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Detection some chemical and biochemical constituents of *Cysticercus tenuicollis* cyst fluid of Iraqi goat

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Abstract
The study was performed to detect some chemical and biochemical constituents in the fluid of *Cysticercus tenuicollis* cysts the larval stage of tapeworm *Taenia hydatigena.* Twenty two cysts were obtained from infected carcasses of Iraqi goat during a period of six months from April to September 2016.Cysts were found attached to the liver, lungs and fat of great omentum. The cysts fluid was collected and subjected to different conventional laboratory methods using commercial kit reagents to analyze and measure the concentration of some chemical and biochemical constituents. The results show that the concentrations of calcium ranged between (2.31-5.70 mg//dl), sodium (81.00-138.00 mmol/l), potassium (6.70-11.40 mmol/l) and phosphorus (0.330-1.460 mg /100ml). Glucose ranged between (16.00-103.00 mg /dl), urea nitrogen (18.00-63.20 mg /dl), uric acid (0.20-28.00 mg /dl) and creatinine (0.180-0.67 mg/dl).Total protein concentration was between (0.60-108.00g/l). Furthermore, cholesterol and triglyceride concentrations were (0.00-17.008 and 20-67.00 mg/dL), respectively. The activity of aspartate aminotransferase was (1.10-3.20U/L),alanine aminotransferase was (1.70-16.00 U/L) and lactate dehydrogenase was (3.00-249.00 U/L).In conclusion the decrease or increase in the chemical and biochemical SE values provide the preventive role of the parasite cyst membrane to exchange these substances. The high rate of SE values of some parameters studied may suggest a various degree of parasitic growth rate or may reflect activity of the cyst's metabolism which needs to be studied in future.

Keywords: *Cysticercus tenuicollis*, fluid, detection, chemical, biochemical constituents, goat

1. Introduction
Cysticercus (C) tenuicollis is the larval stage of the parasite *Taenia hydatigena* tapeworm belongs to the family Taeniidae [1]. The domestic life cycle of parasite is maintained through definitive host, domestic and wild carnivorous, which harbor the adult *Taenia hydatigena* in the small intestine and a wide range of intermediate host including domestic ruminants and wild ones, pigs, squirrels and humans which are infected with larval stage. The definitive host infection occurs when the offal and meat infected with *C. tenuicollis* is fed raw [2]. While intermediate host acquires infection by ingestion of the eggs of *Taenia hydatigena* during grazing or food contamination [3] Eggs hatched in the small intestine and the onchosphere penetrate the blood circulation, and then reach to the liver and other vital organs in the peritoneum. In heavy infection when entire tapeworm segments are ingested, a large number of onchospheres migrate to the liver parenchyma to developed into cysticerci leading to destroying the hepatic cells causing hemorrhagic tracts, eosinophilia infiltration and sever inflammation resulting in acute atraumatic hepatitis (cysticercosis). This condition resembles acute faschilosis and anaerobic condition, which lead to black disease because of generation of *Clostridium novyi* bacteria. Later, fibrotic tracts and serofibrinous peritonitis results in ascites, high temperature and death [4]. The larva *C. tenuicollis was* found attached to different visceral organs such as serous surface of liver, spleen, lung, greater omentum, intestinal mesentery, kidney, heart and unusual location have been described [5]. Cysticercosis is a prevalent disease; causes considerable economic losses due to high degree of morbidity and mortality in livestock and condemnation of infected meat at meat inspection [6]. Cysticercosis is endemic in Iraq. The prevalence of disease has been studied in sheep and goat [7].

However, no work has been done on the analysis of C. *tenuicollis* cysts fluid in Iraq. Therefore, the present study was undertaken to detect some chemical and biochemical constituents of *C. tenuicollis* cysts fluid in Iraqi goats.

