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# COMPARATIVE LIVER HISTOPATHOLOGICAL AND HISTOCHEMICAL STUDIES IN HYDATID CYST DISEASE WITH MEAN OPTICAL TRANSPARENCY

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including cloudy swelling, fatty changes and necrosis.

Hydatid cyst disease is one of the intense parasitic diseases which will cause death and solely may

be treated surgically. The current study was aimed to perform comparative histopathological changes

to reveal the effect of the parasites on host liver tissues. These changes were recorded using digital

Image Analysis Software on the section's digital images of the infected liver tissues, and it have been

demonstrated many significant changes on the host liver tissues. The parasite's histopathological

effects on the liver were presence of granuloma, eosinophilia and some changes in the cells

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# ABSTRACT

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*Keywords:* Hydatid cyst disease, hydatidosis, Histopathology, histochemistry, parasitology, sheep

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#### Introduction

Hydatid disease is a zoonosis [1] caused by Echinococcus *granulosus*; carnivores like dogs and foxes usually function final host; it can be transmitted to herbivorous mammals as intermediate hosts (like man, sheep, camels, cattle and pigs) [2] through the fecal-oral route by ingesting the eggs passed in the final host's stool. The parasite develops in the organs of intermediate host including liver or lung [3]. The hydatid cyst (Metacestode stage or larval stage) varies considerably in size; it grows very slowly and reaches the size of 1 cm by the fifth month [4]. Unilocular cysts are usually less than 5 cm in diameter but may reach 20 cm. In humans, the most common site for hydatid cyst is liver (50-70%) followed by the lungs (25%) and less frequently the spleen, kidneys, heart, bones, C.N.S., viscera, as well as other organs [5-9]. The fully developed hydatid cyst is filled with about 15 quarts of fluid (hydatid fluid) and some of them are sterile [10]. *E. granulosus* cysts of sheep and goats mainly infect the liver and lungs. Sheep showed hypersensitivity reaction, although there was no sudden death in sheep or other animals [11]. Cysts are often multiple and unilocular with liver and lung infection in cattle, but if cows are infected with cattle, the lungs are the dominant infected organs. Cysts grow slowly and even long-life cysts in the liver of the horses could stay little and symptomless [12]; although giant cysts often stay symptomless [13], clinical manifestations are recorded. In one case reported in Switzerland a nine-year-old Irish horse presented with large liver enlargement, raised levels of liver enzymes, liver dysfunction, obstructive lung disease, intermittent pain, eating disorder and emaciation.

Local pathological effects depend on the site of the hydatid cyst. Ruptured liver cyst through the diaphragm can produce a pleural effusion or bronchobiliary fistula [14]. Daali *et al.* (2001) [15] reported that hydatid cysts of the liver opened into the biliary tract in 25% of cases and demonstrated that jaundice, biliary colic and eosinophilia are characteristics of rupture into the biliary tree. [16] reported a case with acute calculus cholecystitis due to hepatic hydatid cyst rupture into the gall bladder, a daughter cyst obstruction by the cystic duct. The liver has over two thirds of cysts with percentage of 66%, where the lungs are infected in about 10-20% of cases; other organs' involvement is less than 10% of cases, and infections with echinococcosis were recorded in the muscle [17-19]. The host reaction to unilocular hydatid cyst is slight: there's some granulation and a relatively thin fibrous wall. Eosinophils aren't conspicuous however if a cyst has died or burst, they may be

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more evident, along with giant-cell granulomas. Deeply stained nuclei (pyknotic) of hepatocytes, Kupffer cells, bile ducts and walls of the blood vessels are usually observed with increased lymphocytes in and around the portal area and Kupffer cells. Also numerous karyolytic nuclei are seen [20- 27] distorted portal area with thickened arterial wall, and ruptured endothelial lining of the hepatic portal vein also can be noticed. [27, 28] noticed many similar deleterious changes in infected liver of various animals included camels, pigs and sheep.

