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Physiological and histological alterations in laboratory rabbits infected with *Necator americanus* (Nematoda: Ancylostomatidae)

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ABSTRACT

The present study was conducted for the period from April to October 2022 in University of Samarra laboratories, Iraq. The study included 20 male laboratory rabbits, whose weight ranged from 1.4 to 1.5 kg, ten of them were as a control group and the other ten were infected with *N. americanus* parasite. Some biochemical tests were carried out such as lipid profile (HDL, Cholesterol, and Triglycerides) and liver enzymes (GOT and GPT), as well as tissue sections of the liver, lung and small intestine to see the alterations due to infection. The present results showed significant differences for total cholesterol between control and infected groups (88.40 and 71.80 mg dL^{-1}, respectively). In the case of triglycerides, non-significant variances were recorded between control and infected groups (68.80 and 69.80 mg dL^{-1}, respectively), whereas in the case of HDL, the present results showed significant differences between control and infected groups (27.40 and 22.60 mg dL^{-1}, respectively). The current results showed significant increase in concentration of GOT of infected group compared to control group (27.20 and 15.60 mg dL^{-1}, respectively). As for GPT, non-significant variances were recorded between infected and control groups (17.80 and 14.60 mg dL^{-1}, respectively), while for Iron, significant differences occured between infected and control groups (74.00 and 101.40 mg dL^{-1} respectively). The histological study showed the presence of histological alterations in the members of infected group.

Key words: *Necator americanus*, Rabbit, Physiology, Histology.
Article type: Research Article.

INTRODUCTION

The Ancylostomidae family includes what are referred to as hookworms. They cling to the mucosa of the host's gut and dwell there, feeding on the blood and bodily fluids that are sucked out of it. hookworms develop and reproduce in their host's small intestine. Embryos have grown by the time they are passed with faeces. It is impossible to identify species that infect humans with certainty from just their eggs. Warmth, shade, and moisture are necessary for the continuing development of eggs. Newly hatched J1s have a rhabditiform oesophagus. In two to three days, juveniles shed their cuticle and live in the faeces, where they consume faecal detritus, continue feeding and growing as a rhabditiform oesophagus until it moults to the third stage, which can infect a host, after about five days. The hookworm's pointed tail helps distinguish filariform J3s from other types of worms. Living in the upper few millimetres of soil. Freezing or desiccation kills them quickly. Skin fatty acids and heat stimulate penetration behaviour (Haas *et al.* 2005). J3s that come into contact with a host's skin get infected (Roberts *et al.* 2013). Juveniles are transported from a blood or lymph channel to the heart and subsequently to the lungs. They enter the alveolar air gaps and move up the respiratory tree via ciliary activity to the glottis. The small intestine is where they eventually end up after being eaten. There, they connect to the mucosa, develop, and moult to the fourth stage, which has a larger buccal capsule. The sexual development of worms occurs after additional growth. The worms consume a lot of blood (Williamson *et al.* 2003; Hossain & Bhuiyan 2016). From the time of infection

to the start of egg production, at least five weeks must pass (Roberts *et al.* 2013). *Necator americanus*, the "American killer," was first discovered in Brazil and then Texas, but it was later determined that it was actually indigenous to Africa, India, Southeast Asia, China, and the islands in the southwest Pacific. It most likely travelled to the New World on slave ships. The evolution of the southern United States' economy and culture, as well as those of other parts of the world where it exists, has been significantly influenced by the worm. There are no established international surveillance systems that provide precise statistics on the prevalence of hookworm infection, which makes it difficult to implement policies to stop the spread of the parasite (Roberts *et al.* 2013).

MATERIALS AND METHODS

The present study was conducted for the period from April to October 2022 in University of Samarra laboratories, Iraq. The study included 20 male laboratory rabbits, whose weight ranged from 1.4-1.5 kg, ten of them were as a control group and the other ten were infected with *N. americanus* parasite. Some biochemical tests such as lipid profile (HDL, Cholesterol, and Triglycerides) and liver enzymes (GOT and GPT), as well as tissue sections of the liver, lung and small intestine to see the changes that occur due to infection.

Microscopic examinations

The microscopic examinations were included direct wet mount method of rabbit faeces for detecting parasitic infection with *N. americanus* (WHO 1991).

