

## PATHOLOGICAL AND BIOCHEMICAL STUDY OF ACUTE FASCIOLIOSIS IN CATTLE SLAUGHTERED AT KIRKUK ABATTOIR, KIRKUK, IRAQ

AHMED ABDULLAH SULTAN

Department of Pathology and Poultry Diseases, Faculty of Veterinary Medicine, Tikreet University

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### ABSTRACT

Liver fluke disease (fascioliosis) is an important parasitic disease may accountable for morbidity and mortality in most species of animals, like cattle, sheep and goat as well as other domestic ruminants. It occurs worldwide due to infection with liver fluke species. The study aimed to notice the gross, microscopic lesions and measured serum biochemical changes accompanied with acute bovine liver Fascioliosis of cattle in Kirkuk. From March to April 2018, fifteen infected bovine livers of different ages were collected from Kirkuk abattoir, and examined grossly. A noticeable rise in the level of serum AST, ALP and also ALT were observed. In gross examination several short vermiform cords were observed on the hepatic surface and the surface had a rough appearance. Some immature, wandering flukes were noted on the cut surface. Histopathologically, a wide range of liver lesions were found. The most important lesions were moderate to severe perihepatitis and haemorrhagic tracts on the hepatic surface. These lesions related to migratory channels filled with blood, fibrin and cellular debris. The present study results indicated that serum biochemical alterations were consistent with pathological lesions; hence the analysis of serum biochemical could be used in diagnosis of acute bovine fascioliosis with other tests.

**Key words:** Acute Fascioliosis, cattle, liver flukes, serum biochemical, liver lesions, histopathological examination.

### INTRODUCTION

Liver fluke infection was caused by *Fasciola hepatica* and *F. gigantica* and it remains economically important parasite of livestock and is emergent zoonotic infection. In most mammalian species liver fluke causes morbidity and mortality and by far important in cattle (Hodzic *et al.*, 2013). A study accompanied by Keyyu *et al.* (2006), stated up to 100% liver attack rates in slaughter slabs in Tanzania in cattle. The prevalence of cattle fascioliosis in Ethiopia was as high as 83.08% (Mulualem, 1998). The variant in conditions of climato-ecological such as temperature, rainfall and altitude, and management system of livestock influences the incidence of fascioliosis together with distribution and survival of the parasites as well as snails (the intermediate host). Clinical examination of the infected animal with the *Fasciola* showed anemia or pale visible mucous membrane (Radostits *et al.*, 2017). On post mortem of infected liver with fascioliosis was an irregular outline, firm and pale. Acute fascioliosis is associated with the migrating of immature flukes through the parenchymal liver and make migratory tracts. In this case, grossly the liver becomes haemorrhagic and

enlarged with fibrinous to fibrous exudates on the surface of liver capsule. Several haemorrhagic spots and focal necrosis were set up on the cut surface of parenchymal liver. The tracts of migratory from direct trauma of the *Fasciola* is macroscopically seen as dark acute haemorrhagic lines of typical post necrotic granulation and scarring (Affroze *et al.*, 2013).

Histopathologically the migratory tracts formed by migrating of immature flukes through the hepatic parenchyma were seen as tracts of necrosis (Steyl, 2009). In the liver, the lesions are only partially a result of liver fluke mechanical action, because the liver injury can be induced by *Fasciola* excretory products, decomposed products of *Fasciola*, bile and liver tissue. Hence the tests of serum biochemical including enzymes of serum liver are also supportive to evaluate the severity of injury of hepatic cells and to monitor the evolution of the disease in animals (Lee *et al.*, 2005). The analysis of serum biochemical also used to assess the degree of cholestasis and the capacity synthesizing of the liver (Hodzic *et al.*, 2013). So, the study's objectives were to measure pathological variations of the liver, hepatic lymph nodes and gall bladder; to assess serum biochemical variations associated with acute infection with liver fluke, and to start association between pathological changes and serum biochemical parameters of the cattle liver.

