Research Article

Biochemical and Histopathological Changes Associated with *Fasciola* spp. in Slaughtered Buffaloes at Al-Muthanna Province, Iraq

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Abstract | The aim of this study was to determine the biochemical changes in the infected buffaloes with fasciolosis. Also, the current study recognized the gross and histopathological changes resulting from the invasive *Fasciola spp.* in the liver and gallbladder. The biochemical results showed statistically significant increases in the serum total bilirubin, creatinine, and active serum enzymes ALT, AST, ALP, and GGT. In contrast, the serum total albumin showed a significant decrease in infected buffaloes. Moreover, the results of the gross pathological examination revealed that cirrhosis and paleness of the liver with multiple abscesses appearing as pale necrotic areas, as well as thickness and calcification with fibrinous exudates of bile ducts, were the most frequent gross lesions. Meanwhile, the histopathological examination showed hyperplasia, fibrous proliferation in the portal area and coagulative necrosis of hepatocytes with large numbers of inflammatory cell infiltration such as eosinophil, Kupffer cell and scattered lymphocytes in the area previously migrated by young flukes as well hepatocytes displayed a fatty change with dilated hepatic sinusoids. Furthermore, our findings show that the bile ducts have hyperplasia, which is associated with fibrous cholangitis and an abundance of inflammatory cells with accumulated bile pigment (hemosiderin) in the dilated bile ducts.

Keywords | Biochemical, Histopathological, *Fasciolosis* in *Bubalus bubalis*

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INTRODUCTION

Water buffaloes are physiologically and anatomically adapted to survive rivers, marshes, and areas subjected to flooding. Thus, they are most well suited for the rivers and marshes, particularly in the Baghdad countryside and the southern marshes in Basrah, Missan, and Thi-Qar provinces of southern Iraq (AlSaedy, 2007). Besides, buffaloes play an important role in the lives of people who live along the marshes of central and Southern Iraq because they are primarily dairy animals that produce thick butter fat and are never used for work, have high production rates in milk and meat conversion from low-quality forage (Minervino et al., 2020). Fasciolasis is one of the most important parasitic zoonotic diseases in the world, posing a serious health threat and causing enormous economic losses in livestock industries through death of infected animals, decrease in production, growth retardation, condemnation of affected livers, costs of treatment, and expense for control measures in ruminants caused by endoparasitic trematodes of the genus *Fasciola,* which belongs to the Phylum Platyhelminthes Class Trematoda, with two important species, *F. hepatica* and *F. gigantica* (Al-Sultan et al., 1999; Abdulwahed and Al-Amery, 2019). Moreover, Fasciolasis has a wide geographic spread as food-borne zoonotic disease. *F. gigantica* is responsible for the large liver fluke in tropical areas, which is particularly prevalent in Asia and Africa due to the wide distribution of its intermediate host

Lemnia natalensis. while, *F. hepatica* is found in temperate regions like Europe, Americas and Australia, with limited distribution of its intermediate host *Lemnia truncatula* (Kanyari et al., 2010; Hassone and Salah, 2019). On the other hand, the invasive or acute stage, the prepatent stage, and the chronic or obstructive stage are the three clinical manifestations of Fasciolasis in animals (Mikaeel, 2020). Acute Fasciolosis result into high mortality especially in sheep, but sub-acute and chronic Fasciolasis cause fever, anemia, icterus, bottle jaw and reduction in production and fertility (Anne and Gary, 2006). A variety of laboratory tests based on biochemical analysis of serum parameters are available includes determination of serum alanine transaminase, aspartate transaminase (ALT, AST), which are the most sensitive indicators of hepatocellular injury. The degree of cholestasis and the liver's synthetic capacity are also assessed by using Alkaline Phosphatase (ALP), gamma-glutamyl tranferase (GGT), and bilirubin (Okoye et al., 2013; Oleiwi et al., 2017).

MATERIALS AND METHODS

Study area and sample collection

The study was conducted randomly on buffaloes slaughtered at the abattoirs of Rumaitha and Samawah in Al-Muthanna province, south of Iraq. The sample collection time was extended from March 2022 to December 2022. Three times a week, the abattoirs were visited, and the number of buffaloes slaughtered range from 2 to 5 every day. Visceral organs infected with Fasciolosis were put in plastic bags and were brought to Parasitology Laboratory, College of Veterinary Medicine, University of Al-Muthanna to identify any gross pathology changes by visualization and palpation by making numerous incisions in various parts to confirm the presence of *Fasciola spp*. in the liver and gall bladder.

