In Vitro Determination of AChE Enzyme Activity from Coenuriasis III Sheep to Some Drugs

Coenuriasis Hastalıklı Koyunlarda Asetilkolinesteraz Enzimi Aktiviteleri Üzerine Bazı İlaçların İn Vitro Etkilerinin Araştırılması

Research Article

M. Şirin Bala, Nurhayat Atasoy*, Vedat Türkoğlu
Yuzuncu Yıl University, Sciences Faculty, Department of Chemistry, Van, Turkey

ABSTRACT

In this study, the blood plasma of healthy sheep and those with ''coenuriasis'', (popularly known as ''gid''), were examined and a comparison of the activities of brain homojenizat acetylcholinesterase have been made. Lastly, a kinetic study was done. The effects of drugs such as Ricobendazole, Amikacin, Gentamicine, Clindamicine and Ceftriaxone, on both healthy and diseased sheep's enzymes were examined. The drugs Ricobendazole and Clindamicin can inhibit the symptoms but it was observed that the other drugs had no effect. While the I_{50} blood plasma value of Ricobendazole and Clindamicin drugs over the healthy sheep were $10.19x10^{-3}$ M and $36.14x10^{-3}$ M, the values of these drugs over the unhealthy sheep were found as $18.51x10^{-3}$ M and $6.90x10^{-2}$ M. Further K_i value of Ricobendazole and Clindamicine drugs over the healthy sheep were $3.00x10^{-8}$ M and $7.36x10^{-7}$ M respectively and the same values of these drugs over the unhealthy sheep were found as $2.62x10^{-8}$ M and $8.50x10^{-7}$ M. The I_{50} value of Rizkobendazol and Clindamicin drugs over the healthy sheep's brain were $6.27x10^{-7}$ M and $7.81x10^{-7}$ M, and the same values of these drugs over the unhealthy sheep's brain were found as $27.75x10^{-7}$ and $7.67x10^{-7}$. Finally, K_i value of Rikobendazol and Clindamicine drugs over the healthy sheep's brains were $3.06x10^{-8}$ M and $1.82x10^{-6}$ M and the same values of these drugs over the unhealthy sheep's brains were found as $1.02x10^{-7}$ M and $1.95x10^{-6}$ M. Changes in the enzyme activity of brain groups was statistically significant ($p<0.05$).

Key Words
Acetylcholinesterase, Inhibition, Plasm, Brain, Coenuriasis ''gid'', Sheep

ÖZET

Bu çalışmada, sağlıklı ve halk arasında delibaş ''coenuriaiasis'' olarak bilinen koyunların kan plazması ve beyin homojenatı asetilkolinesteraz enzimi aktivitelerinin karşılaştırılması yapılmış ve gerek sağlıklı ve gerek hastalıklı koyun asetilkolinesteraz enzimi üzerine bazı parazikuantal ilaçların ve geniş spektrumlu antibiyotiklerin in vitro etkileri araştırılmıştır. Hem sağlıklı hem de hastalıklı koyun enzimleri üzerine Rikobendazol, Amikasin, Gentamisin, Klindamisin ve Seftriakson ilaçlarının etkileri incelendi. Adı geçen bu ilaçlardan Rikobendazol ve Klindamisin bu enzimleri inhibe ederken Amikasin, Gentamisin ve Seftriakson ilaçlarının herhangi bir etkisinin olmadığı bulundu. Sağlıklı koyun üzerine uygulanan Rikobendazol ve Klindamisin ilaçlarının kan plazması I_{50} değerleri; $10.19x10^{-3}$ M ve $36.14x10^{-3}$ M olarak, hastalıklı koyun üzerine uygılanan Rikobendazol ve Klindamisin ilaçlarının kan plazması I_{50} değerleri ise $18.51x10^{-3}$ M ve $6.90x\ 10^{-2}$ M olarak bulundu. K_i değerleri sırasıyla sağlıklı koyun üzerine uygulanan Rikobendazol, Klindamisin; hastalıklı koyun üzerine uygulanan Rikobendazol, Klindamisin ilaçlar için sırasıyla $3.00x10^{-8}$ M, $7.36x10^{-7}$ M; $2.62x10^{-8}$ M ve $8.50x10^{-7}$ M olarak bulundu. Beyinde ise sağlıklı koyun üzerine uygulanan Rikobendazol ve Klindamisin ilaçlarının beyin homojenatı I_{50} değerleri $6.27x10^{-7}$ M ve $7.81x10^{-7}$ M hastalıklı koyun beyin homojenizatı I_{50} değerleri $27.75x10^{-7}$ M ve $7.67x10^{-7}$M olarak bulundu. Koyun beyni üzerine uygulanan Rikobendazol ve Klindamisin ilaçların K_i değerleri ise sağlıklı koyunda $3.06x10^{-8}$ M ve $1.82x10^{-6}$ M olarak, hastalıklı koyunda $1.02x10^{-7}$ M ve $1.95x\ 10^{-6}$ M olarak bulundu. Beyin gruplarının AChE enzim aktivitelerindeki değişimin istatistiksel olarak önemli olduğu saptanmıştır ($p<0.05$).

Anahtar Kelimeler
Asetilkolinesteraz, İnhibisyon, Plazma, Beyin, Coenuriasis (Delibaş Hastalığı), Koyun

Article History: Received: Mar 18, 2014; Revised: Jul 1, 2014; Accepted: Aug 19, 2014; Available Online: Sep 15, 2014.

