STUDIES ON SOME ENZYME ACTIVITIES IN LAMINATED AND GERMINAL LAYERS OF HYDATID CYSTS ISOLATED FROM DIFFERENT INTERMEDIATE HOSTS IN ZAKHO, DUHOK PROVINCE, KURDISTAN REGION OF IRAQ.

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Abstract:

The current study deals with some enzyme activities in laminated and germinal layers of hydatid cysts isolated from liver and lungs of infected sheep, goats and cattle slaughtered in Zakho abattoirs and cysts isolated from humans. The activities of the enzymes, acid phosphatases (ACP), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), glutamate oxaloacetate transminase (GOT) and glutamate Pyruvate transaminase (GPT) were measured in cysts isolated from both liver and lungs of infected sheep, goats, cattle and humans. The activities of all of these enzymes were higher in laminated layer as compared with their activities in germinal layer, however in general infected host tissue showed the highest enzymatic activities as compared with hydatid cyst.

I. INTRODUCTION

Cystic Echinococcosis (CE) or hydatid cyst (HC) is a zoonotic disease of cosmopolitan distribution caused by the larval stage of the *Echinococcus granulosus* (Schantz, 1991, Eckert *et al.*, 2000 and Eckert & Deplazes, 2004). Human can be infected with the larval stage when ingests the eggs of the parasite either with food or drinks (Thompson, 1986). There are six species belonging to the genus *Echinococcus*. Four of them are infective to human causing various forms of Echinococcosis (WHO, 2001). These are *E. granulosus*, causing cystic Echinococcosis (CE); *E. multilocularis*, causing alveolar Echinococcosis (AE); *E. vogeli* and *E. oligarthrus* both causing polycystic Echinococcosis (D'Alessandro, 1997). *E. granulosus* is the most common of the four species. *E. multilocularis* is rare but is the most virulent; and *E. vogeli* is the most rare (Moldovan *et al.,* 2012). Despite to the economical and medical importance of hydatidosis, little attention has been paid to the comparative study of the parasite and its host metabolism. The metabolic pathways may vary in different parasitic species and in their hosts, MacPherson *et al.* (1985) proposed a strategy for the chemotherapy of infectious diseases utilizing biochemical differences, and stated that the inhibition of enzyme systems that are crucial to the parasites but not the host may be the basis of rational approach to the chemotherapy of the parasite. However, biochemical studies are also useful in differentiating strain variations of *E. granulosus* in different countries (Radfar and Iranyar, 2004). The strain characterization is particularly important in regions where more than one species of livestock intermediate host exists and where there is the possibility of different cycles of transmission and sources of infection for humans (Thompson and Lymbery, 1995). The present study was designed to evaluate the activities of some enzymes in laminated and germinal layers of the cyst and the host tissues which are in direct contact with the cyst and those which are at a distance of 5 cm from the cyst wall.

II. MATERIALS AND METHODS

During this study, a total of 40 HCs along with the tissues of infected organs were collected from infected animals (14 sheep, 14 goat and 12 cattle). In addition, 14 humans HCs were obtained after surgical removal of cysts from liver and lungs of patients at Azadi Teaching Hospital in Duhok city and Zahko hospital. Also 12 samples from organs (liver and lung) of uninfected sheep, goats and cattle were collected to be used as control. The activities of the following enzymes were studied acid phosphatases (ACP), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), glutamate oxaloacetate transminase (GOT) and glutamate Pyruvate transminase (GPT) using enzyme kits supplied by Biolabo reagents (France). The Buffer systems (Citrate buffer, Carbonate-Bicarbonate buffer and Tris-HCl buffer). For tissue homogenization 0.5 gram of germinal and laminated layers, infected tissues in direct contact with cyst wall, tissues at 5 cm distance

of the cysts and uninfected tissue were used. The sample were cut in to small pieces homogenized with 5 ml of buffer solution using glass homogenizer connected to a variable speed stirrer and placed in a beaker containing crushed ice (Mero *et al.*, 1988). After complete homogenization, the extract was centrifuged at 4000 rpm for 15 minutes and the supernatants were kept in labelled sample tubes and stored in a deep freezer at -40°C until used. The activities of the enzymes were determined using spectrophotometric method (Jenway 6300, England).

III. RESULTS AND DISCUSSION

1. The result of the activity of Acid phosphatase (ACP) in hydatid cyst isolated from sheep, goats, cattle and human liver and lungs are shown in Table (1). It is obvious that the activity of Acid phosphatase (ACP) in laminated layer was higher than in germinal layer in all cysts, isolated from both liver and lungs of the studied hosts with the highest being in infected tissue as compared with hydatid cyst and uninfected tissue.

Table (1): The activity of ACP enzyme in HC layer and host tissues.