2. Materials and Methods

2.1. Collection and processing of cyst

C*ysticercus tenuicollis* cysts were collected from visceral organs liver, lung, great omentum, intestinal mesentery of infected carcasses of Iraqi goats which slaughtered at the main abattoir Alshuala in the south east region of Baghdad city in Iraq during period of six month from April to September 2016. *C. tenuicollis* was transferred in sterile container in cold box to the laboratory of Parasitology in Veterinary Medicine College of Baghdad University for further examined. Each cyst was processed as an individual isolate, under aseptic condition the surface of cyst cleaned with normal saline then with70% alcohol. The fluid were aspirated using sterile syringe, transfer to a clean test tubes and centrifuged at 15000 rpm at 4 °C for 30 minutes. The supernatants were analysis for measured the concentration of some chemical and biochemical constituents such as calcium, sodium, potassium and phosphorus, glucose, urea nitrogen, uric acid, creatinine, total protein, triglycerides, cholesterol and enzyme Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Lactate dehydrogenase (LDH) using different conventional laboratory methods and commercially available kits reagents (w.w.w. Biolabo. Franc) followed manufacturer's instructions. The concentration of calcium was estimated by the method of CPC (O_ Cresol Phtalein Complexion in alkaline solution) according to [8].The absorbance's was measured at 570 nm and concentration was expressed in mg/dl. Sodium was photometric determination by Mg-Uranylacetate method. Sodium concentration in the fluid expressed in mmol /l [9]. Likewise potassium was performed used flame photometric Turbidimetic test. The turbidity is proportional to the potassium concentration which read photometrically and expressed in mmol/l. Phosphorus was estimated by ammonium molybdate U.V method. The absorbance's measured at 340 nm the produced is proportional to the concentration of phosphate ions which expressed in mg /100 ml [10]. Measurement of glucose is performed by enzymatic colorimetric (oxidase) method according to[11], in the reaction the glucose is oxidzed to D_ gluconate by the glucose oxidize (GOD)with formation of hydrogen peroxide in the presences of peroxidase(POD) a mixture of phenol and 4_aminoantiynne(4_AA) is oxidize by is hydrogen peroxide to form a red quinoneimine dye the absorbance of colored complex measured at 500nm is proportional to the concentration of glucose**(**mg/dl**).** Uric acid (mg/dl**)** was estimated by the phosphotungstic acid (PTA) [11]. Urea nitrogen (mg/dl**)** is determined by using enzymatic colorimetric (diacetyl monoxim) method of [12] Urea is hydrolyzed by urease in to ammonia and carbon dioxide. The ammonia generates react with alkaline hypochlorite and sodium salicylate in the presences of sodium nitroprusside as coupling agent to yield a green cromophore. The absorbance of color intensity formed read at 600nm is proportional to the amount of urea**.** Creatinine (mg/dl) were measured by kinetic colorimetric method based on procured of picrate reaction [13] under alkaline condition reacts with picrate ions forming a reddish complex measured of absorbance at 510 in a pre-fixed interval of time is proportional to the amount of creatinine in the fluid. Samples were analyzed for total protein using the method of Biurat [8] and expressed in (g/l).

Cholesterol (mg/dl) measured by enzymatic colorimetric method of [14] involving the use of three enzymes, cholesterol esterase (CE). cholesterol oxidase (CO) and peroxidase (POD) in the presences of former the mixture of phenol and 4_aminoantiynne(4_AA) is condense by hydrogen peroxide to form quinoneimine dye the absorbance of colored complex measured at 500 nm is proportional to the concentration of cholesterol in the sample. Triglycerides (mg/dl) was measured based on the enzymatic procedure of [15].Hydrolysis of triglyceride to glycerol and free fatty acids by lipoprotein lipase.The glycerol is phosphorelated by adenosine triphosphate in the presences of glycerolkinase to form glycerol_ 3_ phosphate (G_3_P) and adenosine diphosphate.G_3_P is oxidized by glycerophosphate oxidase to form dihydroxyacetone and hydrogen peroxide. a red chromogen is produced by the peroxidase catalyzed coupling of 4__aminoantiynne(4_AA) and phenol with hydrogen peroxide proportional to the concentration of triglyceride in the sample. Enzymes like Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Lactate dehydrogenase (LDH) activities were estimated by the colorimetric method of Reitman and Frankel [8] using commercially available kit (www, Biolabo Franc.) using Semi-autoanalyser as described by manufacturer. Enzyme level was expressed in U/L.

3. Statistical analysis

Data were analyzed using spss software. Results expressed as Mean-standard (SE) [16].