Correspondence to: N. Atasoy, Yuzuncu Yıl University, Sciences Faculty, Department of Chemistry, Van, Turkey
Tel: +90432 225 1707 Fax: +90432 225 1188 E-Mail: nurhayatatasoy@ymail.com

INTRODUCTION

Acethylcholinesterase is mainly found in brain, nerve cells (especially end plates), in muscle and erythrocytes. The main role of AChE is to catalyze the hydrolysis of acetylcholine into choline and acetic acid at cholinergic synaptic sites [1]. The reaction catalyzed by AChE (EC 3.1.1.7) occurs enzymatically in two steps. In the first step, the enzyme plays a role as a strong nucleophile .In the second step, the enzyme mediated as a very important destructor in conjunction with the nucleophilic hydroxyl group of a specific serine residue [2]. The acetylcholinesterase enzyme (AChE) is found in the brain, nerve cells, muscles, and erythrocytes. This enzyme is also common in the animal kingdom. Cholinesterase found in human serum, where it was first discovered. In recent years, specific AChE AChE in erythrocytes from different was found in serum. After the electrophoretic separation of serum proteins, acetylcholinesterase activity occurs in a different fraction than butyrylcholine and tributyrin activity. The optimum pH of the serum and erythrocyte AChE is approximately 7.2. However, both enzymes have different optimum substrate concentrations. Serum is used measurement AChE in the diagnosis of diseases. Various diseases are diagnosed through AChE activity. Normal value because of changes to various diseases, for example, hepatitis, malignant tumor patients, occurs in asthma and pulmonary tuberculosis. AChE activity are affected by testosterone and insulin while serum AChE activity is replaced by sex hormones and several drugs[3].

Due to the numerous warnings from nerve endings at the end of acetylcholine molecules goes into intercellular gap being filled with liquid the synapse. Due to stimulus at the nerve end, numerous acetylcholine molecules pass through the synaptic space that is filled with intercellular fluid. Acetylcholine reaches the postsynaptic membrane without being broken down by the cholinesterase enzyme that is present in the synaptic space due to its short path Ca^{++} plays an important role in the release of acetylcholine. Calcium channels are opened at the nerve end membrane that is depolarized by action potential. Ca^{++}, an extracellular ion, enters the cell, diffuses in the axoplasm due to the difference in density, and provides attachment of acetylcholine vesicles on the membrane. vesicles are opened, here and many acetylcholine molecules appear in the synaptic space [4].

In the present study, the effect of drugs such as Ricobendazole, Amikacin, Gentamicine, Clindamicine and Ceftriaxone, on both healthy and diseased sheep's AChE were examined. Benzimidazole and probenzimidazole antelmintics are widely used in veterinary and human antelmintic therapy [5]. Ricobendazole, benzimidazole carbamate derivates, which called albendazole sulfoxid is a wide spectrum anthelmentic. This drug inhibits polimerisation of βTubulin of parasites, therefore damages energy methabolism of them so, kill the parasites [6]. This drug have been used at 3.75 mg kg^{-1} dose subcutanously against gastrointestinal parasites [7]. After SC injection, ricobendazol is widely distributed from blood to other tissues, especially to the gastrointestinal tract [8]. Amikacin is most often used for treating severe, hospital-acquired infections with multidrug resistant Gram negative bacteria such as Pseudomonas aeruginosa, Acinetobacter, and Enterobacter. Serratia marcescens and Providencia stuartii are also included in the spectrum. Amikacin can also be used to treat non-tubercular mycobacterial infections and tuberculosis (if caused by sensitive strains) when first line drugs fail to control the infection. Amikacin may be combined with a beta-lactam antibiotic for empiric therapy for people with neutropenia and fever. Liposomal amikacin for inhalation is currently in late stage clinical trials for the treatment of respiratory diseases, such as cystic fibrosis Pseudomonas aeruginosa, non-tubercular mycobacterial infections and bronchiectasis [9]. Gentamicin is an aminoglycoside antibiotic composed of a mixture of related gentamicin components and fractions and is used to treat many types of bacterial infections, particularly those caused by Gram-negative organisms. However, Gentamicin is not used for Neisseria gonorrhoeae, Neisseria meningitidis or Legionella pneumophila. Gentamicin is also ototoxic and nephrotoxic, with this toxicity remaining a major problem in clinical use [10]. Clindamycin is used primarily to treat anaerobic infections caused by susceptible anaerobic bacteria, including dental infections, and infections of the respiratory tract, skin, and soft tissue, and peritonitis. In patients

with hypersensitivity to penicillins, clindamycin may be used to treat infections caused by susceptible aerobic bacteria, as well. It is also used to treat bone and joint infections, particularly those caused by Staphylococcus aureus. Ceftriaxone is often used (in combination, but not direct, with macrolide and/or aminoglycoside antibiotics) for the treatment of community-acquired or mild to moderate health care-associated pneumonia [11].

In this study, healthy sheep's and β βcoenuriasis'', which is known as ''gid'' among the public, blood plasm and the comparision of the activities of brain homojenizat acetylcholinesterase have been made. Coenurosis is a disease of the central nervous system in sheep, common worldwide [12-14]. The disease is caused by *Coenurus cerebralis*, the larval stage of Taenia multiceps, a tapeworm, which infests the small intestine of carnivores. Contamination of pastures grazed by sheep with dog faeces can result in larval invasion of the central nervous system and clinical disease. The life cycle is completed when the carnivorous definitive hosts ingest an infested sheep brain.

Both acute and chronic forms of coenurosis have been described, although chronic disease is more readily identified and more frequently reported.

Acute coenurosis has been reported in a flock of sheep introduced in a pasture heavily contaminated by dog faeces (Clinical signs appeared within 10 days, which ranged from mild to severe with death occurring within 3–5 days after onset of neurological dysfunction [15-16]. Acute coenurosis has also been reported in 6–8 week-old lambs, where clinical signs ranged from pyrexia, listlessness and head aversion to convulsions and death within 4–5 days.

Chronic coenurosis is more commonly reported in growing sheep aged 6–18 months, where it presents as a slowly progressive focal lesion of the brain, typically involving one cerebral hemisphere. Chronic coenurosis has rarely been reported in sheep older than 3 years. The time taken from larval hatching, migration to the brain and evidence of neurological dysfunction varies between 2 and 6 months [15,16].

MATERIALS AND METHODS

Obtaining Sheep Blood Plasma and Brains

Municipalities in the province of Van in slaughterhouse of sheep brought to slaughter healthy and showing no signs of disease Coenuraiasis staggers centenary of sheep blood and skulls were diagnosed at Yuzuncu Yil University's Medical Faculty, Department of Pathology. Brain samples were photographed. Brain samples of 6 healthy sheep and 6 sheep with Coenuraiasis were photographed and blood plasma and brain homogenate were held in the deep freeze to detect acetylcholinesterase enzyme levels.