Corresponding author: Dr. Ahmed Abdullah Sultan  
E-mail address: [alsultan5877@tu.edu.iq](mailto:alsultan5877@tu.edu.iq)  
Present address: Department of Pathology and Poultry Diseases,  
Faculty of Veterinary Medicine, Tikreet University

## MATERIALS AND METHODS

### Samples Collection

In this study, fifteen livers of cattle were examined in Kirkuk abattoir. A weekly visit was made to the abattoir at the period of the examination that extended from March to April 2018. The age of animals ranged between months to up 3 year for both sexes.

### Blood samples and Separation of serum

Four hundred blood samples were collected from cattle before slaughtering. Blood sample was collected from the jugular vein in vacutainers without anticoagulant for biochemical analysis including AST, ALP and ALT values were determined by Hitachi 919 (Japan) autoanalyser.

### Pathological examination and sampling of tissue

This study involved collection fifteen (15) samples of bovine livers which were showed sign of infection with flukes were collected from Kirkuki abattoir. This study was carried out from March to April, 2014. During that, fifteen samples from acute bovine liver Fascioliasis at different ages were collected after examination of livers grossly, palpation, incision and noted any changes which detected in the tissue texture of hepatic parenchyma and bile duct. Specimens were fixed with 10% neutral buffered formalin for 72 hours and then routinely processed. These specimens tissues were embedded in paraffin and sectioned at 5µm thickness and stained sections with the Haematoxylin and Eosin stain then examined microscopically and histological features were recorded. The histopathological sectioning and staining by H&E stain was performed in tissue process laboratory, Faculty of veterinary medicine, university of Tikrit.

### Statistical analysis

The Statistical Analysis System-SAS (2012) program was used to explain the differences factors in this study parameters. For significant comparison between percentages, Chi-square test has been used to explain results of this study.

## RESULTS

### a) Pathological Lesions

In grossly examination several short vermiform cords were illustrious on the liver surface and the surface had an uneven appearance (Fig. 1). A numerous of

immature, wandering flukes were noted on the cut surface (Fig. 2). Migrational tracks were randomly distributed through all areas of the livers, then were most common in the central and left lobes. Color progressively changed from brown-red in the smallest tracks to gray surrounded by a red zone of hyperemia in the larger ones. Tense of the liver capsule with fibrous exudates on the liver capsular surface.

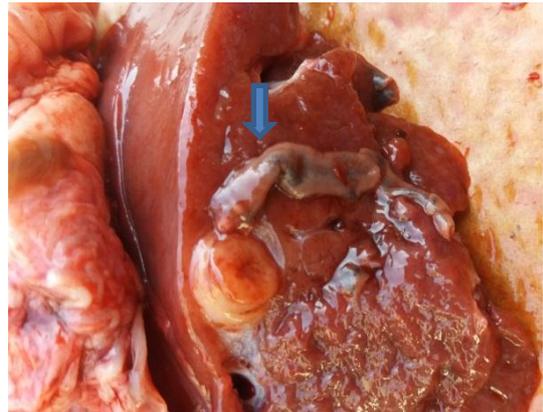
Histopathologically, a wide variety of hepatic lesions were noted. The most important lesions were moderate to severe perihepatitis and haemorrhagic tracts on the liver surface (Fig. 3). These lesions related to migratory channels filled with blood, fibrin and cellular debris (Fig. 4). Migrational tracks were mostly composed with eosinophilic debris of hepatocytic disintegrated and infiltration and aggregation of many eosinophils and neutrophils, macrophages with pigment of haemosiderin and lymphocytes and fewer plasma cell (Fig. 5). Frequently tissue elements surrounding the tracks were affected by a evident coagulative necrosis. (Fig. 6) the neighboring portal areas and the congested sinusoids were plentifully infiltrated by eosinophils, lymphocytes, and macrophages (Fig. 7). Blood vessels thrombosis was frequent in areas adjacent to migrational tracks (Fig. 8). Mural thrombi were less common in arteries than in veins. Central veins thrombosis in migratory areas was moderately general. Arteritis and phlebitis were recurrent in the migratory areas and not necessarily attendant with thrombosis. The biliary tract were less affected than the parenchymal liver in acute form of the *Fasciola* infection. Multifocal hepatitis & the necrotic lesions with deep eosinophilic cytoplasm, karyorhexis and karyolysis were observed (Fig. 9 & 10). There were also mild fibrosis and proliferation of bile duct and distortion of the hepatic cords in some areas (Fig. 11 & 12). Also, congestion with extensive focal necrosis in parenchyma & the degenerative changes characterized by vacuolation of the hepatocytes were noted particularly around central vein (Fig. 13).