## **Materials and Methods**

## Histopathological and histochemical studies:

Samples of the infected liver with CE were collected from infected livestock slaughtered in Al-Madina Al-Munawwara abattoirs. Two small pieces of the chosen tissue (liver, heart and skeletal muscle) were fixed in 10% neutral buffered formal for 24 hours, then washed under slow running tap water for 24 hours, dehydrated in ascending grades of ethyl alcohol (50%, 70% and 95% and 100%), for an hour in each grade. Clearing was done in xylol. Once the specimens became translucent, they were embedded in paraffin wax using three changes of wax. Tissue blocks were then made and serial sections (5-6  $\mu$  each) were cut using a rotator microtome [29]. All serial sections were treated and prepared chemically in the laboratory using various chemicals and stains for the following studies:

#### Histopathological examination:

Harris hematoxylin and eosin stains were used to demonstrate the general histological structure. Periodic acid Schiff's Method (PAS) was used to detect polysaccharides; glycogen and polysaccharides (neutral) appeared magenta red. Mercuric bromophenol blue was used to demonstrate the total proteins. Feulgen method was used to detect DNA materials. Mallory's trichorome stain was used to demonstrate the collagen fibers according to Bancroft and Gamble (2008) [30].

#### Image analysis:

BEL micro Image Analyzer software for microscopy ver. 2.3 was used for the digital image analysis of normal and infected Liver.

#### Statistical analysis:

All the observed data of MOT microscopic optical transparency (pexil) for PAS+ stain, DNA content and the total protein were recorded and statistically analyzed by using T-test on Microsoft Excel, to demonstrate the significant changes on the infected tissues.

#### Results

The control liver tissue is shown in Figs.1 and 2, where cords of hepatocytes are radiating from the central vein (cv) to the periphery of the lobule, and between the hepatocytes blood sinusoids are detected with numerous Kupffer cells. The portal area contains branches of the hepatic portal vein (hpv), hepatic artery and bile ducts. Figs. 3 and 4 show thin collagen bundles supporting walls of the hepatocytes, blood vessels, bile ducts and blood sinusoids. Normal distribution of total protein in the central and portal areas can be noticed in Figs. 5 and 6. Dense stain affinity is realized in wall of the central vein with noticeable stain affinity in hpv, bile ducts and less stained hepatocytes.

Dense stain affinity of polysaccharides is detected in walls of the bile ducts, hepatocytes and walls of blood vessels in the central and portal areas (Figs.7 and 8). Deeply stained nuclei of hepatocytes, Kupffer cells, bile ducts and walls of the blood vessels are observed in Figs. 9 and 10. Wall of the hydatid cyst is demonstrated in Figs. 11 and 12. It consists of outer granular layer and inner laminated layer which is encircled by thick fibrous layer. Many dystrophic changes were observed in liver tissue infected with the hydatid cyst (Figs. 13, 14 and 15). Increased lymphocytes in and around the portal area and Kupffer cells were noticed with numerous pyknotic and karyolytic nuclei. The observed results were confirmed by MOT mean optical transparency. The values showed significant increase for pyknosis and reached  $154.36\pm13.95$ ,  $156.60\pm12.36033$  and  $158.53\pm15.78$  in infected samples S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub> respectively compared with the non-infected control group  $121.43\pm6.88$  (Table 1).

Also, the MOT values showed both significant and non-significant decrease for karyolysis and reached  $121.1\pm7.98927$ ,  $156.6\pm12.3603$  and  $117.5\pm5.72224049$  in infected samples S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub> respectively compared with the non-infected control group  $121.43\pm6.88$  (Table 2). Highly distorted portal area with thickened arterial wall and ruptured endothelial lining of the hpv were also detected. Distribution of collagen fibers in the infected liver tissue could be observed in Figs. 16, 17 and 18. Dense stain affinity of collagen fibers could be realized in the laminated layer, distorted portal area with fibrotic red stained arterial wall. MOT values showed both significant and non-significant increase and reached  $160.73\pm12.33$ ,  $173.60\pm21.43$  and  $182.36\pm17.45$  in infected samples S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub> respectively compared with the non-infected control group  $116\pm18.26$  (Table 3).

Concerning total protein, the granular acquired deep blue coloration with less stained laminated layer (Figs.19, 20 and 21), and poorly to moderately stained hepatocytes, MOT values showed both significant and non-significant decrease and increase and reached  $71.66\pm14.14$ ,  $152.26\pm11.86$  and  $51.46\pm8.24$  in infected samples S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub> respectively compared with the non-infected control group  $84.76\pm13.28$  (Table 4). Distribution of polysaccharides in the infected liver tissue was observed in Figs. 21, 22, 23 and 24, where deeply stained germinal layer, fibrotic areas, walls of the blood vessels and some