Determination of lipid profiles concentration

Estimating lipid profiles concentration in serum using a commercial kit, equipment from Randox England Company.

Determination of both GOT and GPT concentrations

GOT and GPT concentrations in serum were estimated using a commercial kit, equipment from Randox England Company.

Determination of Iron concentration

Estimating iron concentration in serum using a commercial kit, equipment from Biolabo France Company.

Histological preparations

Histological sections were prepared based on Bancroft and Stevens (1987) method for detecting the histological alterations to each liver, lung and small intestine.

Statistical analysis

Data analysis was performed according to the t-test; the means of the groups were measured with $P \leq 0.05$ and using the SPSS program (Cleophas & Zwinderman 2016).

RESULTS AND DISCUSSION

Microscopic examinations

The microscopic examinations were included direct wet mount method of rabbit faeces using eosin stain for detecting parasitic infection with *N. americanus* (Fig. 1).

Lipid profiles

The present results showed significant differences in total cholesterol between control and infected groups (88.40 and 71.80 mg dL^{-1}, respectively). In the case of triglycerides, non-significant differences were recorded between control and infected groups (68.80 and 69.80 mg dL^{-1}, respectively), whereas in the case of HDL the present results showed significant differences between control and infected groups (27.40 and 22.60 mg dL^{-1}, respectively; Table 1).

Liver's enzymes and Iron

The present results showed significant increase in concentration of GOT of infected group compared to control group (27.20 and 15.60 mg dL^{-1}, respectively). In the case of GPT, non-significant differences were recorded

between infected and control groups (17.80 and 14.60 mg dL^{-1}, respectively), while in the case of iron, significant differences occurred between infected and control groups (74.00 and 101.40 mg dL^{-1}, respectively; Table 2)