Obtaining Brain Samples and Homogenates

Put with the diagnosis obtained from the skull opened and the brain was taken from the pair of scissors 10 g samples. In order to prepare a watery solution of the brain, 0.02 M sodium phosphate buffer (pH 7.4) containing 0.356 g Na_2HPO_4 and 8.25 g sucrose was added to 8-10 g thinned brain in a volume of 100 ml. This mixture was exposed to break down for 3 minutes with the aid of a mixer. Meanwhile prevent overheating of the mixer was placed around the ice. A further degradation resulting homogenate was kept for 10-15 minutes in a ultrasonic homogenizer. Again, ice was placed around the case to prevent heating.

The homogenates were then placed in centrifuge tubes and were centrifuged for 20 minutes at 1500 rpm. The sediment that contained blood cells and cellular debris was discarded. Supernatants were collected and centrifuged at 1200 rpm, +4°C for 60 minutes. The clear homogenate above was carefully obtained, and the remaining sediment was discarded. This homogenate was used to measure enzyme activity.

Determination of Activity of the Acetylcholinesterase Enzyme

Acetylcholinesterase enzyme activity in sheep blood plasma and brain was determined by the Ellman method. This method is based on measurement of yellow colored anion (2-nitro-5 thiobenzoate) during the reaction at pH 8 in a spectrophotometer at 412 nm. Thiocholine ester was used as the substrate [17]. Acetylcholinesterase enzyme activity was

Table 1. Statistical Values in Healthy Sheep and Sheep with Coenurosis.

	Sheep Brain AChE Activity Values		Sheep Plasma AChE Activity Values	
Sample	A Healthy	A Diseased	A Healthy	A Diseased
a	0.343566176	0.491360294	1.083883002	1.043382353
b	0.322058824	0.692647059	1.527897924	0.799632353
c	0.502941176	0.392647059	0.866133218	0.539338235
d	0.443933824	0.521139706	1.149572881	1.009191176
e	0.858088235	0.397058824	0.875865052	1.008088235
f	0.475736029	1.818529412	4.011461938	1.134926471
Gid 'Coenuriasis' diseased and healthy sheep brain Statistics Results				
Healthy Brain Average			0.4911 ± 0.07908 N=06	
Diseased Brain Average			0.7189 ± 0.2244 N=06	
P value			0.039	
T			0.9576	

determined as follows: 0.1 mL DTNB marker and 0.1 mL appropriately diluted enzyme was added to one of the tubes. The blind tube was kept at 65 °C for 3 minutes in a water bath and the enzyme was inactivated. Next, 2.7 mL of 0.05 M sodium buffer at pH 8 was added to each tube. The tubes were mixed and pre-incubated at 37 °C for 5 minutes. The reaction was started by adding 0.1 mL of 3 mM substrate (acetylthiocholine iodide) solution to each tube. Tubes were mixed again and left to incubate at 37 °C for 10 minutes. Absorbance at 412 nm was measured 10 minutes later and an increase in absorbance was found.

Studies on Determining I50 and Ki Values for Drugs Demonstrating an Inhibitory Effect

Inhibitory activity for the study of different inhibitor concentrations were measured. % Activity-[I] graphics of drugs demonstrating inhibitory effect were plotted. I_{50} values were calculated from the graphics equation.

Activity measurements with 5 convenient substrate concentrations at two constant drug concentrations equal to, above, and below the value that lowers enzyme activity of sheep blood plasma AChE were conducted to determine the K_i values of the drugs demonstrating an inhibitory effect. With the values obtained, Lineweaver-Burk plot graphics were drawn for each inhibitor. Incubation type was determined from the graphics. From the expression of $K_M = V_{max}(I + [I] / K_i)$, which was equal to competitive inhibition, was determined while for non-competitive inhibition; the $V_{max} = V_{ımax}(I + [I] / K_i)$ formula was used to determine K_i values.

Chemical Substances and Materials Used

Sodium phosphate, sodium chloride, sodium bicarbonate, sodium phosphate dihydrate, sodium hydroxide, 5, 5' – dithiobis (2-nitro benzoic acid) (DTNB), acetylcholine iodide, and hydrogen chloride were obtained from Sigma-Aldrich Chemie GmbH and Merck A.G. All solutions in this study were prepared with bi-distilled water.

RESULTS

Statistical analysis

Data were presented as means ± standard deviation (S.D.). Three parallel measurements were analyzed by Student's t-test. SPSS (Version 11.5 for Windows, SPSS Inc.) Analysis of Variance was used for comparisons of means 9. P values < 0.05 were accepted as statistically significant. Drug concentrations that produced 50% inhibition (I_{50}) were calculated from [Drug] activity % graphs [18].

Healthy Sheep Blood Plasma Ricobendazole

% activity values of acetylcholinesterase that varies with Ricobendazole concentration.

E.U	0	1.15	10.66	18.40
%Activity	100	58.75	32.86	20.55

Table 2. K_i and I_{50} Values of the Inhibitory Drugs in Sheep Blood Plasma.

	Healthy Sheep Plasma Ricobendazole	Diseased Sheep Plasma Ricobendazole	Healthy Sheep Plasma Clindamycin	Diseased Sheep Plasma Clindamycin
K_i ($mol^{-1} min^{-1}$)	$10.19x10^{-3}$	$18.51x\ 10^{-3}$	$7.36x10^{-7}$	$8.50x10^{-7}$
I_{50} (M)	$3.50x10^{-8}$	$2.62x10^{-8}$	$4.08x10^{-7}$	$6.64x10^{-7}$

Table 3. K_i and I_{50} Values of the Inhibitory Drugs in Sheep Brain Homogenate.

	Healthy Sheep Brain Ricobendazole	Diseased Sheep Brain Ricobendazole	Healthy Sheep Brain Clindamycin	Diseased Sheep Brain Clindamycin
K_i ($mol^{-1} min^{-1}$)	$3.06x10^{-8}$	$1.02x10^{-7}$	$1.82x10^{-6}$	$1.95x10^{-6}$
I_{50} (M)	$6.27x10^{-7}$	$2.78x10^{-6}$	$7.81x10^{-7}$	$7.67x10^{-7}$

I_{50} value found for Ricobendazole using regression equation in Figure 1 and Figure 2 is $10.19x10^{-3}$ M. K_i value is $3.00x10^{-8}$ $mol^{-1}min^{-1}$.