### b) Serum Biochemical Analysis

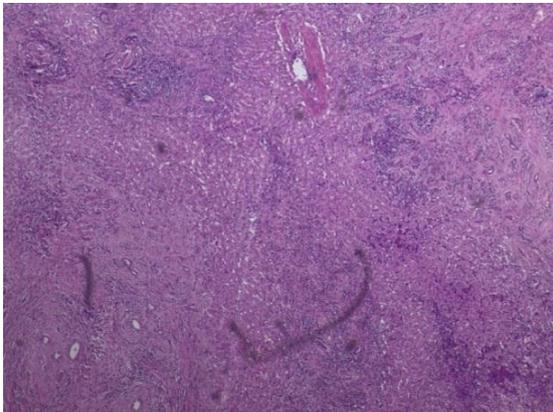
Parameters of serum biochemical were compared among animals without visible gross lesion and no *Fasciola* (group A) and animals with acute fasciolosis (group B), When comparing mean values with that of group A, animals with acute fasciolosis have significantly raised activity of serum ALT, ALP & AST (Fcalcul.>Ftabul.; P<0.05).



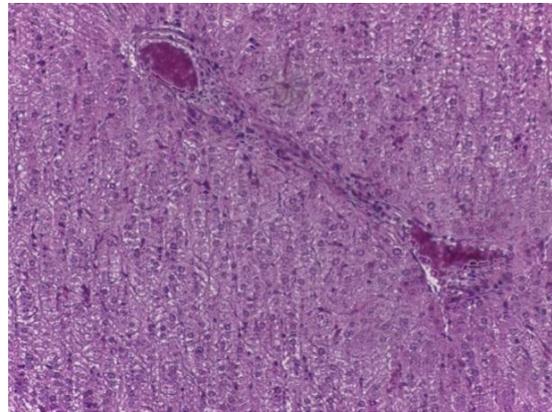
**Figure 1:** Numerous short vermiform cords on the liver surface with uneven appearance (arrows).



**Figure 2:** Immature, wandering flukes on the cut surface (arrows).



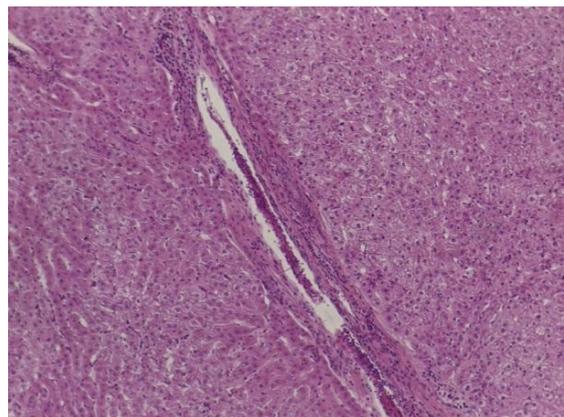
**Figure 3:** Liver of Cattle showed moderate to severe perihepatitis and haemorrhagic tracts on the liver surface.



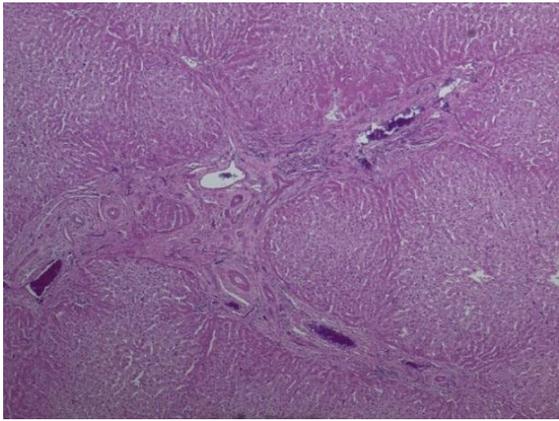
**Figure 4:** Liver of Cattle showed migratory channels filled with blood, fibrin and cellular debris.