Blood samples and biochemical examination

Blood samples were drawn from two groups of buffaloes from different age groups and both sexes. Fasciolosis-infected buffaloes make up the first group, while the second group includes healthy buffaloes as a control group. Before slaughtering buffaloes, blood samples were collected by puncturing of the jugular vein, after cleaning and disinfectant the area by the using ethyl alcohol (70%) through using sterilized 10 ml syringes. The blood samples were put in tubes without EDTA and send directly to laboratory by cooled box to separate the sera according to the method previously described (Singh et al., 2011), and stored at -20 °C in Eppendorf tubes for subsequent biochemical tests including total Bilirubin(TB), Creatinine (Cr), Total Albumin (ALB), alanine transaminase (ALT), aspartate transaminase (AST), Alkaline Phosphatase (ALP) and Gamma-Glutamyl Transferase (GGT), were estimated using commercial kit provided by BIOLABO (France).

Histopathological examinations

After recording the gross pathology changes, a pieces of liver and gall bladder from the infected buffaloes with *Fasciola* spp. were collected and wash several times with normal saline (0.9%) and then place immediately into 10% formalin for histopathological examination. The histopathological sections were processed according to (Al-Kubaisee et al., 1999).

RESULTS AND DISCUSSION

The biochemical results showed statistically significant increases in the serum total bilirubin, creatinine and active serum enzymes ALT, AST, ALP and GGT when compared to healthy control animals. In contrast, the serum total albumin showed significant decrease in infected buffaloes during the period post infection, as shown in the Table 1. The biochemical results of the current study affirmed many previous studies by Hashem and Mohamed (2017), Nasreldin and Zaki (2020), Brahmbhatt et al. (2021), concerning effects of Fasciolosis on serum total Bilirubin, Albumin, Creatinine level and active serum enzyme ALT, AST, GGT and ALP during the period post infection. But our results study were conflict with the findings obtains by Rajamanickam et al. (1987), Matanović et al. (2007), who found a significant decrease in biochemical parameters in infected animals such as creatinine and active serum enzymes AST and ALP during the post-infection period, as well as disagreements with the findings of Ganguly et al. (2016), that found a significant increase in the albumin level of infected animals. This variation might be attributed to the migration of juvenile flukes through the liver parenchyma, which causes disruption of hepatocellular integrity, fibrosis, and necrosis, thus, enzymes from damaged cells are released into the blood and their concentration increases in serum AST and ALT in the infected animals (Brahmbhatt et al., 2021). Also, the cellular changes from parasitism increased the permeability of the hepatic cells, which in turn resulted in the release of the enzymes into the serum (Hashem and Mohamed, 2017). In addition, the serum GGT levels found in our study is an indicator of chronic changes, cholestasis, and epithelial damage in bile ducts that may be due to the presence of adult flukes in biliary tract (Hodzic et al., 2013). Our findings, on the other hand, revealed a significant increase in ALP, which is indicative of parenchymal and hepatobiliary damage or obstruction of the hepatobiliary system and may be caused by liver flukes present within the biliary network, resulting in hyperplastic obstructive and cholangitis (Edith et al., 2010). While the decrease in albumin concentrations can be attributed to either the fluke's loss in the gastro-

intestinal tract via the bile duct or the parasite's destructive effects on hepatic parenchyma caused by migratory flukes, which impair liver function and insufficiencies the ability to retain and use nitrogen and produce albumin (Oleiwi et al., 2017), In contrast, elevations of creatinine levels in our study may be attributed to renal problems according to the previous study by Nasreldin and Zaki (2020), who reported that glomerulopathy is associated with fasciolosis, which may be due to the deposition of granular and pesudolinear immunoglobulin (IgG) in the mesangial region of the glomeruli that results in membranoproliferative and mesangioproliferative glomerulonephritis. Furthermore, a significant increase in bilirubin was found in our study, which could be caused by bile duct obstruction, which causes bilirubin to return to the hepatocyte and then increase in serum (Kiladze et al., 2000).

Table 1: The effects of Fasciolosis in buffaloes on some biochemical parameter by T test (Mean±SE).