Sheep Blood Plasma Ricobendazol

With varying concentrations of acetylcholinesterase activity % Rikobendazol values.

E.U	0	1.15	10.66	29.66
%Activity	100	67.22	40.14	32.18

I_{50} value found for Ricobendazole using regression equation in Figure 3 and Figure 4 is $18.51x10^{-3}$ M. K_i value is $2.62x10^{-8}$ $mol^{-1}min^{-1}$.

Healthy Sheep Blood Plasma Clindamycin

With varying concentrations of clindamycin concentration % activity values

E.U	0	8.91	17.82	26.73
%Activity	100	94.16	69.54	64.72

I_{50} value found for Clindamycin using regression equation in Figure 5 and Figure 6 is $36.14x10^{-3}$ M. K_i value is $4.08x10^{-7}$ $mol^{-1}min^{-1}$.

Diseased Sheep Blood Plasma Clindamycin

% activity values of acetylcholinesterase that vary with Clindamycin concentration.

E.U	0	8.91	17.82	26.73
%Activity	100	97.75	89.24	78.11

I_{50} value found for Clindamycin using regression equation in Figure 7 and Figure 8 is $69x74x10^{-3}$M. K_i value is $6.64x10^{-7}$ $mol^{-1}min^{-1}$.

Healthy Sheep Brain Homogenate Ricobendazole

% activity values of acetylcholinesterase that varies with Ricobendazole concentration.

E.U	0	2.43	4.46	7.29
%Activity	100	81.8	67.8	56.5

I_{50} value found for Ricobendazole using regression equation in Figure 9 and Figure 10 is $6.27x10^{-7}$M. K_i value is $3.06x10^{-8}$ $mol^{-1}min^{-1}$.

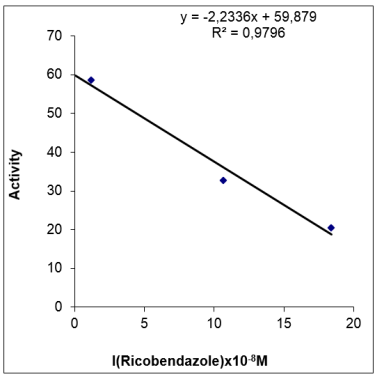

Figure 1. Inhibitor concentration graphics of Ricobendazole against % activity.

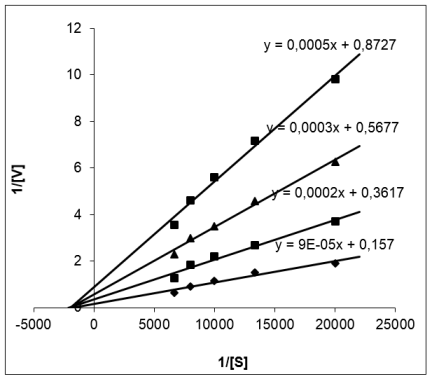

Figure 2. Lineweaver-Burk plot for Ricobendazole.

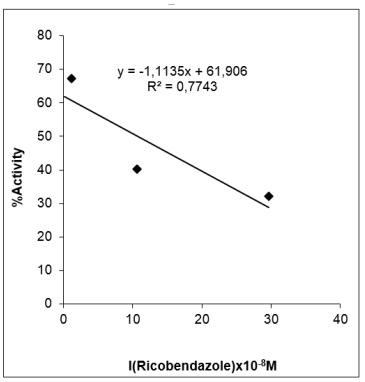

Figure 3. Inhibitor concentration graphics of Ricobendazole against % activity.

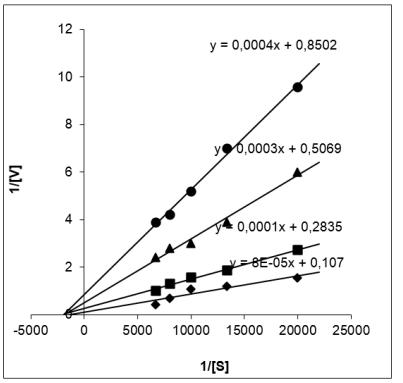

Figure 4. Lineweaver-Burk plot for Ricobendazole.

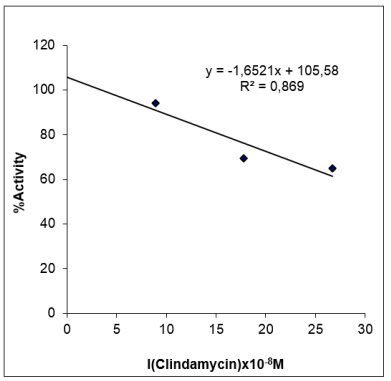

Figure 5. Inhibitor concentration graphics of Clindamycin against % activity.

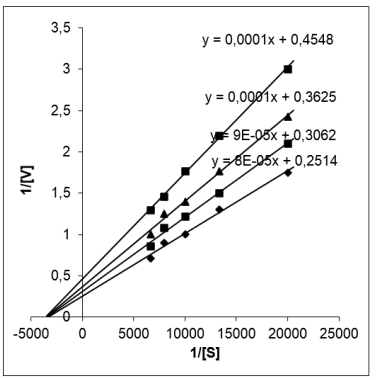

Figure 6. Lineweaver-Burk plot for Clindamycin.

Diseased Sheep Brain Homogenate Ricobendazole

% activity values of acetylcholinesterase that vary with Ricobendazole concentration.

E.U	0	1.18	4.73	10.66
%Activity	100	92.1	74.1	58.1

I_{50} value found for Ricobendazole using regression equation in Figure 11 and Figure 12 is $27.50x10^{-7}$M. K_i value is $2.62x10^{-8}$ $mol^{-1}min^{-1}$.

Healthy Sheep Brain Homogenate Clindamycin

% activity values of acetylcholinesterase that vary with Clindamycin concentration.

E.U	0	2.43	4.46	7.29
%Activity	100	81.8	67.8	56.5

I_{50} value found for Clindamycin using regression equation in Figure 13 and Figure 14 is $7.81x10^{-3}$M. K_i value is $18.20x10^{-7}$ $mol^{-1}min^{-1}$.