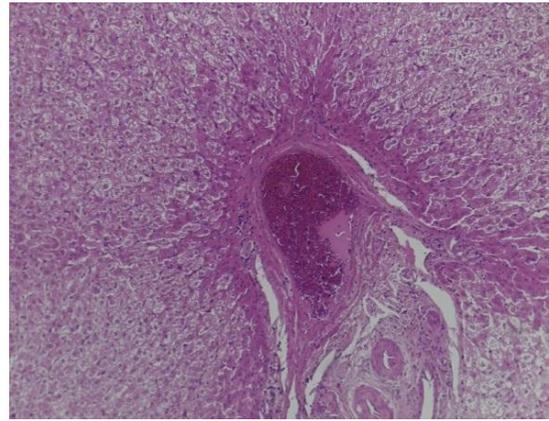
**Figure 5:** Liver of Cattle showed migrational tracks were mostly composed of eosinophilic debris of hepatocytic disintegrated and infiltration and aggregation of many eosinophils and neutrophils, macrophages with pigment of haemosiderin and lymphocytes and fewer plasma cell



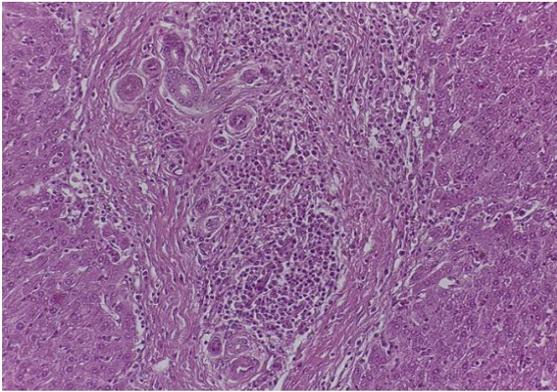
**Figure 6:** Liver of Cattle showed tissue elements surrounding the tracks were affected by a evident coagulative necrosis



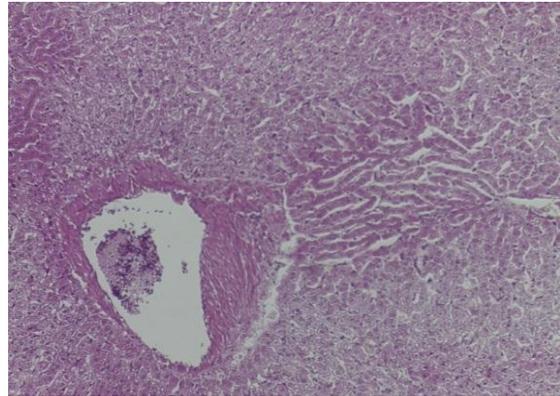
**Figure 7:** Liver of Cattle showed The neighboring portal areas and the congested sinusoids were plentifully infiltrated by eosinophils, lymphocytes, and macrophages.



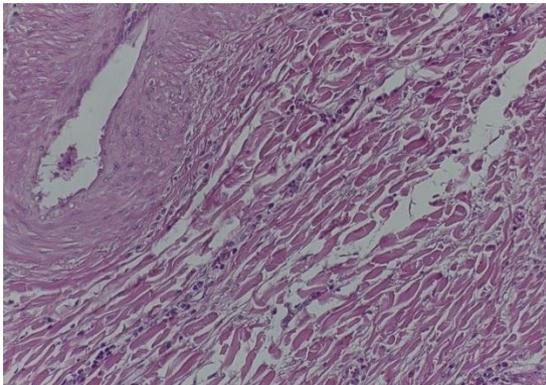
**Figure 8:** Liver of Cattle showed Blood vessels thrombosis was frequent in areas adjacent to migrational tracks.