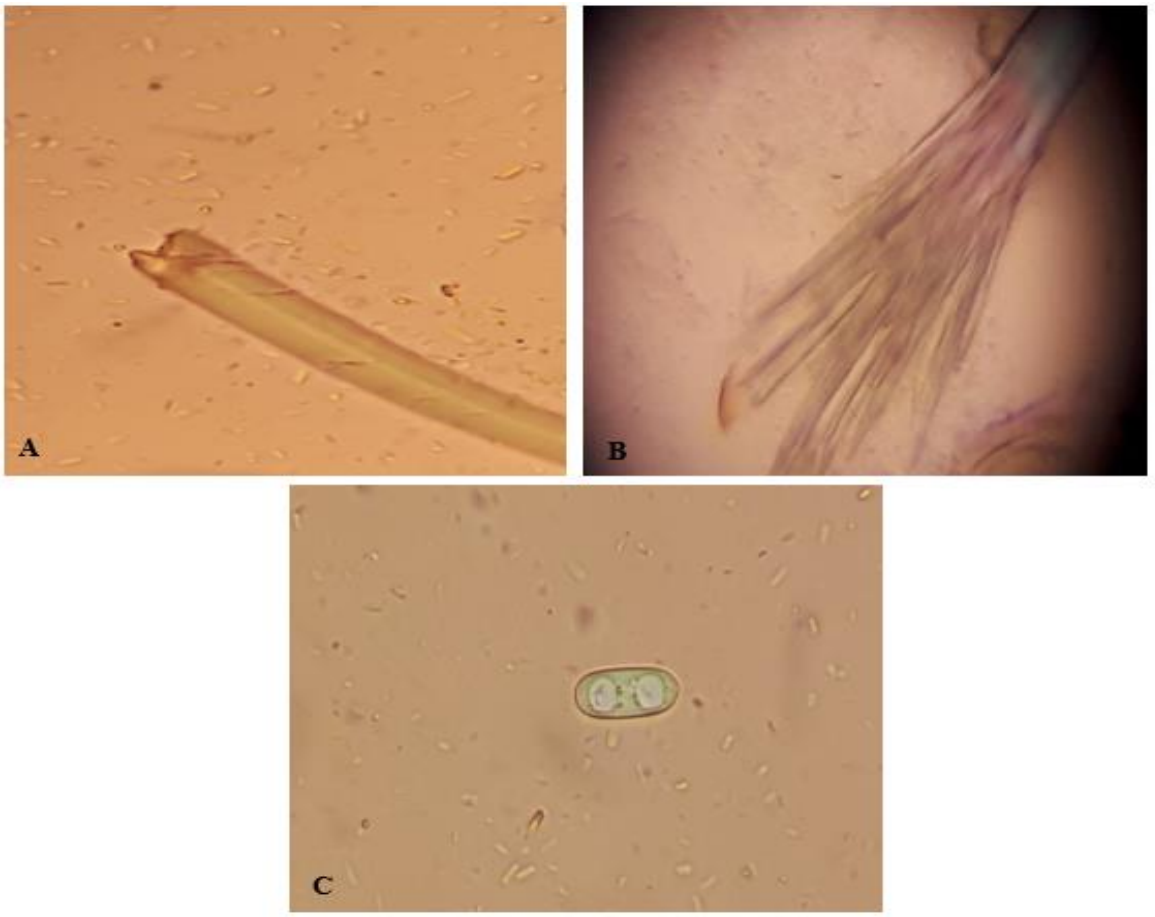

Fig. 1. *N. americanus*; (A): Head of adult worm, (B): Tail of adult worm, (C): Egg of the worm × 40.

Table 1. The levels of lipid profiles of each laboratory rabbits infected with *N. americanus* and normal groups.

Parameters \ Groups	-ve Ctrl	+ve Ctrl
	Mean ± SD (mg dL^{-1})	
Total Cholesterol	8.11 ± 88.40	5.76 ± 71.80*
Triglycerides	7.85 ± 68.80	9.01 ± 69.80 $^{n.s}$
HDL-C	2.07 ± 27.40	1.34 ± 22.60*

Note: ($^{n.s}$) non-significant difference, (*) significant difference.

Table 2. The levels of liver enzymes and Iron of each laboratory rabbits infected with *N. americanus* and normal groups

Parameters \ Groups	-ve Ctrl	+ve Ctrl
	Mean ± SD (mg dL^{-1})	
GOT	2.70 ± 15.60	4.60 ± 27.20*
GPT	2.88 ± 14.60	3.90 ± 17.80$^{n.s}$
Iron	11.93 ± 101.40	13.56 ± 74.00*

Note: ($^{n.s}$) non-significant difference, (*) significant difference.

Pierce *et al.* (2019) reported that both genders with *N. americanus* infection showed dyslipidaemia (higher than normal levels of total cholesterol, triglycerides, LDL, or the total cholesterol/HDL ratio, low HDL), as well as abnormal liver function tests (abnormal alanine transaminase, aspartate transaminase), whereas Umbrello *et al.* (2021) reported that liver enzymes were normal during the infection with *N. americanus* Examining the concentrations of two liver enzymes in blood serum helps us to infer the presence or absence of damage or a specific injury (Nelson & Cox 2008). As the increase in the concentration of these two enzymes above the normal limit for them (in the blood) indicates the presence of a certain pathological condition (Al-Ammash 2012). GOT enzyme is present in liver cells, heart muscle, and skeletal muscle cells, and to a lesser extent in the kidney and pancreas, and when the cells of these types of tissues are damaged, the GOT is released, and its increase depends on the number of damaged cells (Penman *et al.* 2022). GOT enzyme is similar to GPT, however, due to the presence of the first in other tissues, it is not considered specialized for damage to the liver (Pratt & Kaplan 2000). In the case of GPT, it is present in the liver cells and is present in small quantities in the kidneys, heart, and skeletal muscles. Any damage to the visceral tissue in the liver leads to the release of this enzyme, and thus a rise in its concentration (Pagana *et al.* 2022). In general, any damage that occurs in the liver cells as a result of the invasion of the parasite that possesses some tissue-dissolving enzymes, leads to the liberation of the enzyme into the blood and thus an increase in its concentration (Sabaa 2021). Heavy infections in malnourished hosts are associated with anaemia and protein loss. Protein-losing enteropathies may also result from the inflammatory changes induced by other intestinal hook worms (Wakelin 1996). The primary harm caused by hookworms in humans is intestinal blood loss brought on by mature parasites. The anaemia caused by iron shortage that develops after a moderate to severe infection is referred to as "hookworm illness" in most cases. Blood loss happens when the worms contract their muscular oesophagi to create negative pressure, which draws a plug of tissue into their buccal capsules, and then utilize their cutting apparatus to adhere to the intestinal mucosa and submucosa (Hotze *et al.* 2004). Haemoglobin is released when some red blood cells lyse, by a series of haemoglobinases in the parasite's gut (Williamson *et al.* 2003). Depending on the status of host iron, a hookworm burden (i.e., the intensity of infection, or number of worms per person) of 40 to 160 worms is associated with haemoglobin levels below 11 g dL^{-1} (Lwambo *et al.* 1992; Bundy *et al.* 1995). However, other research has demonstrated that anaemia may manifest with a lower hookworm burden **(**Olsen *et al.* 1998). Depending on the species, hookworms can cause varying degrees of iron-deficiency anaemia (Albonico *et al.* 1998). For instance, in Zanzibar, children with only *N. americanus* infections had a prevalence of hypoferritinemia (ferritin level < 12 μg L^{-1}) of 33.1%, whereas children with both *N. americanus* and *A. duodenale* infections had a prevalence of 58.9% **(**Stoltzfus *et al.* 1997). There is a direct relationship between the decline in haemoglobin, serum ferritin, and protoporphyrin levels and the severity of hookworm infection, which is commonly assessed by quantitative egg counts, when the host's iron stores are depleted **(**Hotze *et al.* 2004). One of the most significant neglected tropical diseases in humans is hookworm infection, which affects 576-740 million people worldwide and results in intellectual and physical growth retardation as well as iron deficiency anaemia (Chang *et al.* 2020).

