frame the potential mechanism of abamectin toxicity in live (Zanoli *et al*., 2012).

In recent studies it was reported that animals exposed to avermectins shows poisoning reactions indicative of central nervous system depression (Li *et al*,. 2013). Therefore, enzymatic studies regarding the use of pesticide are showing attractions. Another study investigating the effect of treating cows infected with Dictyocaulus viviparous by eprinomectin antiparasitic drug on BChE enzyme shows that the enzyme activity was decreased (Pereira et al., 2017).

BChE enzyme activity is reported to be increase in several diseases, furthermore, its activity progressively increase in severity of dementia progression while AChE enzyme remain deny (Geula *et al*., 2004).

The main characteristic feature of Alzheimer disease are plaques (Daffner *et al*., 2000); the extracellular deposit mainly composed of amyloid β peptides, the b amyloids indicate as the primary evidence of pathogenic in Alzheimer disease (Hardy *et al*., 2002). The second pathologic feature of AD disease is the neurofibrillary tangle is a deposit primary composed of phosphorylated tau, tangle are results as degradation of neurons, leaving behind as clamed ghost tangle (Deffner *et al*., 2000). Level of the

butyrylcholinesterase enzyme in Alzheimer significant increase, the high values associated with plaques and tangles (Geula *et al*., 1995; Geula, *et al* 2004).

The G1 molecule form of BChE is predominant in develop brain and it has been shown to be dramatically increased in cerebral cortex of AD brains and that demonstrate a significant positive correlation with number of plaques in AD cortex. The G4 form of BChE is predominantly in mature brain (Arendt *et al*., 1992).

Further clue uphold a possible role for BChE in pathology, that BChE enhance aggregation of amyloid B peptide which are significant step in plaques formation (Barber *et al* 1996). It has been demonstrated that specific inhibitors of BChE reduce the level of amyloid precursor which are the provenance of amyloid B peptide the component of plaques in Alzheimer disease (Geula *et al* 2004). Beside there is still no cure for Alzheimer disease, therefor its compulsory to discover new drugs against Alzheimer disease.

The docking methods save, economize in time and in the huge amount of money that are spent specifically in drug industry to generate new drugs, by using this computational methods we can predict optimize and have a closer image of the interaction that may occur between a protein and a ligand based on the massive data that have been synthesized and deposited for different protein which are available in the web servers, screening those data of an predicted interaction, led to decline down to small number that can be carried out for the experiment level, due to the conformational change that happen to the component, the experimental part elucidate the exact interaction.

The three-dimensional crystal structure of human butyrylcholinesterase (*h*BChE) was downloaded from RCBS PDB database (Protein Data Bank) with PDB ID: 3O9M; and resolution of 2.98 Å, consisting of the protein crystal structure complexed with benzoic acid (the hydrolyzing product of cocaine). From the tetrameric crystal structure of *h*BChE, three of the subunits were deleted. Only one monomeric subunit in complex with benzoic acid was used because the enzyme structure is composed of four identical

subunits. The *h*BChE was used in the molecular studies since the 3D structure of the horse BChE was not available on protein data bank and is closely related to horse BChE than other BChE with 90% identity (Alpi *et al.*, 2015).

Avermectin B_{1a} the predominant compound in abamectin structure with 95%, was used as the ligand, the 3D structure was generated using a 3D Structure Predictor, COSMOS.

Molecular docking was carried out to elucidate the protein–ligand interaction. This was performed between the horse BChE and avermectin b_{1a} as a ligand using a docking algorithm, PatchDock (Schneidman *et al*., 2005). The solution with the highest geometric score = 7416 was selected. To analyze the interatomic association, Protein Ligand Interaction Profiler (PLIP) was used (Salentin *et al.*, 2015). Then a molecular visualizing system, PyMOL, was used to obtain desired images from the outcome file. To illustrate the amino acids of the active side to BChE, indicating the noncovalent interaction of the inhibitor with the amino acids of the enzyme active site. Docking outcome it shows that three hydrogen bonds were formed with these amino acids Asn68 (distance = 2.69 Å), Glu276 (3.08 Å) and His438 (2.91 Å). Followed by hydrophobic bonds, associates corresponding amino acids, Asn68 with a distance (3.14 Å), Trp82 (3.79 Å), two bonds with Gln119 (3.44 Å and 2.33 Å), Val277 (2.26 Å), Aln328 (3.15 Å), and four bonds through Try332 in different distances (3.23 Å, 2.62 Å, 1.83 Å, 3.53 Å).

Although the avermectin b_{1a} is a large molecule it interact with His438 one of the catalytic triad of enzyme active site with a hydrogen bond, and with Trp82 amino acid of anionic site with hydrophobic bond, this may show the competitive inhibition.

Molecular docking exhibit that avermectin b_{1a} binds with number of strong hydrogen bonds with several important amino acids residue of BChE and numerous of hydrophobic interactions which may elucidate the inhibitor potency.

6. Conclusion:

The inhibitory effect of the pesticide abamectin on horse serum butyrylcholinesterase was investigated in this study. In dose dependent manner the Abamectin was found to inhibit BChE, the half maximal inhibitory concentration IC_{50} and K_i value was 10.6 µM and 2.26 ± 0.35 µM, respectively. The inhibition type was competitive, the V_m and K_m was calculated as 252.59 ± 7.11 U mg^{-1} protein and 0.155 ± 0.020 µM, respectively. The molecular docking results elucidate the inhibitory potency of avermectin B_{1a} whom interact with numerous of hydrogen and hydrophobic bonds with amino acids of the enzyme active site.

Humans are exposed to very little amount of abamectin from treated crops, abamectin residues less than 0.025 ppm can be in the crop, also due to the abamectin characteristic of not persist in the environment, maintenance the minimal side effect that my occur of undesirable inhibition to the BChE enzyme and incapacitating the enzyme activity.

It is suggested that further studies need to be conducted on butyrylcholinesterase, as this enzyme is competitively inhibited by abamectin thereby increasing its importance because of its elevated level in several pathological diseases specifically in Alzheimer disease, as a result of future studies abamectin will be available for treatment of Alzheimer patients in near future.

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