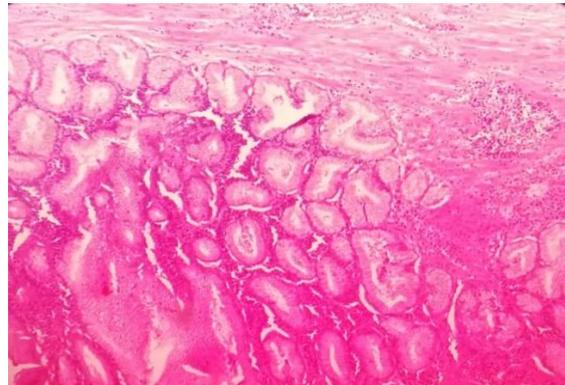
**Figure 9:** Liver of Cattle showed Multifocal hepatitis & the necrotic lesions with deep eosinophilic cytoplasm, karyorhexis and karyolysis were observed



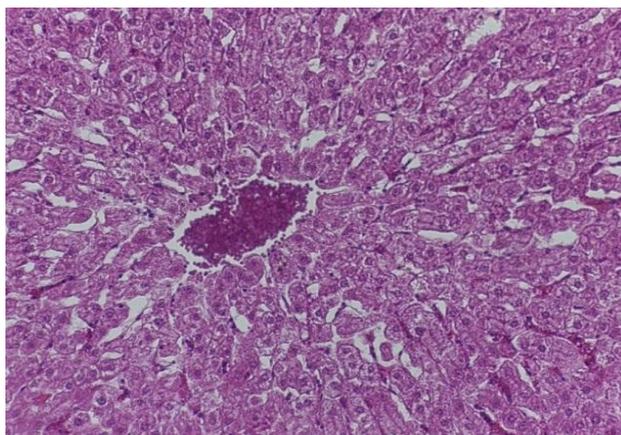
**Figure 10:** Liver of Cattle showed Multifocal hepatitis & the necrotic lesions with deep eosinophilic cytoplasm, karyorhexis and karyolysis were observed.



**Figure 11.** Liver of Cattle showed mild fibrosis and proliferation of bile duct and distortion of the hepatic cords in some areas



**Figure 12.** Liver of Cattle showed mild fibrosis and proliferation of bile duct and distortion of the hepatic cords in some areas



**Figure 13:** Liver of Cattle showed congestion with extensive focal necrosis in parenchyma & the degenerative changes characterized by vacuolation of the hepatocytes were noted particularly around central vein

**Table 1:** Comparisons of some serum liver enzymes among group-A/controls, group-B/ infected Acute Fasciolosis (Mean±S.E).

Groups	Lesion condition	AST(U/L)	ALT(U/L)	ALP(U/L)
Group A	No Visible Lesion	56.78 ± 1.08	14.7 ± 0.51	147.99 ± 4.11
Group B	Acute Fasciolosis	74.00 ± 12.40	21.30 ± 7.74	277.45 ± 90.55

## DISCUSSIONS

According to Adrien *et al.* (2013), there is enlargement of liver in response to acute inflammation as the traveling juvenile fluke mechanically injures the parenchyma. Molina *et al.* (2005), also reported at the cut surface, the liver was irregular, firm & edematous with hemorrhagic channels and adhered fibrosis in the liver parenchyma & also fibrinous tags on the capsule & exudates between organs. These authors defensible fibrinous tags on the capsule are due to reaction of capsule to the flukes penetrating it. Supporting the current finding Adrien *et al.* (2013) and Kitila & Megersa (2014), stated the hepatic and mesenteric lymph nodes are reactive, enlarged and they oozed edematous material up on opening. Liver abscesses are usually caused by bacterial infections and subsequent lysis of the neutrophils & are then surrounded by a fibrous capsule. Therefore, multiple soft liver abscesses in our study might be resulted from secondary complication of the bacteria. On the other hand, this may be due to response of the host against fluke infection and continuous mechanical irritation along with parenchymal damage accompanied by severe haemorrhagic lesions & immunological reactions (El-Hallawany and Abdel-Aziz, 2012). Also, invasion of the liver by migrating immature liver fluke injures the tissue and results in decrease of the oxygen tension (anaerobic condition), that allowable the germination & proliferation spores of Closteridia with associated release of its toxins and prompt hepatocellular necrosis (Jones *et al.*, 1997; Sayed *et al.*, 2008). In our present study, the histopathological investigation also exposed the presence of numerous eosinophils mixed with few lymphocytes & accompanied by hemorrhage &