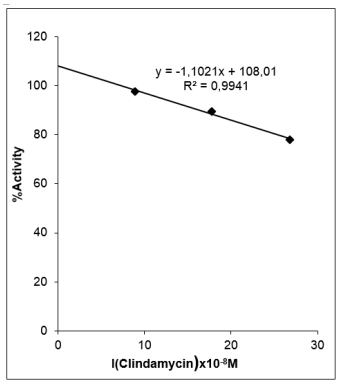

Figure 7. Inhibitor concentration graphics of Clindamycin against % activity.

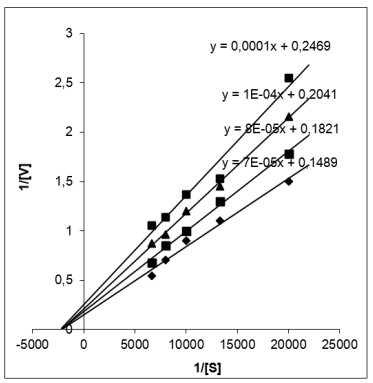

Figure 8. Lineweaver-Burk plot for Clindamycin.

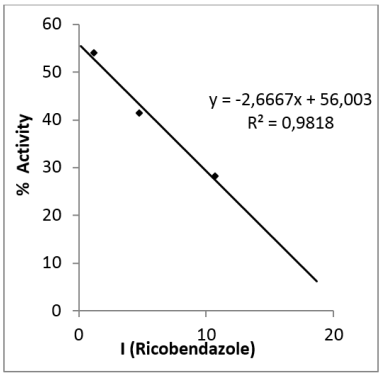

Figure 9. Inhibitor concentration graphics of Ricobendazole against % activity.

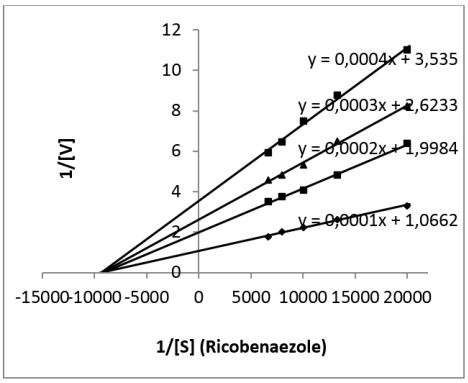

Figure 10. Lineweaver-Burk plot for Ricobendazole.

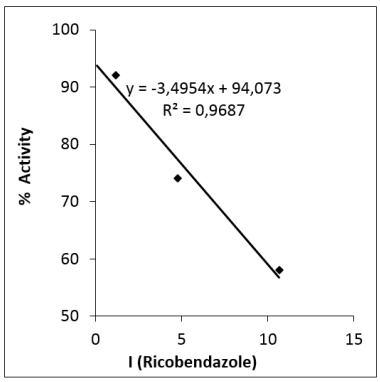

Figure 11. Inhibitor concentration graphics of Ricobendazole against % activity.

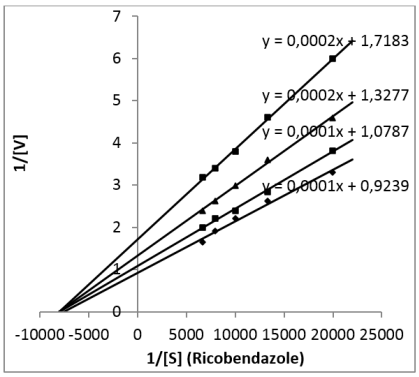

Figure 12. Lineweaver-Burk plot for Ricobendazole.

Sheep Brain Homogenate Clindamycin

% activity values of acetylcholinesterase that vary with Clindamycin concentration

E.U	0	2.43	4.46	7.29
%Activity	100	82.3	68.2	54.8

I_{50} value found for Clindamycin using regression equation in Figure 15 and Figure 16 is 7.67x 10^{-3}M. K_i value is 19.47x10^{-7} $mol^{-1}min^{-1}$.

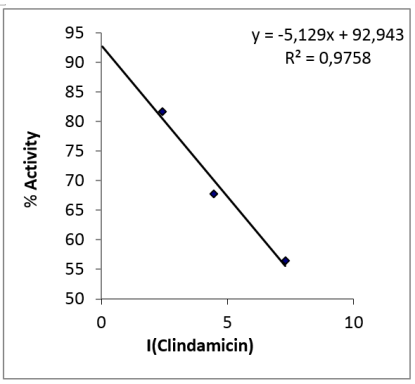

Figure 13. Inhibitor concentration graphics of Clindamycin against % activity.

DISCUSSION

In this study, we have both healthy and ''coenuriasis'' diseased sheep staggers in the brain and blood samples were taken during the slaughter of Van Large City slaughterhouse. Acetylcholinesterase activity of these samples was measured and compared to each other. According to Ellman method, no statistically significant relationship was found among the plasma

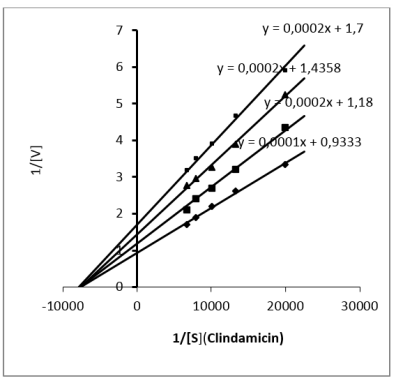

Figure 14. Lineweaver-Burk plot for Clindamycin.

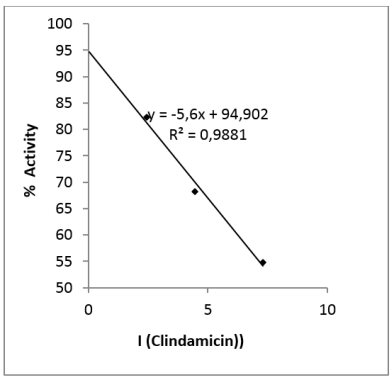

Figure 15. Inhibitor concentration graphics of Clindamycin against % activity.