# Khadija Abdul Jalil Faddladdeen, 2019

Pharmacophore, 10(2) 2019, Pages 51-62

hepatocytes were deeply stained, while the remnant hepatocytes and the laminated layer were less stained. MOT values showed significant decrease and reached  $80.13\pm10.42$ ,  $81.93\pm15.36$  and  $44.86\pm9.15$  in infected samples  $S_1$ ,  $S_2$ , and  $S_3$  respectively compared with the non-infected control group  $113.66\pm18.10$  (Table 5). Deeply stained nuclei of cells of the germinal layer, lymphocytes and thickened arterial walls were detected in Figs. 25, 26 and 27, but hepatocytes and delaminated endothelial lining of the blood vessels were less stained. MOT values showed significant increase and reached  $73.83\pm10.76$ ,  $77.63\pm15.35$  and  $71.33\pm24.44$  in infected samples  $S_1$ ,  $S_2$ , and  $S_3$  respectively compared with the non-infected control group  $38.93334\pm9.12$  (Table 5 and histo. 5).



**Fig. 1:** Central area of the liver tissue of a control animal showing: Hepatocytes (h), sinusoidal spaces (s), central vein (cv) and kupffer cells (k). (H&E



Fig. 3: Thin collagen bundles supporting wall of the central vein  $(\Box)$  (cv), hepatocytes, sinusoidal spaces, hepatic portal vein (hpv) hepatic artery (ha) and bile ducts (bd). (Mallory's trichorome stain  $\times 100$ ).



**Fig. 5:** Normal distribution of total protein in the central area of the liver tissue. Notice: dense stain affinity in the wall of the central vein  $(cv) (\Box)$  and blood cells inside it with less stained hepatocytes

(h). (Mercuric bromophenol blue  $\times 100$ ).



**Fig. 2:** The portal area showing: branch of the hepatic portal vein (hpv) \* branch of the hepatic artery (ha), bile duct (bd) (H&E ×100)



**Fig. 4:** Thin collagen bundles supporting wall of the central vein (□) (cv), hepatocytes, sinusoidal spaces, hepatic portal vein (hpv) hepatic artery (ha) and bile ducts (bd). (Mallory's trichorome stain x50).



**Fig. 6:** Normal distribution of total protein in the portal area. Notice moderate stain affinity in hepatocyte with increased stain affinity in walls of hepatic portal vein (hpv) hepatic artery (ha). (Mercuric bromophenol blue ×50).

#### Khadija Abdul Jalil Faddladdeen, 2019 Pharmacophore, 10(2) 2019, Pages 51-62



**Fig.7:** Normal distribution of polysaccharides in the central and portal areas. Notice: dense stain affinity in hepaotcyets and walls of the blood vessels.



Fig. 9: Showing normal DNA content in the central and portal areas. Notice: deeply stained nuclei of: kupffer cells (k), most hepatocytes, walls of the blood vessel and bile ducts. (Feulgen's reaction ×100)



**Fig. 11:** Showing wall of the hydatid cyst with its well-developed granular layer (g), thin laminated layer (L) and thick fibrous layer encircles it (f).(H&E



Fig. 8: Normal distribution of polysaccharides in the central and portal areas. Notice: dense stain affinity in hepaotcyets and walls of the blood vessels. (PAS×50)



Fig. 10: Showing normal DNA content in the central and portal areas. Notice: deeply stained nuclei of: kupffer cells (k), most hepatocytes, walls of the blood vessel and bile ducts. (Feulgen's reaction ×100



**Fig. 12:** Showing wall of the hydatid cyst with its well-developed granular layer (g), thin laminated layer (L) and thick fibrous layer encircles it (f). (H&E x



**Fig. 13:** Revealing increased lymphocytic infiltration in and around the portal area, increased kupffer cells (K) with sings of karyolysis (ka) and pyknosis (>) in nuclei of hepatocytes. (H&E ×100)

#### Khadija Abdul Jalil Faddladdeen, 2019 Pharmacophore, 10(2) 2019, Pages 51-62



**Fig. 14:** Well developed germinal (g) layer, laminated (L) layer with highly aggregated lymphocytes (ly) beside the wall of the hydatid cyst with thick fibrous layer (F). (H&E  $\times$ 50)



**Fig.16:** Showing laminated layer of the hydatid cyst with dense stain affinity of collagen fibers and they also increased in and around the portal area. (Mallory's trichorome stain x50)



**Fig.18:** Highly distorted portal area with increased collagen fibers. Notice: thickened arterial wall which acquired brightly red stain. (Mallory's trichorome stain x50)



**Fig.20:** Deeply stained thickened arterial wall (a). (Mercuric bromophenol blue ×50)



**Fig.15:** Highly distorted portal area, highly thickened arterial wall (□) with narrowed lumen of it (>) and ruptured endothelial lining of the hepatic portal vein (\*). (H&E ×50)



Fig.17: Highly distorted portal area with increased collagen fibers (Mallory's trichorome stain x50)



**Fig.19:** Deeply stained total protein in the granular layer (g) less stained laminated layer (>) moderate to deeply stained fibrotic layer which encircles the hydatid cyst with poorly stained hepatocyts (h). (Mercuric bromophenol blue ×50).