edema in acute fasciolosis. These lesions somewhat correlated with the results of Talukder *et al.* (2010), from liver of goats and completely agreed with state of Borai *et al.* (2013) and Kitila & Megersa (2014). Eosinophilia happens due to sensitivity to the parasitic foreign protein, which may be a part of immune phenomena. Therefore, eosinophilia is likely to be seen when flukes are migrating through the tissue in large animals (Kerr, 2002; Borai *et al.*, 2013). Edema & excessive fluid in interstitial space of hepatocyte may be due to reduction in plasma colloidal osmotic pressure as a result of hypoalbuminemia. In this study the acute cases also shown dilated blood vessels with disorganized & distortion of the hepatic cords while the hepatocyte revealed variable degrees of necrosis & degeneration, which are in agreement with Mac Gavin *et al.* (2017), Sayed *et al.* (2008), Sohair and Eman (2009) and Kitila & Megersa (2014). These authors support the consequence as it might be due to mechanical and toxic effects of liver fluke plus immunological reactions affecting the complex vascular & biliary systems in the liver accompanied by vascular obstruction and distension. In some areas of biliary tract proliferation and mild fibrosis in acute fluke infection of our study might show the previous infection as asymptomatic infection can occur. Adrien *et al.* (2013), stated extensive damage of liver with the hemorrhagic dark red tracts of necrotic hepatic parenchyma, presence of immature flukes & thrombosis of the hepatic vessels through the migratory stage are constant features of the liver fluke infection in all species, & thrombosis happens over areas of localized phlebitis formed by fluke migration.

## a) Serum Biochemical Analysis

Fasciola causes the release of reactive oxygen types that produce destruction to cell wall & hepatic tissue necrosis. These changes have an effect on biochemical parameters in serum including liver specific enzymes (Hodzic *et al.*, 2013). There were differences in activities of the liver enzymes like AST, ALT, GGT, ALP, LDH & GLDH in serum are usually show pathological alterations of tissue & organ (Tanritanir *et al.*, 2009). In agreement to these reports, the results of the present study revealed a variation in ALT, AST & ALP values among cattle affected by acute fasciolosis in comparisons with animals with no fasciolosis (Tables:1). When the liver is injured or damaged, the liver enzymes leak into the blood, causing raises of serum liver enzyme (Mert *et al.*, 2006) and Kitila & Megersa (2014). Different mean values of AST, ALT & ALP were also measured may arise from physiological adaptations, growth effects, maturation of metabolic pathways, body composition, & or nutrition (Sharon, 2013). Also, in "Overview of hepatic disease in large animals" these authors stated the levels of serum of liver enzymes differ even with ages, breeds & sexes. In current study, the mean value of serum liver AST & ALT were adequately higher ( $P < 0.05$ ) with acute lesions like degeneration of hepatocyte, telangiectasis & eosinophilic hepatitis which are in agreement with reports of (Yasuda, 1988), in large animals, serum concentrations of liver enzymes like transaminases are usually higher in acute liver disease than in chronic liver disease. This increase of serum transaminases at the early stage of the infection could be connected to the hepatocellular necrosis & degenerative changes created by migrating juvenile flukes through the hepatic parenchyma (Dias, 1996). The raise of serum AST & ALT activity in affection with hepatic parenchyma than in biliary tract destructs in our study also partly agreed with the results of Al-Quraishy and Al-Moussawi (2001). AST is current in both the cytoplasm & mitochondria of hepatocytes and will raise together with ALT in states of changed membrane permeability (Yasuda 1988), however the mitochondrial AST isoenzyme is fewer likely released with most of the conditions which result in raised membrane permeability (Kerr and Steiner, 2012). ALT is current in high concentration in the cytoplasm of hepatocyte & is considered to be liver exact in small animals & ruminants like camel. Its plasma concentration rises with hepatocellular degeneration or damage/necrosis and hepatocyte proliferation (Hodzic *et al.*, 2013). The previous findings of Mbuh and Mwaye (2005), similarly detected the increase of ALT in fasciolosis may be due to destruction of hepatocyte since ALT is mainly found in the hepatic parenchyma. On the other hand, the elevation in mean value of ALT in this study may be due to death of hepatocyte from liver fluke infection causing complete or partial obstruction of bile ducts and then recurring of bilirubin to hepatocyte (Dias, 1996; Kilad *et al.*, 2000).