Hosts	Organs	ACP (N=5)				
		Germinal (IU/L)	Laminated (IU/L)	Tissue near (IU/L)	Tissue 5cm (IU/L)	Control tissue (IU/L)
Sheep	Liver	1.69±0.10	10.69±0.39	35.30±1.35	32.07±0.60	21.42±2.22
	lung	1.62±0.12	11.30±0.47	31.06±0.98	30.99±3.00	26.01±1.92
Goats	Liver	1.75±0.19	11.70±0.82	43.10±1.75	33.15±2.63	21.11±3.36
	lung	1.35±0.10	11.30±0.61	36.87±1.13	32.82±1.38	23.61±0.69
Cattle	Liver	1.35±0.15	16.98±2.05	34.27±2.53	30.08±2.31	23.81±2.43
	lung	1.35±0.10	12.31±1.14	35.86±1.25	34.38±0.68	24.74±2.14
Human	Liver	1.15±0.08				
	lung	1.28±0.12				

2. The result of the activity of glutamate pyruvate transminase (GPT) in hydatid cyst isolated from sheep, goats, cattle and human liver and lungs are shown in Table (2). The activity of GPT in laminated layer was higher than that of germinal layer in all cysts isolated from both liver and lungs of all intermediate hosts with the highest being in infected tissue as compared with cyst and uninfected tissue.

Table (2): The activity of GPT enzyme in HC layer and host tissues.

Hosts	Organs	GPT (N=6)				
		Germinal (IU/L)	Laminated (IU/L)	Tissue near (IU/L)	Tissue 5cm (IU/L)	Control tissue (IU/L)
Sheep	Liver	14.80±0.81	20.63±0.80	39.70±2.51	37.75±2.19	25.95±2.18
	lung	12.88±0.71	19.68±0.15	29.23±0.70	28.20±1.44	22.90±0.73
Goats	Liver	11.15±0.60	18.78±0.55	36.48±0.93	33.85±1.33	26.78±1.37
	lung	10.80±0.72	16.43±0.84	31.00±1.82	27.40±2.39	23.25±2.05
Cattle	Liver	12.05±1.25	18.53±0.80	33.80±2.02	27.38±1.50	21.53±1.38
	lung	11.48±0.72	20.52±0.76	30.18±1.42	26.84±1.10	22.96±1.65
Human	Liver	12.70±1.17				
	lung	10.96±0.55				

3. The result of the activity of glutamate oxaloacetate transminase (GOT) in hydatid cyst isolated from sheep, goats cattle and human liver and lungs are shown in Table (3). The activities of GOT in laminated layer was higher than that of germinal layer in all cysts isolated from both liver and lungs of all intermediate hosts with the highest being in infected tissue as compared with cyst and uninfected tissue.

Table (3): The activity of GOT enzyme in HC layer and host tissues.

Hosts	Organs	GOT (N=6)				
		Germinal (IU/L)	Laminated (IU/L)	Tissue near (IU/L)	Tissue 5cm (IU/L)	Control tissue (IU/L)
Sheep	Liver	13.90±1.49	31.96±0.71	136.4±12.06	125.8±11.10	68.20±4.90
	lung	13.04±1.26	34.56±1.49	115.8±6.67	110.2±9.92	69.38±2.77
Goats	Liver	23.63±1.33	34.48±1.68	158.5±14.60	117.9±8.14	80.35±8.16
	lung	24.08±1.09	38.30±2.23	119.6±1.79	102.2±5.00	71.02±4.14
Cattle	Liver	16.70±2.21	33.35±0.98	182.3±12.28	136.4±16.53	84.00±4.03
	lung	15.80±1.46	35.40±1.28	147.6±10.44	112.9±4.57	77.83±2.47
Human	Liver	22.53±4.09				
	lung	22.73±3.63				

4. The result of the activity of alkaline phosphatase (ALP) in hydatid cyst isolated from sheep, goats, cattle and human liver and lungs are shown in Table (4). The activity of ALP in laminated layer was higher than that of germinal layer in all cysts isolated from both liver and lungs of all intermediate hosts with highest being in infected tissue as compared with cyst and uninfected tissue.

Table (4): The activity of ALP enzyme in HC layer and host tissues.