**Fig.21:** Poorly to moderately stained hepatocyts in the central and portal areas of the liver tissue of the infected animal. (Mercuric bromophenol blue ×50)



Fig.22: Deeply stained PAS +ve materials in the germinal layer with less stain laminated layer. Fibrotic areas and highly thickened elongated arterial walls acquired deep red coloration. (PAS×50)





**Fig.24:** Highly distorted portal area with deeply stained walls of blood vessels. (PAS×50)



**Fig.25:** Faintly stained nuclei of hepatocytes, deeply stained nuclei (□) of the germinal layer, poorly stained laminated layer and deeply stained nuclei of the thickened arterial wall (\*). (Feulgen reaction ×50)



Fig. 26: The portal area showing deeply stained nuclei of the blood vessels and lymphocytes, delaminated endothelial lining of the central vein showed poorly stained DNA content (\*)(Feulgen reaction ×50)



**Fig. 27:** The portal area showing deeply stained nuclei of the blood vessels and lymphocytes, delaminated endothelial lining of the central vein showed poorly stained DNA content (\*) (Feulgen reaction ×50)

Table 1: Showing MOT	values of pyknosis ir	the liver of the control	and infected animals.
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	control	Sample 1	Sample 2	Sample 3
Mean	121.4333	154.3667	156.6	158.533336
S.D.	6.88898	13.95827	12.36033	15.78497572
% of change		27.1205	28.95967	30.55174025
T test		0.000343**	0.000471**	3.4751E-05**

\* Significant (P<0.05) \*\* Highly significant (P<0.01)

Pharmacophore, 10(2) 2019, Pages 51-62



Histogram 1: Revealing MOT values of pyknosis in the liver of the control and infected groups.

Table 2: Showing MOT	values of karyolysi	s in liver of the	e control and	infected a	animals
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	control	Sample 1	Sample 2	Sample 3
Mean	121.433	121.1	156.6	117.5
S.D.	6.88898	7.98927	12.3603	5.72224049
% of change		-0.2745	28.9597	-3.2390924
T test		0.452452	0.000471**	0.194620551

<sup>\*</sup> Significant (P<0.05) \*\* Highly significant (P<0.01)



Histogram 2: Revealing MOT values of karyolysis in liver of the control and infected groups.

Т	Table 3: Showing MOT values of collagen in liver of the control and infected animals						
		control	Sample 1	Sample 2	Sample 3		

	control	Sample 1	Sample 2	Sample 3
Mean	116	160.7333	173.600001	182.366667
S.D.	18.26524	12.33964	21.43667021	17.45797273
% of change		38.56322	49.65517457	57.21264532
T test		0.001377	9.13641E-06	5.96857E-06

\* Significant (P<0.05) \*\* Highly significant (P<0.01)



Histogram 3: Revealing MOT values of collagen in livers of the control and infected groups

# Khadija Abdul Jalil Faddladdeen, 2019

Pharmacophore, 10(2) 2019, Pages 51-62

	control	Sample 1	Sample 2	Sample 3
Mean	84.76667	71.66667	152.266662	51.466669
S.D.	13.28436	14.14292	11.86737444	8.246211521
% of change		-15.4542	79.6303474	-39.28430879
T test		0.224658	1.41053E-06**	5.03962E-06**

Table 4: Revealing MOT values of total protein in liver of the control and infected groups.

\* Significant (P<0.05) \*\* Highly significant (P<0.01)



Histogram 4: Showing MOT values of total protein in liver of the control and infected animals.

Table 5: Showing MOT	values of pol	lysaccharides in	liver of the control	ol and infected animals.
0		2		

	control	Sample 1	Sample 2	Sample 3
Mean	113.6667	80.13334	81.933329	44.866671
S.D.	18.10098	10.42048	15.36319456	9.155987722
% of change		-29.5015	-27.91789259	-60.52785554
T test		0.004832**	0.008914277**	0.000168295**

\* Significant (P<0.05) \*\* Highly significant (P<0.01)



Histogram 5: Revealing MOT values of polysaccharides in liver of the control and infected groups.