Histological study

1. The liver

The results of this study showed larva of worm among hepatocytes (Fig. 1), vacuolated degeneration and necrosis of hepatocytes, infiltration of lymphocytes, fibrinoid and congestion of blood vessel (Fig. 2) and disappeared sinusoids (Fig. 3).

Some of these findings concur with those of Uduchi *et al.* (2022), who found that *N. americanus* infection in albino rats led to inflammatory alterations, hepatocytic degenerative changes, necrosis, distortion of fragmented hepatocytes, and distortion of fragmented hepatocytes. The liver is known as the major organ of detoxification and biotransformation, due to its location and blood supply (Uduchi *et al.* 2022). Perhaps since the location of the liver is close to the lung, the larva may reach the liver during its migration inside the host's body, and this is indicated by the current study, which indicated the presence of the larva within the liver tissue section.

2. The lung

The present study showed diapedesis of lymphocytes, leakage of plasma proteins, congestion of blood vessel and aggregate of lymphocytes (Fig. 4), degeneration of alveolar cell, necrosis of alveolar cells (Fig. 5) and larva of worm among the lung cells (Fig. 6).

Wilkinson (1990), noted that lung sections from mice infected with *N. americanus* displayed intra-alveolar oedema, sections of larvae, and an inflammatory infiltrate primarily consisted of acute inflammatory cells with sporadic lymphocytes and sparse eosinophils in the peri-bronchial region. The larvae were mostly discovered in the previously described tiny bronchi and alveolar ducts, as shown, and they did not appear to have caused any localized cellular inflammation. Infected groups exhibited an increase in the intensity of the cellular inflammatory infiltrate, and eosinophils were observed as a higher proportion of the infiltrate on tissue sections, in addition to the polymorphonuclear leukocytes, lymphocytes, and macrophages that were noticeable with the formation of giant cells in some sites. Although some larvae (or detritus) in the lung parenchyma were surrounded by inflammatory cells, it was also possible to see larvae that had no adhering cells. The peribronchial infiltrate's inflammatory cells surrounding the larvae exhibited the similar variety of cell types. As Wilkinson (1990) indicated to possibly implicating vessel leakage during the earlier stages of infection and in some cases infected mice had observable worms in their alveoli, these were surrounded by collections of inflammatory cells with occasional giant cells. Migration of larvae after entering the host's body and reaching the lung, which is the normal larval path through which it continues to develop to reach the adult stage after reaching the intestine may cause significant damage to the lung tissue (Bouchery *et al.* 2020). In addition, the presence of larvae in the lung tissue was observed, as shown in the results of the current study.