Biochemical parameters	Group	Mean ± SE	P value
Albumin (g/dl)	Control	3.395±0.009*	0.00
	Infected	2.899±0.045	
Creatinine (mg/dl)	Control	0.740±0.107	0.00
	Infected	1.841±0.032*	
Bilirubin TSB(mg/dl)	Control	0.201±0.026	0.05
	Infected	1.310±0.069*	
AST/GOT (IU/L)	Control	101.916±0.340	0.00
	Infected	190.304±3.219*	
ALT / GPT (IU/L)	Control	20.508±0.166	0.04
	Infected	77.214±8.605*	
ALP (IU/L)	Control	118.303±0.147	0.03
	Infected	181.230±0.298*	
GGT (IU/L)	Control	26.317±1.155	0.00
	Infected	71.281±0.849*	
No. of animals	Control	15	*= p ≤0.05
	Infected	30	

On the other hand, the results of gross pathological examination showed that the most commonly found gross lesion was cirrhosis, paleness of the liver, and thickness of the bile ducts associated with the presence of an adult parasite, as shown in Figure 1. Besides, the liver were appear to be tougher with irregular surface due to parenchymal congestion with multiples abscesses formation appear as a pale necrotic areas, as showed in Figure 2. While advanced lesions lead to nodule formation and fibrosis, as well as enlargement, thickening, and calcification of the bile duct walls, all these changes have been noticed in buffaloes infected with chronic fasciolosis, as seen in Figure 3. Besides, the gallbladder was congested and dilated, full of dense matter of bile juice, as showed in Figure 4.

Figure 1: Gross examination of infected liver with Fasciolosis showed cirrhosis and paleness of the liver and enlargement of bile ducts.

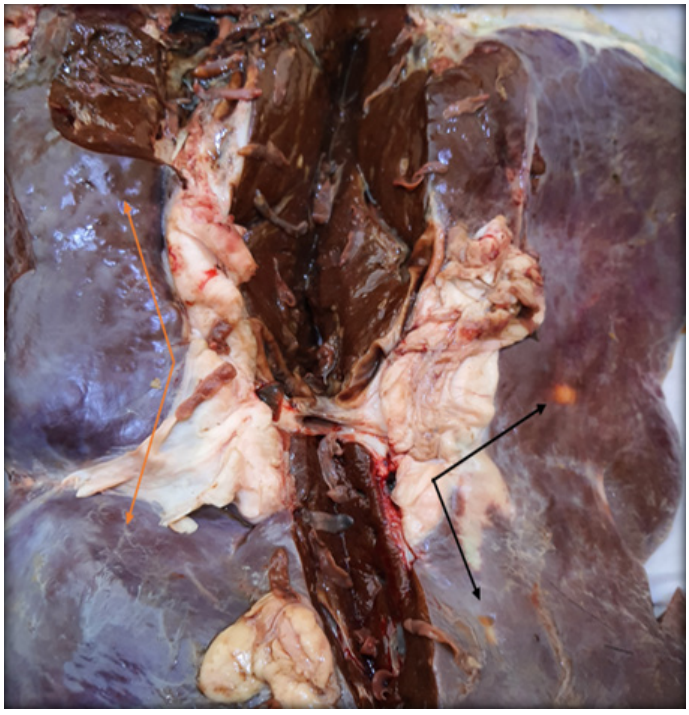

Figure 2: Gross examination of infected liver with Fasciolosis showed congestion with multiples abscesses formation on the hepatic capsule.

Furthermore, *Fasciola* spp. infection caused varying degrees of histopathological changes in infected buffaloes liver and gallbladder. The liver histopathological changes in the present study included hyperplasia, fibrous proliferation in the portal area, and coagulative necrosis of hepatocytes with large numbers of inflammatory cell infiltration, such as