Table 6: Showing MO	T values of DNA	contents in liver	r of the control	and infected	animals.

	control	Sample 1	Sample 2	Sample 3
Mean	38.93334	73.83334	77.633336	71.333329
S.D.	9.120252	10.76947	15.35220254	24.44898738
% of change		89.64039	99.40065764	83.21913558
T test		8.79E-06**	0.000120091**	0.001174164**

\* Significant (P<0.05) \*\* Highly significant (P<0.01)

# Khadija Abdul Jalil Faddladdeen, 2019

Pharmacophore, 10(2) 2019, Pages 51-62



Histogram 6: Revealing MOT values of DNA contents in liver of the control and infected groups.

#### Discussion

In the liver, lungs and occasionally other organs of the intermediate host, the larval stages (hydatid cysts) developed. Humans are infected with hydatid cyst when they accidentally ingest *Echinococcus* eggs [31-33].

Eggs of the classic *E. granulosus* hatch to larva called oncospheres. Usually after ingestion, oncospheres passes to the liver by the portal vein and develops one or more cysts in the right lobe liver of 75% of the infected individuals. The cysts grow slowly, about 1 cm a year, and the hydatid cysts are asymptomatic usually discovered incidentally on X-ray.

The hydatid cyst has a histopathological effect on host's organs tissues including granulation around the cyst and a relatively thin fibrous wall, eosinophils are not observed much unless if a cyst has died or ruptured, in that case they may be more evident, along with giant-cell granulomas [5, 34], the effects on the liver are usually same as hepatitis including growing of granulation tissue, eosinophils, and some changes in the cells including cloudy swelling, fatty change and cell necrosis [23, 27].

Normal structure of the liver tissue is observed in the present study, where cords of hepatocytes are radiating from the central vein to the periphery of the lobule, and between the hepatocytes blood sinusoids numerous Kupffer cells are detected. The portal area contains hepatic artery, branches of the hepatic portal vein and bile ducts.

Thin collagen bundles are supporting walls of the blood sinusoids, blood vessels, hepatocytes and bile ducts.

Liver tissue infected with hydatid cyst showed that it consists of outer laminated and inner layer granular layer which is encircled by thick fibrous layer. In the liver tissue infected with the hydatid cyst many dystrophic changes were observed and many authors studied the hydatid cyst in different animals [20, 21, 23- 27, 34].

Ruptured endothelial lining of the hepatic portal vein and highly distorted portal area with thickened arterial wall were also detected. Results of this study are in agreement with those of Ciftcioglu et al., (1997); Stevens et al. (2002); Kumar et al. (2005) and Abd El Hamed, 2009) [20, 23, 28, 35]. Many authors including Kumar et al. (1999); Conway (2002); Stevens et al. (2002); Lucas et al. (2012) and Soaresa, et al., 2013) [23, 27, 34-36] described the histopatholical changes and reaction of the host tissues against hydatid cyst in human's liver; this effect included granulation tissues, eosinophiles, if the cyst has died or ruptured it will be more evident along with giant-cell granulomas in portal the areas with a dense fibrous capsule around the cyst. The present study observed similar results. Also Abd El Hamed (2009) [28] noticed many similar deleterious changes in infected liver of various animals including camels, pigs and sheep. In camels the author noticed widened and destructed hepatic portal vein with highly atrophied bile ducts; also small hemorrhagic areas and complete fibrous layer which encircling the laminated layer of the cyst were detected. In pig granuloma was observed beside the cyst wall with many degenerated changes in the parenchymal cells and highly dilated sinusoidal spaces. Irregular and elongated hepatic portal vein which contained haemolysed blood corpuscles in the highly disturbed portal area was realized. Also disturbed portal area was observed in the liver of infected sheep beside the cyst wall. This portal area contained thickened blood vessels with many hemorrhagic areas in between the hepatocytes. In this respect, coagulative necrosis was noticed in human liver infected with the cyst of E. maltilocularis [20]. Homogenous thin cystic structures of various dimensions with many giant cells at the periphery of the lesion were observed around the cyst, and reported by Kumar et al. (1999); Conway (2002); Stevens et al. (2002) and Lucas et al., 2012) [23, 34-36].