Hosts	Organs	ALP (N=6)				
		Germinal (IU/L)	Laminated (IU/L)	Tissue near (IU/L)	Tissue 5cm (IU/L)	Control tissue (IU/L)
Sheep	Liver	16.46±0.86	26.70±1.33	295.4±2.21	285.0±5.28	189.1±2.03
	lung	14.31±1.30	27.93±0.68	279.3±17.99	273.2±5.92	177.1±17.41
Goats	Liver	15.26±2.03	28.16±2.87	405.0±10.12	351.0±6.52	101.9±21.03
	lung	15.26±2.03	29.97±2.63	276.9±50.70	259.8±16.58	191.3±1.80
Cattle	Liver	18.00±3.41	29.43±3.15	512.3±10.20	458.9±16.70	104.6±12.71
	lung	16.89±2.77	34.25±2.31	283.4±17.51	243.1±7.48	105.7±1.01
Human	Liver	15.26±1.84				
	lung	15.80±1.80				

5. The result of the activity of lactate dehydrogenase (LDH) in hydatid cyst isolated from sheep, goats, cattle and human liver and lungs are shows in Table (5). It is obvious that the activity of LDH in laminated layer was higher than in germinal layer in all cysts, isolated from both liver and lungs of the studied hosts with the highest being in infected tissue as compared with hydatid cyst and uninfected tissue.

Table (1): The activity of LDH enzyme in HC layer and host tissues.

Hosts	Organs	LDH (N=5)				
		Germinal (IU/L)	Laminated (IU/L)	Tissue near (IU/L)	Tissue 5cm (IU/L)	Control tissue (IU/L)
Sheep	Liver	24.28±3.62	142.5±13.68	416.2±15.08	370.7±17.58	189.4±19.27
	lung	24.28±2.56	188.3±4.21	320.6±14.16	263.9±14.62	202.4±3.62
Goats	Liver	27.52±1.98	165.1±11.05	511.5±15.02	479.2±10.98	169.8±13.56
	lung	25.90±4.71	186.2±22.47	275.2±7.24	231.5±11.92	160.3±16.48
Cattle	Liver	27.47±4.09	147.3±6.47	520.8±24.04	459.5±38.87	215.3±21.66
	lung	29.14±4.12	119.8±16.62	395.0±19.06	336.8±14.16	178.1±23.33
Human	Liver	19.42±5.49				
	lung	22.66±3.96				

The current study indicated that the activities of all studied enzymes were high in laminated layer in all studied hosts as compared to germinal layer. Regarding host tissues, the tissues in contact with hydatid cysts showed the highest enzymatic activities. As hydatid cysts are space occupying lesions, their growth exerts pressure on the surrounding tissues which leads to tissue damage and the leak of enzymes from damaged cells to the surrounding tissue (Zeheer,1997, Nyblom *et al*., 2004).

So far there is no any available studies concerning the activities of these enzymes in cyst layers, howevere, the available studies were performed on protoscolices and hydatid fluid in which high enzymatic activities were observed (MacManus and Bryants (1995), Frayha and Haddad (1980), Izadi & Ajami (2006), Rahdar *et al*.(2008) and Rouhani and Vatankhah (2008) on HF and Abdullah (2010) on protoscolices).

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كورتى:

ئەڤ ڤكولێنە هاتيە ئەنجامدان سەر جستيا هندەك ئەنزيما لسەر تيخىٚ پەردار و خەرزەدار ييّت كيسكيت ئاڤىٚ ييّت مشەخور ئەويّت هاتينە خرڤەكرن ژ سيھ و كەزەب يت (پەز، بزن و چيل) ئەوين هاتينە سەربرين ل گوشتاركەها زاخو و هەروسا لە مروڤا ژىٚ هاتين كومكرن. و جستيا ئەڤ جورە ئەنزيمە هاتنە بكارئينان (ACP, GPT, GOT , ALP وLDH) . و وەسا ديار بو كو چستيا ڤان جورە ئەنزيما لتيخىٚ پەردار بلند تربو ژ تيخىٚ خەرزدار. و ديسا ئەڤ چستيە بلند تربو لناڤ شانيّت دەوروبەرى كيسكىٚ ئاڤىٚ ژ كيسكىٚ ئاڤىٚ.

الخلاصة:

تظمنت هذه الدراسة قياس فعالية بعض الإنزيمات في الطبقات الصفائحية و الجرثومية للأكياس العدرية المعزولة من الاكباد و الرئات المصابة للأغنام والماعز والأبقار المذبوحة من مسلخ زاخو و كذلك الأكياس المعزولة من الانسان . تم قياس نشاط الأنزيمات (ACP, ALP , LDH , GOT ,GPT)

في الاكياس المعزولة من كل من الكبد والرئتين وكذلك في الأنسجة من الأغنام المصابة والماعز والأبقار والإنسان. وكانت أنشطة جميع هذه الإنزيمات أعلى في الطبقة الصفائحيه بالمقارنة مع أنشطتها في الطبقة الجرثوميه. ولكن بصفة عامة أظهرت أنسجة المضائف المصابة أعلى الأنشطة الأنزيمية بالمقارنة مع الاكياس العدرية.