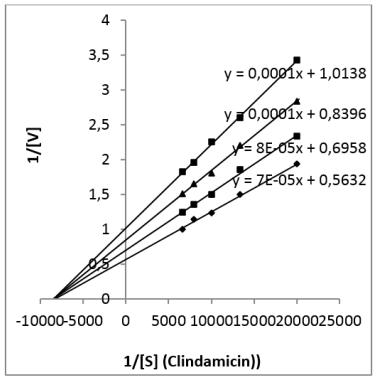

Figure 16. Lineweaver-Burk plot for Clindamycin.

groups in these activity measurements done by the Ellman method, altought a statistically insignificant increase was observed when the values were compared. In this study, the effects of 5 drugs on the activity of sheep plasma and brain homogenate acetylcholinesterase enzyme were investigated.

These used drugs are Amikacin Sulphate, Cefaday, Gentamycin, Clindamycin and Ricobendazole. Lineweaver–Burk plot graphics from the 1/V vs. 1/S inhibition graphics were plotted for these drugs to determine the inhibition type. Charts were drawn in Excel program using inhibitor-free control values. Curves were lengthened with extrapolation and graphics were created whit determining the plots in which curves cross the x axis.

For this purpose, enzyme activities were measured in 5 different substrate and 3 different inhibitor concentrations. Concentrations of the inhibitors used were as follows; concentrations of inhibitors used for healthy sheep plasma, Ricobendazole; $3.00x10^{-8}$M, Clindamycin; $4.08x10^{-7}$ M, concentrations of inhibitors used for diseased sheep plasma, Ricobendazole; $4.08x10^{-7}$ M, Clindamycin $6.64x10^{-7}$ M. inhibitor concentrations of drugs that show sheep brain homogenate inhibition; healthy sheep brain homogenate: Ricobendazole; $6.27x10^{-7}$ M, Diseased sheep, Ricobendazole; $2.76x10^{-6}$ M, inhibition concentrations of healthy sheep Clindamycin; $7.81x10^{-7}$ M, inhibition concentrations of diseased sheep Clindamycin; $7.67x10^{-7}$ M. Although given in when plasma and brain homogenate samples were compared, it was seen that plasma AChE activity was higher. Initial activation measurements obtained in the study are shown in (Table 1). There is difference among these values. It is believed that the reason for this difference is the stages of the disease in sheep, i.e. it was either an initial or terminal stage of the disease. As is already known, acetylcholinesterase has two types. Type 1 is real cholinesterase that exists in neurons and brain whereas Type 2 is also known as pseudocholinesterase. Acetylcholine is the substrate for both. However, it shows structural differences. This was reflected in the results. This method is the most common and most important of the methods preferred based on scientific studies [17].

In the past, studies about AChE were done by many scientists and AChE was isolated and purified from various materials (plants, tissues, animals, etc.) and characterization was done. The first time, isolation of this enzyme was done by Stedman and Strelitz purified the enzyme from horse serum [18, 19]. Main et al. [20], isolated, purified AChE and made the characterization of the enzyme from horse serum. After the initial isolation and purification of acetylcholinesterase enzyme, other scientists [21-24]. also purified the enzyme and made the characterization.

Additionally In 2010, [25] measured the activity of AChE and some of its serum biochemical parameters in rat brains in order to detect safety and to measure anxiolytic effects of the "kava" plant. This adult female rats were used and in certain regions of the brain of rats after 4 weeks of AChE have seen a significant reduction in the activity.In addition, this, liver and kidney function parameters have not seen

any adverse effects.

In 1997, [26] used a modified electrometric method to measure AChE in erythrocytes of sheep that were exposed to organophosphates and carbamate insecticides . They observed in vitro inhibition of AChE in erythrocytes of the sheep which were exposed to these chemicals. They concluded that this modified method was especially effective measuring activity of AChE faster in sheep that were exposed to such substances.

Askar and colleagues [27] compared the Ellman and Micheal methods in serum, erythrocyte and tissues of sheep, neat cattle and pig and they found no difference between methods but they observed that variance % coefficient was higher when the Micheal method was used.

Soyer et al. [28] investigated the proposition that inhibition of acetylcholinesterase was one of the suggested treatments for Alzheimer's disease and reported that some drugs such as Tacrine, Donepezil Rivastigmine and Galantamine are acetylcholinesterase inhibitors. They reported that for the treatment Alzheimer's disease should be applied in different fields.

Kahraman et al. [29] made a study on human serum acetylcholinesterase levels in insecticide poisoning that included organophosphate and carbamate in 2008. They reported that the main purpose of this study was to evaluate the relationship between the clinical course and mortality. In this study , patients exposed to organofosfot and karbomatl the intsektisit have looked at the levels of serum cholinesterase. Complications in patients with average serum AChE level found lower than who did not occurred complications. statistically significantly.

In the Blacksea region, The Chronic effects of carbosulfan (250 g/L,EC) that is one of the pesticides used on rainbow trout (oncorhynchun mykiss) enzyme activity was also studied. Fishs were exposed to the toxic effects of carbosulfan for 60 days in the river system (6 L/h). During long-term tests the activity of erythrocyte AChE was measured in rainbow trout and the inhibition rate was determined. Rainbow trout change in enzyme activity was found to be statistically significant. ($p<0,05$). It was reported that the increase in AChE inhibition rate continued until the 3rd week and that the inhibition rate was 41.32% [30]. Alike and Güloglu [31] refined acetylcholinesterase from sheep liver and investigated the effects of several antibiotics and drugs on the enzyme. I_{50} values of Vermidon, Neostigmine and Panalgine drugs that are administered on acetylcholinesterase enzymes purified from liver were $1.27x10^{-3}$ M, $1.02x10^{-4}$ M, and 1.24 M, respectively. K_i values are $1.25x10^{-3}$ $mol^{-1}.min-1$ 4.32 $mol^{-1}.min^{-1}$. Since alfoxyl, another drug studied, did not have any effect on the enzyme, I_{50} and K_i values were not given.

In another study, conducted on the goat's brain acetylcholinesterase inhibition in vitro of pure and some commercial pesticides were investigated. The resulting purity of the enzyme 46-fold, the optimum value of pH was found to be 7.6 and the optimum temperature was found to be 400 C. Substrate value concentration was $4x10^{-4}$ M, K_m value was $3x10^{-5}$ and activator concentration was 5mM for $MgCl_2$, and 30 nM for NaCl. In this study, Furadane (2,3-dihydro-2,2-dimethyl-7-benzofuranil-n-methylcarbomate), Elsan (0,0-dimethyl-etoxy carbonyl benzyl phospodithionate), diisopropylflorophsphate (DFD) and Physostigmin were administered as pesticides. I_{50} value of these pesticides was $5x10^{-6}$ M for Furadane and $6x10^{-10}$ M for Physostigmin [32].