4. Results of the study

Twenty two *Cysticercus tenuicollis* cysts were originated from the infected goat carcasses and found in variable sizes attached to the liver, lung and great omentum. The cysts have characteristic features it looks as long necked bladder worm, consisting of one an invaginated scolex surrounded by a transparent membrane with clear fluid filed cyst cavity plate1(A,B,C,D). Fluid of 22 cysticerci was analyzed for detection of some chemical constituents (calcium, sodium, potassium and. phosphorus). These electrolytes were present at significant levels. The data were illustrated in table [1]. The lowest concentration of calcium was 2.31 mg/dl and the highest was 5.70 mg/dl with average of (4.023 mg/dl). Fluid contained sodium in measurable quantity range from the lowest concentration 81.00 mmol/L to the highest concentration 138.00 mmol/L with average of (118.6 mmol/L). Potassium concentration was low range between 6.70 mmol/L to 11.40 mmol/L with average of (9.17 mmol/L). Fluid also contained phosphorus, the concentration was also low ranged between 0.33074mg/100 ml to 1.460 74mg/100 ml with average of (0.74mg/100 ml). Analysis of 22 cystic fluids was done to measure the average concentration of some biochemical constituents. The results were illustrated in table [2] showing that the glucose was found in considerable amount, the lowest concentration was 16.00 mg/dl and the highest reach to 103.00 mg/dl with average of (48.3 mg/dl). Urea nitrogen ranged from lowest 18.00 mg/dl to highest concentration 63.20 mg/dl with average of (43.6mg/dl**).** The lowest concentration of Uric acid was 0.20 mg/dl and highest was 28.00 mg/dl with average of (4.29 mg/dl). Creatinine ranged 0.67 - 0.180 mg/dl with average of **(**64.8 mg/dl**).** Total protein was 0.60 g/l and reached to 108.00 g/l with average of (2.26 g/l). Cholesterol measured between 0.00-17.00 mg/dl with average of (4.9 mg/dl), while Triglyceride was18.20 mg/dl reached to 67.00 mg/dl with average of (24.16 mg/dl). Analysis of enzymes

present in the fluid to determine the average concentration showed that the lowest level of AST was1.10 U/L and the highest level was 30.20 U/L with average of (23.9U/L). Alanine aminotransferase ranged from lowest 1.70 U/L to 160.00 U/L with average of (89 U/L). Examination of LDH concentration in the cysticerci fluid showed a lowest level of 3.00 U/L and reached to a highest concentration of 249.00 U/L with average of (201 U/L). LDH was found in considerable amounts, whereas AST was present in lower concentration.

Table 1: Chemical components of C *tenuicollis* fluid from liver, lung of goats (Mean ± SE, n=22)

Host origin and site of the cyst				
goats		**Liver and, lung**		
Chemical Components	Units	Range	Average	**Mean ± SE**
Calcium	mg/dl	2.31-5.70	4.023 mg/dl	4.143 ± 0.166
Sodium	mmol /l.	81.00-138.00	118.6 mmol/l	120.78 ± 3.075
Potassium	mmol /l.	6.70-11.40	9.17 mmol/l	9.435 ± 0.276
Phosphorus	Mg/100 ml	0.330-1.460	0.74mg/100 ml	0.775 ± 0.059

Table 2: Biochemical components of C *tenuicollis* fluid from liver, lung of goats (Mean ± SE, n=22)

Host origin and site of cysts				
goats			**Liver and lung**	
Biochemical Components	**Units**	**Range**	**Average**	**Mean ± SE**
Glucose	(mg/dl)	16.00-103.00	48.3 mg/dl	52.52±3.91
Urea	(mg/dl)	18.00-63.20	43.6mg/dl	45.61±2.26
Uric acid	(mg/dl)	0.20-28.00	4.29 l mg/dl	4.114 ± 0.959
Creatinine	(mg/dl)	0.67 -0.180	64.8 mg/dl	0.348 ± 0.023
Total protein	(g/l)	0.60-108.00	2.26 g/l	16.085 ±6.484
Cholesterol	(mg/dl)	0.00-17.00	4.9 mg/dl	4.946 ± 0.534
Triglyceride	(mg/dl)	8.20-67.00	24.16 mg/dl	22.725 ± 2.039
A ST	(U/L)	1.10-3.20	23.9 U/L	2.019 ± 0.116
ALT	(U/L)	1.70-16.00	89 U/L	6.385 ± 0.808
LDH	(U/L)	3.00-249.00	201 U/L	100.78 ± 13.23

Plate 1: Showing variable size and characteristic feature of *Cysticercus tenuicollis cysts*