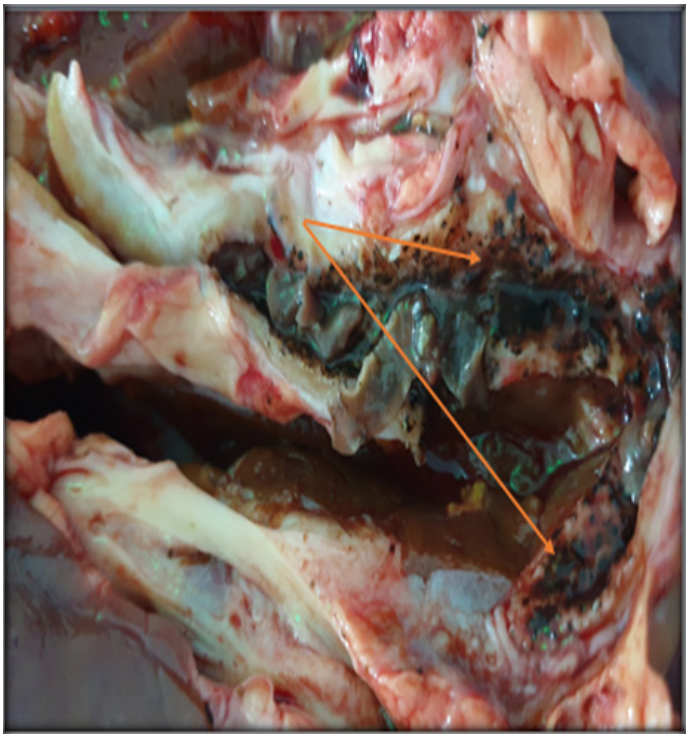

Figure 3: Gross examination of infected liver with Fasciolosis showed Calcified of the bile ducts and fibrinous exudates.

Figure 4: Gross examination of infected gallbladder with Fasciolosis showed congested and dilated full of density matter of bile juice.

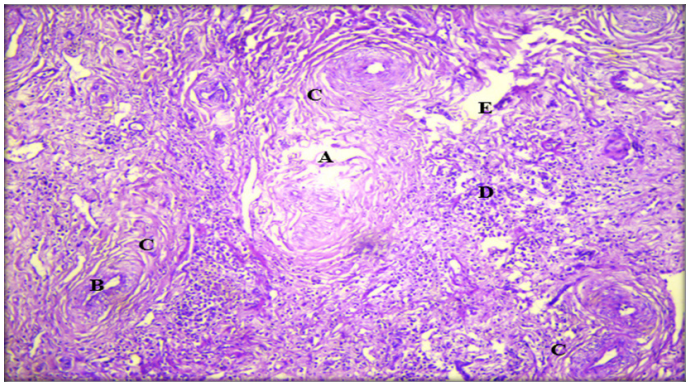

Figure 5: Histopathological section of liver showing; A: destruction of liver tissue; B: thickening of bile duct; C: fibrosis proliferation in the portal area; D: coagulative necrosis of liver cell; E: parasitic tract formed from destructed hepatocytes intermixed with infiltrated inflammatory cell (H & E stain. X10).

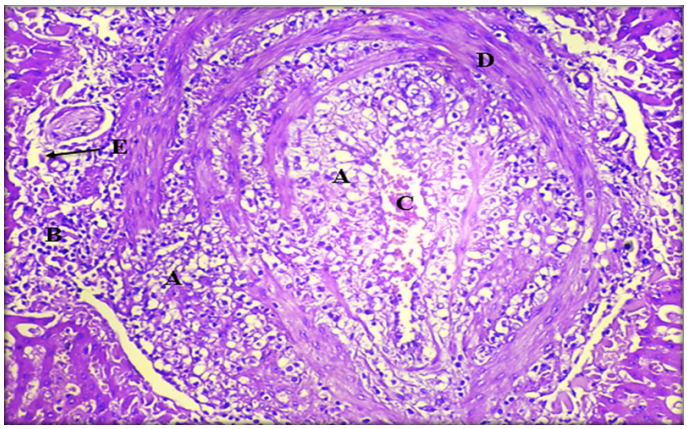

Figure 6: Histopathological section of liver showing; A: fatty change; B: coagulative necrosis; C: hemorrhage; D: fibrous connective tissue capsule; E: Kupffer cells (H & E stain. X20).

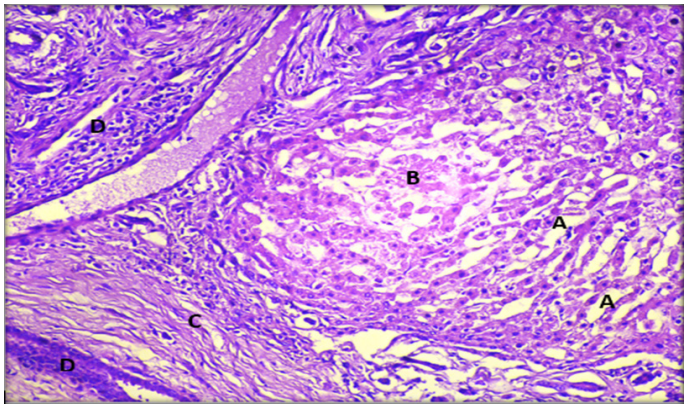

Figure 7: Histopathological section of liver showing; A: dilated sinusoids; B: coagulative necrosis of hepatocytes; C: fibrosis of portal area; D: migratory tract with inflammatory cell infiltration. (H & E stain. X20).