According to Adama *et al.* (2011) and Kitila & Megersa (2014), serum ALT remain elevated for several days after acute liver fluke infection indicating epithelial injury in the bile ducts which agrees with raised serum ALT in hyperplastic cholangitis in our study. So, in hyperplastic cholangitis AST & ALT raise may be due to damage of parenchyma as a secondary effect of cholestasis (Salem and Hassan, 2011) and Kitila & Megersa (2014). Attributing to the report of Kocatepe (2012) and Kitila & Megersa (2014), elevated serum ALP was though, also recorded with necrosis of hepatocytes in this study; so, hepatic ALP can rise following necrosis of hepatocytes due to secondary intrahepatic biliary obstructions and as part of the nodular regeneration process.

## CONCLUSION AND RECOMMENDATIONS

This study attempted to assess the lesions of liver and serum biochemical variation that accompany liver fluke infection in cattle. Grossly livers were: pale, irregular in outline, firm and tough in consistency. On the other hand, multifocal eosinophilic hepatitis, telangechthisand different necrosis of hepatocyte and degeneration were histopathologic changes detected in acute cases.

Concentration of analysis of the serum liver enzyme was moreover used to evaluate liver fluke infection and showed significant raise of serum AST and ALT with acute lesions of parenchyma. Based on our study the following recommendations were forwarded: In these study results of serum biochemical deviations were also constant with pathological findings and so, the analysis of serum biochemical could be used as complementary in diagnosis of cattle hepatic fluke infection. In the study we used cattle with no hepatic fluke and no gross hepatic lesions at all, as control groups for comparisons serum biochemical but added experimental studies with case-control should be done to establish better association between liver pathological alterations and serum biochemical variation.

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## دراسة مرضية وبيوكيميائية عن مرض ديدان الكبد الحاد في الماشية المذبوحة في مسلخ كركوك ، كركوك ، العراق

أحمد عبد الله سلطان

قسم أمراض الامراض وامراض الدواجن ، كلية الطب البيطري ، جامعة تكريت

E-mail: alsultan5877@tu.edu.iq Assiut University web-site: [www.aun.edu.eg](http://www.aun.edu.eg)

مرض ديدان الكبد (اللفافة) هو مرض طفيلي مهم قد يكون مسؤولاً عن الإصابة والنفوق في معظم أنواع الحيوانات ، مثل الأبقار والأغنام والماعز وكذلك الحيوانات المجترة الأخرى. يحدث في جميع أنحاء العالم بسبب العدوى بأنواع ديدان الكبد .. تهدف الدراسة إلى ملاحظة الآفات العيانية والمجهريّة والتغيرات الكيميائية الحيوية المصلية المقاسة المصاحبة لأصابه الأبقار بالنوع الحاد في اللفافة في الماشية في كركوك. خلال الفترة من مارس إلى أبريل ٢٠١٨ ، تم جمع خمسة عشر كبداً من الأبقار المصابة من مختلف الأعمار من مسلخ كركوك ، وتم فحصها بدقة. لوحظ ارتفاع ملحوظ في مستوى مصل AST و ALP وكذلك ALT. في الفحص الإجمالي ، لوحظت العديد من الحبال القصيرة على السطح الكيدي والسطح كان له مظهر تقريبي. ولوحظ بعض الديدان تجول على سطح الكبد ، تم العثور على مجموعة واسعة من آفات الكبد. وكانت أهم الآفات معتدلة إلى حادة التهاب الكبد ونزيف على سطح الكبد. هذه الآفات المتعلقة بقتوات الهجرة المملوءة بالدم والفيبرين والحطام الخلوي. أشارت نتائج الدراسة الحالية إلى أن التغيرات الكيميائية الحيوية في المصل كانت متوافقة مع الآفات المرضية. وبالتالي يمكن استخدام تحليل الكيمياء الحيوية في الدم في تشخيص اللفافة البقرية الحادة مع اختبارات أخرى