5. Discussion

Infection with cysticercosis due to bladder worm *C. tenuicollis* cysts is prevalence in Iraq. The disease in sheep was 14.22% and 16.01% in goat [7]. Morphological, immunological, physiological and some biochemical variation have been described in some Taeniid metacestodes, including *T. taeniaeformis, T. crassiceps, Echinococcus spp* and non Taeniid *Hymenolepis diminuta* [17-21]. In the present study some chemical and biochemical substances have been described as a part of wide study on the *C. tenuicollis* cysts. Chemical substances such as (calcium, sodium, potassium and phosphorus) were found at variable concentration in the studied cysts fluid. These chemical and biochemical substances play definitive role in the physiology, metabolism and immunology of the host, which reflected the relationship between the intermediate host and parasite [20]. The occurrence of calcium ions is important in ATP production and ATPase activities. The means concentration of calcium and Potassium ions was found lower than the mean concentration of sodium, which constituted the major ion in the fluid compared to the mean concentration of phosphate, these findings were agree with **[20,22]** studies**.** The cyst membrane is considered as transport border between the *Cyst* fluid and host serum. Thus, the quantity of chemical composition probably related to species not to cyst location [20] The environment of the infectious stages of parasite is the host and the only way by which macromolecules absorption in immature stage is endocytosis, more important route supporting data comes only from studies on *Taenia crassiceps* [23]. The parasite in its intermediates hosts has various degree of metabolism in various colonized organs and the biochemical parameter is reflecting the quantitative differences in the metabolism of the cyst in respect to the site of parasitism [22]. In this study, the dominant preferred site of *C. tenuicollis* in goats was the liver, lung and great omentum. The concentration of glucose was found in considerable amounts. Rosen *et al*, (1994) [19] mention that the glucose indicate the presence of glycolysis and glyconeogenesis cycles related to energy production with parasite cyst. It has been shown that glucose is absorbed by transport system found in mammals in the external cyst wall of *T. solium* neurocysticerci, and also in the apical membrane of the tegument of adult tapeworm [24]. Metacestode parenchymal tissues contain numerous gap junctions to uptake glucose [24]. Larval cestodes generally show a more constant glycogen content than the corresponding adults [24], this may reflect the more stable intermediate host environment, usually the coelomic cavity or tissues [25]. Dixon *et al* (1973) [26] isolated the glycoprotein from the fluids of cysts, which contain about 7·7% heterosaccharide consisting of glucose, galactose, mannose, fructose, neuraminic acid and glucosamine. It was

suggested that glycoprotein appears to be derived from the parasite, and functions as the osmotically active macromolecule in the cyst fluid maintaining the turgidity of the cyst**.**

Urea and Uric acid occurrence refer to the presence the urea cycle, which is essential to eliminate the toxic level of ammonia through amino acid and nucleotide metabolism.[27].Creatinine level reflects ammonia metabolism and energy production [27].

The concentration of total protein in the cyst fluid of *C. tenuicullis* was found in measurable quantity in the present study which agree with result of [28, 29]. Protein content depends on the age, degree of maturation and previous metabolic history of the worm. Studies have been carried out on larval *E. granulosus* [17] confirmed the presence of several host proteins, including serum albumin and immunoglobulins in hydatid cyst fluid. Host protein may enter the cyst by diffusion through the cyst membranes by endocytosis or by specific filter or transport mechanisms. The ionic nature of proteins may also play an important role in their own absorption [27]. Increasing protein reflects its importance in the catabolism and anabolism (growth) activities and mixed production of it by host and parasite. Taeniid metacestodes are capable of absorbing a variety of proteins; these mcromolecules can retain their structural and functional integrity following transport [17]. The present study was agreed in the concentration of many parameters with previous studies of [28, 29].

The concentrations of cholesterol in the present study disagreement with study of [29]. The occurrence of cholesterol is related with its function as a structural component and to colonization site of parasite because it is increased in the liver more than in lung disorder[18].Triglyceride appearance in high concentration is expected as it the most abundant sterol molecule within parasite, also as an energy source with physical protection role. It is probable that these lipids play a vital role in the establishment and development of the parasite [18].

Among enzymes AST, ALT and LDH exhibited activities in the cyst fluid of *C tenuicullis*. However, the concentrations of LDH were present in considerable amounts. Abidi *et al.* (1989) [28] in his study analyzed the major biochemical components of *Taenia hydatigena* cysticerci collected from goats and pigs and showed marked differences, particularly in glycogen, protein, lipid levels, cholesterol and triglycerides conclusion that the cysticerci of goat and pig origin probably represent two different strains. In conclusion the decrease or increase in the chemical and biochemical SE values provide the preventive role of the parasite cyst membrane to exchange these substances The high rate of SE values of some parameters studied may suggest a various degree of parasitic growth rate or may reflect activity of the cyst's metabolism which needs to be studied in future.

6.References

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