Demir and Türkoğlu [1] have examined the in vitro effect of neostigmine-methylsulphate on serum, plasma, muscle and liver acetylcholinesterase into rats. Neostigmine-methylsulphate was injected once to the rats at 0.05 mg/Kg dosage, methylsulphate was also injected once and differences in plasma, blood and liver acetylcholinesterase activities in rats were observed after the injection. In this study, a control group was also formed and a comparison with the drug-administered group was made. Measuraments made experimentally were done in the blood and tissue samples that were obtained 1-3 and 6 hours after the injection. The enzymatic activity studies were also performed in addition to the protein determination.

According to Habila et al. [33] measured blood and brain AChE activity in rats with Trypanosoma congolense disease using Ellman Method. As AChE activity was compared with the control group, they observed that it was significantly lowered in rats with Trypanosoma congolense disease.

As a result of the work conducted in the results, In this study values between with given values between in this study show some differences vary slightly. Values reported in literature show some differences from those found in this study. The reason for this is that products taking place as a result of hydrolysis of separate substrates by acetylcholinesterase are different and this indicates that acetylcholinesterase in plasma and brain cells are of different types. For instance, acetylcholine in serum cholinesterase is hydrolyzed more readily compared to butyrylcholine. Butyrylcholine is rarely hydrolyzed by the enzyme in red blood cells. Thus, serum and red blood cells have both types of the enzyme; however, their amounts vary. Real cholinesterase, which is a type I enzyme, is present in the neural tissue. Serum and red blood cells have type II cholinesterase (pseudocholinesterase) as well as real cholinesterase [34-35-36]. As a result 4 stock mixtures were obtained, healthy brain homogenate and healthy brain plasma; diseased brain homogenate and diseased brain plasma. This stock mixture used and the effect of 5 praziquantel and antibiotic drugs on AChE was examined. These drugs are as follows: Ricobendazole, Amikacin, Gentamycin, Clindamycin and Ceftriaxone. Clindamycin and Rikobendazol of these drugs showed effect on both normal and diseased sheep plasma and brain AChE percent showed inhibitory effect. Lineweaver-Burk graph was drawn for each to determine the type of inhibition. Inhibition type was "non competitive" in all these four samples. Here is understand to the active site of the unbound enzyme used drugs, bound to a place outside the active site so that the chemical structures of drugs that are substrates of the enzyme naturally acetylcholine are alike. These drugs are as follows: Ricobendazole, Amikacin, Gentamycin, Clindamycin and Ceftriaxone. Of these drugs, Clindamycin and Ricobendazole showed inhibitory effects on sheep plasma and brain AChE in both healthy and diseased sheep. Lineweaver-Burk graph was drawn for each to determine the type of inhibition. Inhibition type was "non competitive" in all these four samples. This implies that these drugs did not bind on the active center of the enzyme, instead they bound on another site than active center, thus chemical structure of these drugs were not similar to that of natural substrate of the enzyme, acetylcholine.

K_i constants of the inhibitors were calculated from Lineweaver-Burk Graphs. Values were given as molar. I_{50} value indicates the inhibitor concentration that causes 50 inhibition. In order to find this value, inhibition against % activity graph was drawn. From the equation of the line I_{50} values were detected, but these values were also evaluated as molar units. In this kinetic study, it was found that Amikacin, Gentamycin, Clindamycin and Ceftriaxone did not demonstrate any effect on the enzyme. Therefore graphs were not drawn and K_i and I_{50} values could not be calculated. Ricobendazole, Clindamycin exhibits a noncompetitive inhibition effect on the enzyme activity, because V_{max} values decrease and KM values not change. When substrate and inhibitor attach to different sides of the enzyme, noncompetitive inhibition occurs and the increase in the substrate concentration does not eliminate inhibition.

This research is an in vitro study. A search of the literature suggests that most of the in vitro studies give results similar to the in vivo studies. In this respect, this study can be considered important because it can be inspiring for and would enlighten an in vivo study. It is known that one of the effects of the drugs is to slow down or accelerate the corresponding metabolic event or pathway. This feature is important for monitoring treatment of a disease or alleviating the disease symptoms.

ACKNOWLEDGEMENT

Authors would like to thank the administrative units of the scientific research projects of Van Yuzuncu Yil University for the financial support.