3. The intestine

The current study revealed necrosis in mucosa layer (Fig. 7), degeneration in Brunner glands and lamina propria, formation of ghost cells, congestion of blood vessel and atrophy of epithelial layer (Fig. 8). Some these findings are in agreement with Uduchi *et al.* (2022) that reported degenerative alterations in intestine section.

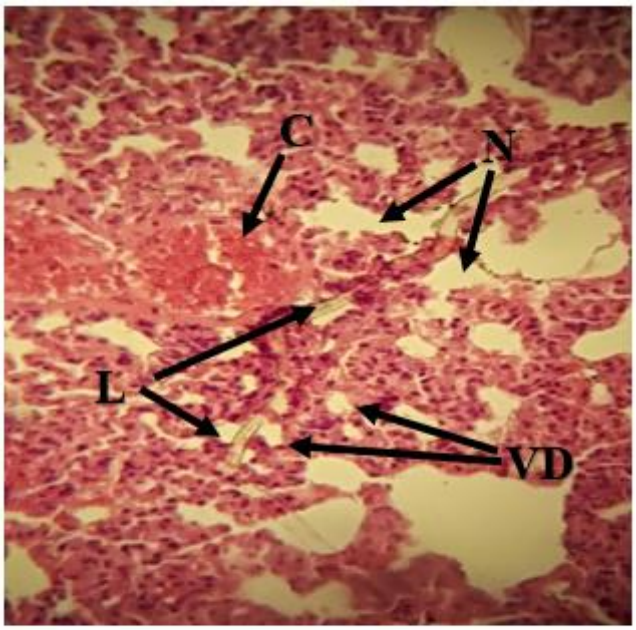

Fig. 1. Liver section of infected group show worm larva (L), vacuolated degeneration (VD) and necrosis of hepatocytes (N), H&E staining, 100X.

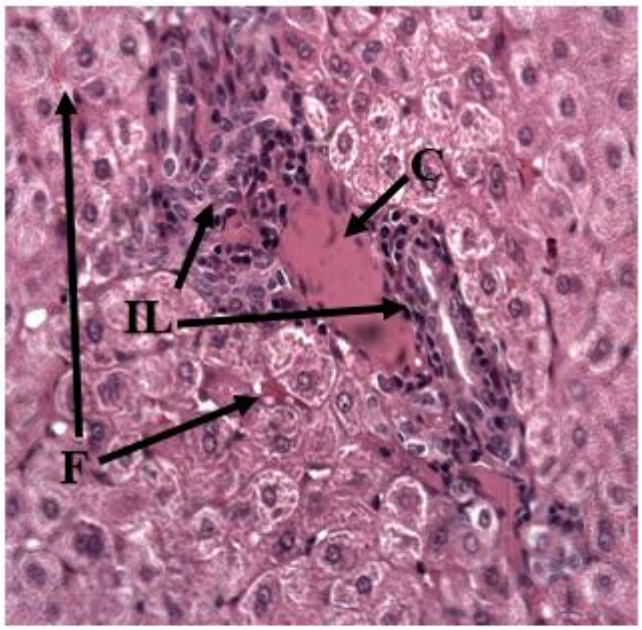

Fig. 2. Liver section of infected group show infiltration of lymphocytes (IL), fibrinoid (F) and congestion of blood vessels (C), H&E staining, 400X.

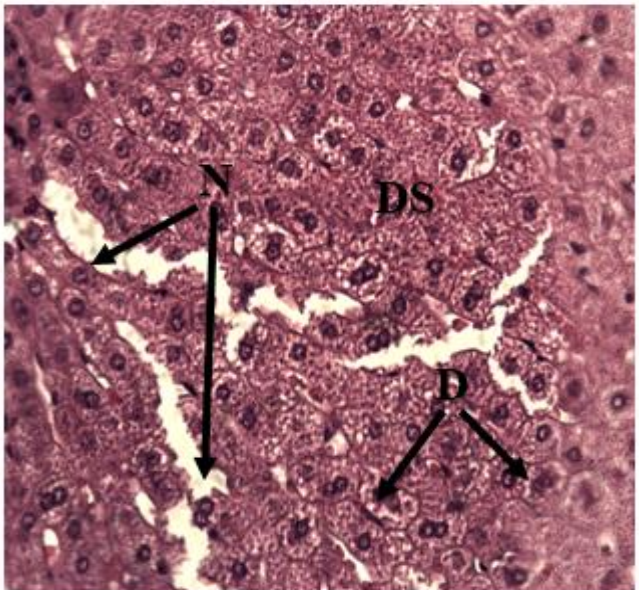

Fig. 3. Liver section of infected group show Disapeared of sinusoids (DS), degeneration of hepatocytes (D) and necrosis of hepatocytes (N), H&E staining, 400X.