eosinophils and macrophages, with scattered lymphocytes in the area previously migrated by young flukes, as shown in Figure 5. In addition, hepatocytes displayed a fatty change that was distinguished from other hepatocytes by separate vacuoles that developed in the cytoplasm and had nuclei that were placed peripherally, as showed in Figure 6. Besides, hepatic sinusoids dilated as a result of their cell walls

degenerating, nuclei deforming, and cytoplasmic contents leaking into the sinusoids as showed in Figure 7. Moreover, the histopathological examination showed that the hepatocytes were hypertrophied (swollen), which is demonstrated by an increase in size, opaque cytoplasm, and centrally located nuclei. It was also accompanied by periportal vein cellular infiltration, which was primarily composed of eosinophils, Kupffer cells, and lymphocytes, as shown in Figure 8. In addition, our results revealed that the bile ducts displayed hyperplasia, which is accompanied by fibrous cholangitis and an abundance of inflammatory cells; as well, bile pigment was accumulated as yellowish brown concretions in the dilated bile ducts (hemosiderin pigmentation), as seen in Figure 9. In contrast, histopathological lesions of the gallbladder showed the same features already described for the liver lesions. Various lesions were found during the histopathological investigation of the gallbladder that had *Fasciola* spp. infection, such as epithelial necrosis, hemorrhage, inflammatory cell infiltration, and hypertrophy and hyperplasia of the gallbladder's epithelium as showed in Figure 10.

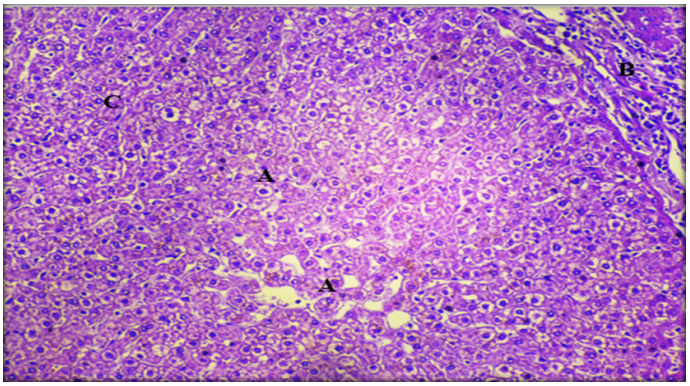

Figure 8: Histopathological section of liver showing, A: hypertrophy of hepatocytes; B: fibrosis of portal area with infiltration inflammatory cells; C: kupffer cells (H & E stain. X20).

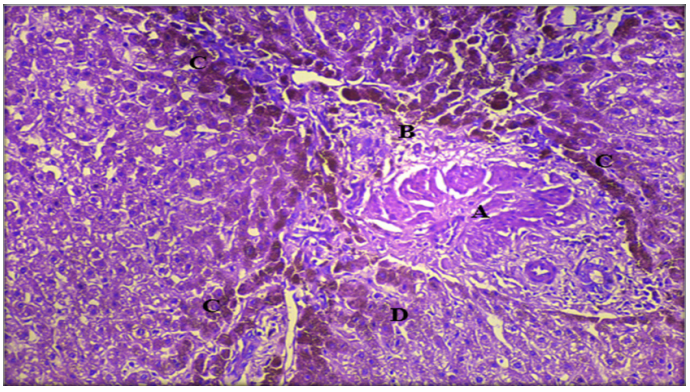

Figure 9: Histopathological section of liver showing A: hyperplasia of bile duct which proliferation of the epithelial lining as gland, B: fibrosis of portal area with infiltration inflammatory cells, C: hemosiderin (Yellowish brown materials accumulated among hepatocytes in bile canaliculi, D: kuffper cells (H & E stain. X20).