Concerning collagen fibers, dense stain affinity of collagen fibers could be realized in the laminated layer and in the distorted portal area with fibrotic red stained arterial wall, MOT values showed both significant and insignificant increase and reached  $160.73\pm12.33$ ,  $173.60\pm21.43$  and  $182.36\pm17.45$  in infected samples S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub> respectively compared to the non-infected control group  $116\pm18.26$ . The presence of collagen has an important aspect of the host immune reaction against parasite. The obtained results for collagen from present study are similar to Guerret *et al.* (1998) [37] who analyzed collagen and other matrix protein deposits in a study on experimental alveolar echinococcosis in mice liver to establish a relationship between resistance/susceptibility to *E. multilocularis* larval growth and fibrogenesis; they evaluated the collagen in the lesions by using a colorimetric method. Also the nature of matrix proteins involved in the peri parasitic fibrosis was assessed

using immunostaining on tissue sections; progressive and significant increase in the collagen-protein content of the parasitic areas of the infected lobe was observed. Lymphocyte follicles were occasionally observed within the granulomatous infiltration. They confirmed that fibrogenesis is an important aspect of the host immune reaction against parasitic development and that both the extent and the course of matrix protein deposition differ in the liver of susceptible and resistant mice, respectively. They also added that the long-lasting expression of actin and lysis oxidase by host cells in NMRI mice suggested that in this resistant strain. The fibrosis observed in this study is in accordance with the results of the previous study.

Concerning total protein, the granular layer of the hydatid cyst acquired deep blue coloration with less stained laminated layer and poorly to moderately stained hepatocytes; MOT values showed both significant and insignificant decrease with also increase results (71.66±14.14, 152.26±11.86 and 51.46±8.24 in infected samples S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub> respectively) compared to the non-infected control group. The obtained results of total protein are similar to that observed by Conga et al. (2008) [38] who explained that the protein has an important role with other components in providing protection to parasite against the host immune system. Dense stain affinity of total proteins was noticed by Abd El Hamed (2009) [28] in the germinal layer of the cyst of infected camel's liver with less stain affinity in the laminated layer. They explained that this dense stain affinity which was observed in the germinal layer may be due to rough endoplasmic reticulum (RER), ribosome and mitochondria found in the cells of that layer. Decreased stain affinity, which was observed in the laminated layer may be due to the non-cellularity of this layer. Remarkable reaction was detected in the fibrous layer which encircling the cyst wall. Parenchymal cells showed decreased stain affinity compared to the control group. Dense stain affinity of total proteins was observed in the liver of the control pig, highly decreased stain affinity of protein was observed in the liver tissue of the infected pig, but moderate stain affinity was recorded in the germinal layer of the cyst. The laminated and fibrous layers showed less stain affinity compared to the germinal layer. Decreased stain affinity of total protein in wall of the hydatid cyst and the surrounding hepatocytes, disturbed portal areas contained faintly stained blood vessels and bile ducts was observed. Her results showed that the cyst wall in the infected camel's liver showed dense reaction for total proteins and so the surrounding hepatocytes, but the hydatid cyst and hepatocytes of the infected pig's liver showed highly decreased stain affinity of total proteins compared to the hydatid cyst of the camel's liver. The wall of cyst and the liver tissue of the infected sheep liver showed the least stain ability for total proteins. These observations are in agreements with the results of this work.

Infected liver tissue showed deeply stained polysaccharides in the germinal layer, fibrotic areas, walls of the blood vessels and some hepatocytes, while the remnant hepatocytes and the laminated layer were less stained. MOT values showed a significant decrease and reached  $80.13\pm10.42$ ,  $81.93\pm15.36$  and  $44.86\pm9.15$  in the infected samples S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub> respectively compared to the non-infected control group (113.66±18.10). The presence of glycogen serves in giving support and protection to hydatid cyst against the immune system and could eliminate some drugs from reaching the germinal layer [39], that makes the treatment more complicated, and this should be taken in concern.

Moderate reaction for polysaccharides was noticed by Abd El Hamed (2009) [28] in the cyst wall of infected liver of camels and sheep with high polysaccharides content in the laminated and fibrous layers of pig's cysts, but the germinal layer accepted less stain affinity. Nearly most hepatocytes showed decreased stain affinity in the liver of the infected pig. Rashed *et al.* (2004) [24] noticed increased polysaccharides and mucopolysaccharides content in the infected Nagdi sheep.

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Pharmacophore, 10(2) 2019, Pages 51-62

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