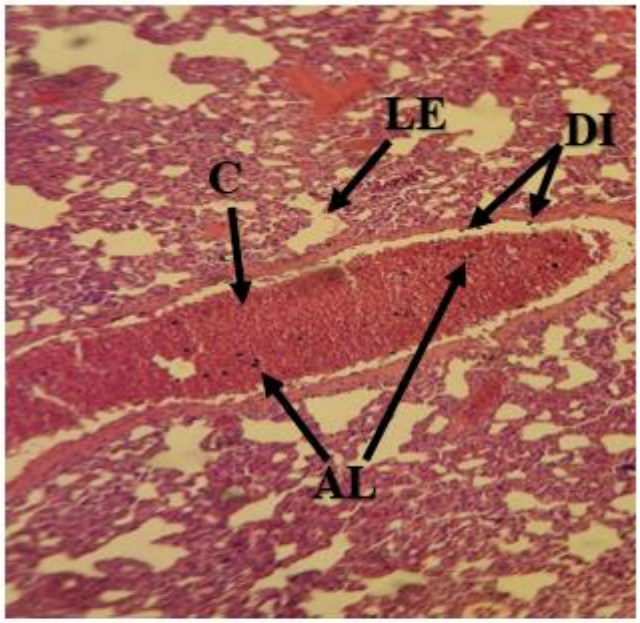

Fig. 4. Liver section of infected group show diapedesis of lymphocytes (DI), leakage of plasma proteins (LE) and congestion of blood vessels and aggregate of lymphocytes (AL), H&E staining, 400X

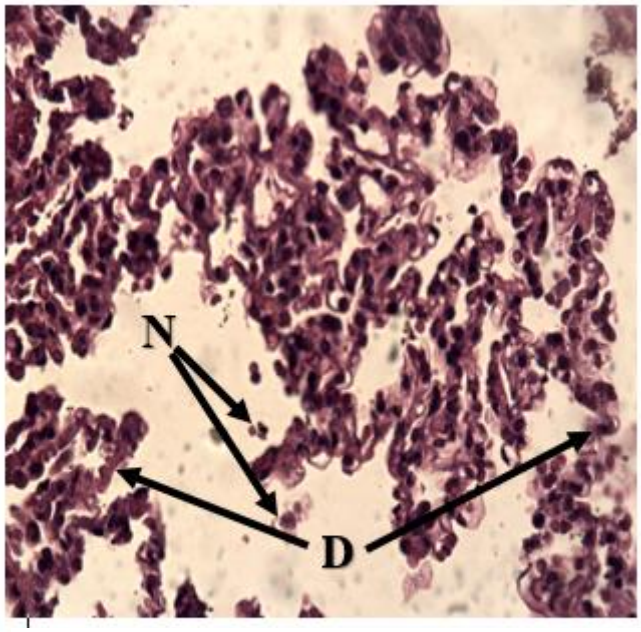

Fig. 5. Lung section of infected group show degeneration of alveolar cells (D), and necrosis of alveolar cells (N), H&E staining, 400X.