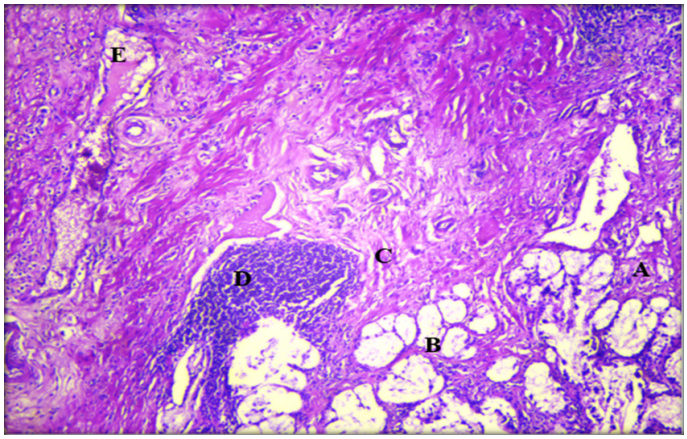

Figure 10: Histopathological section of gallbladder showing A: necrotic tissue; B: destruction of glands; C: fibrosis of mucosa; D: aggregation of lymphocytes cells and E: hemorrhage (H & E stain. X10).

The histopathological findings in the current study were similar to those in the study conducted by Kovorodin et al. (2021), who reported that the histopathological changes in the liver infected with fasciolosis showed atrophy and mild hepatocellular degeneration in the liver tissue around the capsule, infiltration of eosinophils, fibroblasts, and lymphocytes, and dilatation in the sinusoids. The main histopathological changes might be due to the juvenile flukes migrating through the liver capsule and hepatic tissue, which results in necrosis, hemorrhages, fibrosis, and cirrhosis in the liver, or the chemical effects either by these parasites or by the hosts' inflammatory and immune responses, which involve a strong Th2 response, which in turn is associated with the production of cytokines, thereby contributing to the overall pathophysiological condition (Al-Mahmood et al., 2017). Furthermore, the atrophy, necrosis, and fatty changes in the liver tissue may be because of the digestion of the host components by the flukes through the release of proteases, which facilitates their migration, feeding, as well as immune evasion (McGavin et al., 2001). Also, the infiltration of inflammatory cells may occur either due to the migration of immature liver flukes or due to the release of proteases by the flukes, which cause continuous irritation, that leads to hemorrhages and the infiltration of inflammatory cells (Okoye et al., 2015). In addition, the results of the bile duct histopathological examination were similar to those obtained by Talukder et al. (2010), who noted epithelial hyperplasia, ductal fibrosis, and inflammatory cell infiltration with eosinophils, macrophages, and lymphocytes. These lesions might be because suppressing the early peripheral inflammatory response and delaying the hepatic inflammatory response following infection, juvenile flukes change the immune response (Sadeghi-Hashjin and Naem, 2001). On the other hand, the histopathological changes of the gallbladder isolated of the buffaloes infected with *Fasciola spp.* were in agreement with those reported by Kardena et al. (2017),

who revealed that the most common pathological changes were epithelial necrosis, hemorrhage, inflammatory cell infiltration, and hyperplasia of the epithelium of the gall bladder. These lesions may be caused by the migration activity and spiny tegument of *Fasciola* spp. which can irritate the mucous membranes of the gall bladder and is considered to be an indication of a chronic trauma because persistent worms sucking blood at the same location may lead to blood vessel ruptures, which result in bleeding and tissue damage (Lotfollahzadeh et al., 2008). Moreover, the increase thickening the mucous membranes in the lamina propria of the gallbladder which may be associated with the stimulation of proline secreted by worms *Fasciola* spp. (Kardena et al., 2017).

CONCLUSIONS AND RECOMMENDATIONS

According to the data, the present study concluded that fasciolosis is an important veterinary disease that infects buffaloes and causes a significant economic loss. Also, this study revealed that infected buffaloes with fasciolosis experience significant changes in their biochemical parameters. Furthermore, the study found that fasciolosis infection resulted in a high level of pathological changes in the liver and gallbladder.

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NOVELTY STATEMENT

The novelty of the study is focus on determining the biochemical changes in the infected buffaloes with fasciolosis and also, recognized the gross and histopathological changes resulting from the invasive Fasciola spp. in the liver and gallbladder, thus, they pose serious health risk and has a significant negative economic impact on the livestock industry due to animal deaths, decreased production, growth retardation, liver damage, treatment costs, and the high cost of control measures.

AUTHOR'S CONTRIBUTION

All authors contributed equally.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

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