References

1. C.H. Walker, H.M. Thompson, Phylogenetic distribution of cholinesterases and related esterases. In: Mineau, P.(Ed.), Cholinesterase-inhibiting insecticides. Their impact on wildlife and the environment. Elsevier, Amsterdam, (1991) 3.
2. H. Demir, V. Türkoğlu, Effects of neostigmine methylsulphate on enzyme activity of acetylcholinesterase in rat serum, plazma, muscle and liver in vivo. Scand. J. Lab. Anim. Sci., 32 (2005) 25.
3. I. Wilson, B. D. Nachmansohhn, In Ion Transport Across Membranes. Acedemic Pres Inc., New York. 35s, (1954).
4. D.L. Nelson, M.M. Cox, Lehninger Principles of Biochemistry. 4th Edn., WH Freeman and Company, New York, (2000).
5. C. Cristofol, G. Virkel, L.Alvarez, S. Sanchez, M. Arboix, C. Lanusse, Albendazole sulphoxide enantiomeric ratios in plasma and target tissues after intravenous administrarion of ricobendazole to cattle. J. Vet. Pharmacol. Ther., 24 (2001) 117.
6. E.A. Formentini, N. Mestorino, J.O. Errecalde, Pharmacocinetics of ricobendazole after its intravenous, intraruminal and subcutaneus administration in sheep. Vet. Res. Commun., 29 (2005) 595.
7. E.A. Formentini, O.N. Mestorino, E.L. Marino, J.O., Errecalde, Pharmacocinetics of ricobendazole in calves. J. Vet. Pharmacol. Ther., 24 (2001) 199.
8. C. Lonusse, G. Virkel, S. Sanchez, A. Alvarez, A. Lifschitz, F. Imperiale, A. Monfrinotti, Ricobendazole kinetics and availability following subcutaneous administration of a novel injectable formulation to calves. Res. Vet. Sci., 65 (1998) 5.
9. S.J. Leonard, translation: Sb Kolesnikov. Pharmacology 4. Print page No: 295
10. Anti-Infective Agents, (1998).
11. R. Moulds, M. Jeyasingham, "Gentamicin: a great way to start". Australian Prescriber 3 (2010) 134.
12. M. Gladwin, Clinical Microbiology Made Ridiculously Simple 4th ed. Miami, FL: MedMaster (2007) Inc. p. 67.
13. F. Veronesi, E. Lepri, M.C. Marchesi, G. Fillippini, M.T. Mandara, A focus of brain coenurosis in sheep coming from an Italian umbrian stock farm. Large Anim. Rev., 14 (2008), 217.
14. A. Varcasia, G. Tosciri, G.N.S. Coccone, A.P. Pipia, G. Garippa, A. Scala, V. Damien, G. Vural, C.G. Gauci, M.W. Lightowlers, Preliminary field trial of a vaccine against coenurosis caused by Taenia multiceps. Vet. Pathol., 162 (2009) 285.
15. P.R. Scot, Diagnosis and treatment of coenurosis in sheep.Veterinary Parasitology 189 (2012) 75.
16. M.L. Doherty, H.F. Bassett, R. Breathnach, M.L. Monaghan, B.A. McLearn, Outbreak of acute coenuriasis in adult sheep in Ireland. Vet. Rec., 125 (1989) 185.
17. G.L. Ellman, K.D. Courtney, J.R.V. Andres, R.M. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7 (1961) 88.
18. K. Sümbüloglu, V. Sümbüloğlu, Materiality Tests. IN: Biostatistics, Sumbuloglu, K.and V. Sumbuloglu (Eds.). Hatipoglu Publisher, Ankara, (1998), pp: 76-1.
19. E. Stedman, L.H. Easson, Cholinesterase an enzyme present in the blood serum of the horse [J]. Biochem. J., (1932), pp. 121-177.
20. F. Strelitz, Studies on cholinesterase. 4. Purification of pseudocholinesterase from horse serum. Biochem. J., 38 (1944) 86.
21. A.R. Main, W.G. Soucie, L.I Buxton, E. Arinc, The purification of cholinesterase from serum. Biochem. J., 143 (1974) 733.
22. X.C. Xie, S.N. Li, G.N. Zhu, Y.J. Tan, Purification of Bra in Acetylcholinerase (AChE) from Topmouth Gudgeon and Comparative Study between Crude and Purified AChE[J]. Chinese J. Pesticide Sci., 1 (2003) 45.
23. Y. Zhang, Comparison of biochemical characteristics of acetylcholinesterase between Apis cerana cerana Fabricius and Apismellifera ligustica Spinola [D]. Fujian: Fujian Agriculture and Forestry University, (2005), pp. 71-80.
24. H. Wei, J.L. Shen, W. Wu, J.W. Zhao, Z.X. Zhan, Purification,biochemical properties and insecticidessusceptibility of acetylcholinerase from housefly(Musca domesticaL.)[J]. J. Agro-Environ. Sci., 28 (2009) 156.
25. X. Peng, Purification and characterization of acetylcholinesterase, a kind of pesticide target [D]. Sichuan: Sichuan University, (2007) 13.
26. N. Noor, Ankxiolytic action and safety of kava: Effect on rat brain acetylcholinesterase aktivity and some serum biochemicalparameters. Afri. Tournal Pharmocy Pharmacolopy, 4 (2010) 823.
27. F.A. Mohammad, Modified electrometric method for measurementof erythrocyte acetylcholinesterase activity in sheep. Vet Hum Toxicol. 39 (1997) 337.
28. K.A. Askar, A.C. Kdi, A.J. Moody, Comparative analysis of cholinesterase activities in food animals using modified Elman and Micheal assays. The Canad. J. Vet. Res.,75 (2011) 261.
29. Z. Soyer, S. Parlar, V. Alptüzün, Synthesis and acetylcholinesterase (AChE) inhibitory activity of some N-substituted-5-chloro-2(3H)-benzoxazolone derivatives. Marmara Pharmaceutical Journal 17 (2013) 15.

30. N. Kahraman, S. Yantural, S. Kalkan, N.C. Oray, N Hocaoğlu, A. Ugurhan, Evaluating the relationship between serum acetylcholinesterase levels and clinical course and mortality of patients presented with organophosphate and carbamate posinings. Turk. J. Emerg. Med., 8 (2008) 121.
31. E. Çapkın, Effects of carbosulfan on erythrocyte acetylcholinesterase (AChE) activities of rainbow trout (Oncorhynchus mykiss). J. Fish. Sci., 5 (2011) 240.
32. Ö.F. Güloglu, V. Türkoğlu, İ. Çelik, Purification and characterization of Acetylcholinesterase from sheep liver and ınhibition by some Poinkillers. Asian J. Chem., 13 (2006) 1097.
33. S. Guhathakurta, S. Bhattacharya, In vitro inhibition of goat brain acetylcholinesterase by pure and commercial anticholinesterase pesticides. Ecotoxicol. Environ. Safety, 17 (1989) 16.
34. N. Habila, H.M. Inuwa, I.A. Aimola, O.I Lasisi, D.G. Chechet, I.A. Okafor, Correlation of acetylcholinesterase activity in the brain and blood of wistar rats acutely infected with Trypanosoma congolense. J. Acute Dis., 1 (2012) 26.
35. D. Nachmansohn, E. Lededer, Chemical, molecular basis of nevre activity. Bull. Soc. Chim. Biol., 21 (1939) 797.
36. D. Nachmansohn, M.A. Rothenberg, Hydrolyze of the butrylcholine in the serum cholinesterase. Science, 100 (1944a) 454.
37. D. Nachmansohn, M.A. Rothenberg, Hydrolyze of the butrylcholine in the red cell enzyme. J. Biol. Chem., 158 (1944b) 653.