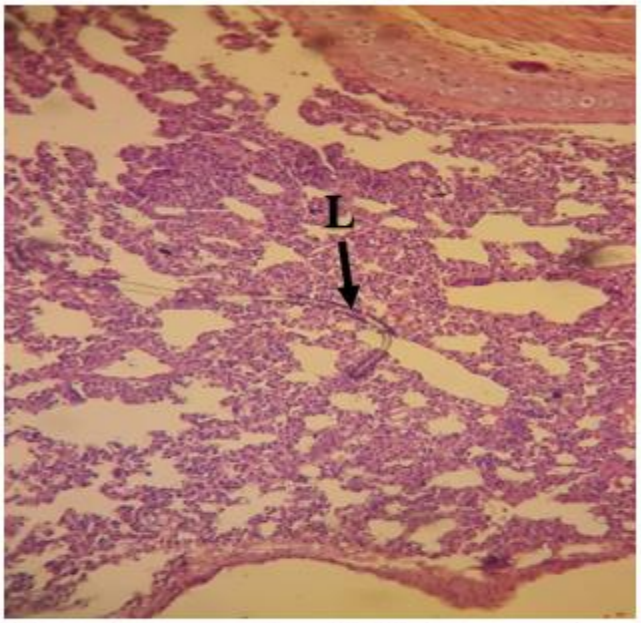

Fig. 6. Lung section of infected group exhibiting worm larva (L), H&E staining, 400 X.

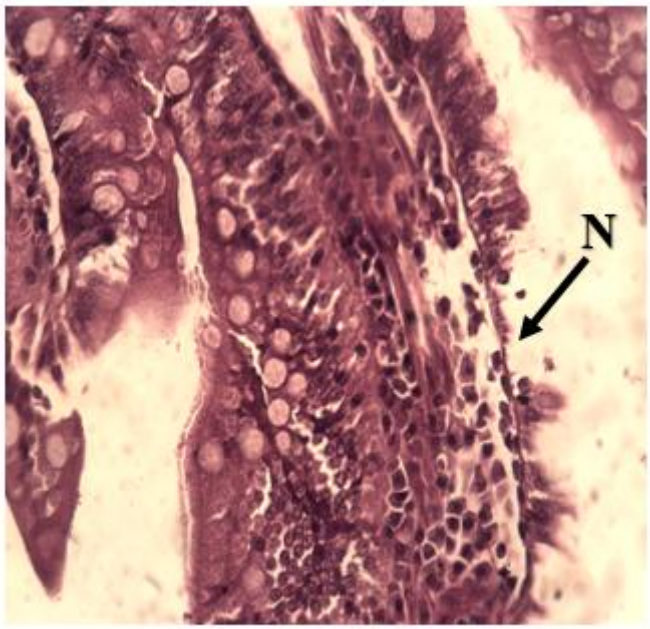

Fig. 7. Intestine section of infected group exhibiting necrosis in mucosa layer (N), H&E staining, 400 X.

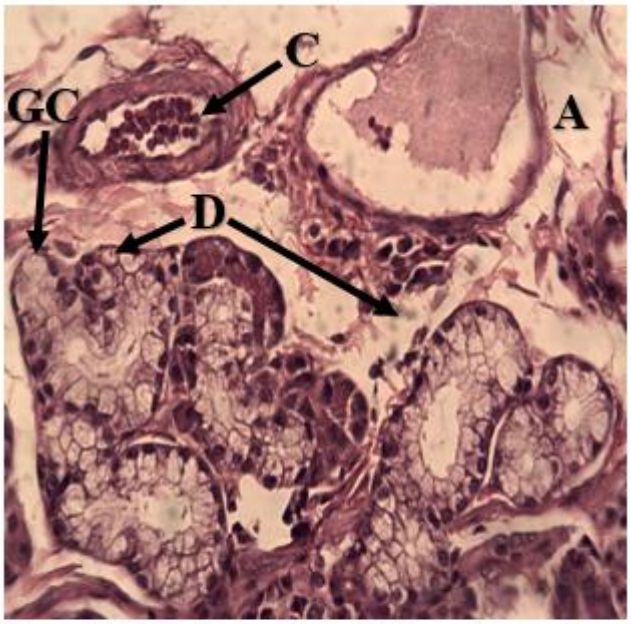

Fig. 8. Intestine section of infected group exhibiting degeneration in Brunner glands and lamina propria (D) and Ghost cells (GC), congestion of blood vessels (C), atrophy of epithelial layer, H&E staining, 400 X.

CONCLUSION

1. The occurrence of significant differences in the levels of lipid profiles (cholesterol and HDL) and non-significant differences of triglycerides in infected group compared to control group.
2. The current study recorded significant differences in GOT and Iron, and non- significant differences in GPT of infected group compared to control group.
3. The occurrence of alterations in histological sections of liver, lung and intestine of infected group compared to control group.

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