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The Hematological and Histopathological changes of *Giardia duodenalis* on the intestine in Diyala Government

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Abstract

Giardia duodenalis is one of the most prevalent human intestine protozoan parasites in the world and infected a broad variety hosts of animals, The goal of the current study was to determine the hematological picture and histopathological changes of intestine in rabbits. 15 rabbits used in this study for 2 months divided into 3 groups: 1st group the control its normal feed and water without any treatment, 2nd group given one dose orally weekly of Giardia cyst for 2 months, 3rd group given two dose orally weekly of Giardia cyst for 2 months. The results of hematological analysis between control group and treated groups at one and two months revealed a statistically significant difference ($P<0.05$) decrease in the mean of the following RBC count, Hb, PCV, platelets and increase of WBC count, monocytes and lymphocytes, also the pathological changes were observed grossly and histopathological in intestine including hyperplasia in colonic glands and mucosa, increase no. of goblet cells and aggregation of parasites in mucosa.

Keyword: Giardia duodenalis, intestine, histopathological changes.

Introduction:

One of the most prevalent intestinal parasites that affects the human and a wide variety of other animals is Giardia according to Feng and Xiao, (2011), the Giardia duodenalis (syn. Giardia intestinalis and Giardia lamblia) has a wide range of hosts that includes humans and domestic,

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farmed, Giardiasis is a serious zoonotic illness that affects both human and veterinary health and is brought on by Giardia duodenalis (Ryan and Cacci, 2013).

According to Xiao and Fayer, (2008) the examinations into outbreaks and case control studies, Giardiasis can be transmit from the human to human (anthroponotic) or from animals to people (zoonotic).

Giardia can spread through the oral route after coming into touch with infected individuals either directly or indirectly (Feng et al., 2011). Members of this genus have been responsible for several outbreaks connected to consuming or surface water sources that have impacted whole towns (Robertson *et al.*, 2010).

Giardiasis is expected to affect 280 million people annually worldwide, with infection rates being greater in poorer nations (Feng and Xiao, 2011; Ryan and Caccio, 2013; Squire and Ryan, 2017).

Infections can become severe and persistent in newborns, the elderly, and those with impaired immune systems, despite the fact that they frequently resolve on their own in immunocompetent adults (Feng and Xiao, 2011). Domestic animals like sheep and cattle are recognized as a key contributor to zoonotic sources of infection since Giardia species and genotypes that infect people have also been found (Xiao & Fayer, 2008).

Typically, Giardiasis is conceder a self-limiting clinical condition marked by the watery diarrhea, cramping in the abdomen, bloating, loss of weight, and nutritional deficiencies (Einarsson *et al.*, 2016). But silent infections happen more often than symptomatic illnesses. (Feng *et al.*, 2011; Rayani *et al.*, 2014; Wegayehu *et al.*, 2016). El-Hady *et al.*, (2019) reported that clinically diarrhoea was the first complaint that affected all cases, secondly abdominal colic 84 (90.3 %), then failure to thrive affecting 32 (34.5 %) cases, also abdominal distension affecting 26 (28 %) cases, finally vomiting affecting 6 (6.6 %) of cases.

The disease has pathological changes includes presence the trophozoites of *Giardia spp*. in the lumen of the gallbladder and attach to the mucosal epithelium, presence of

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trophozoites of *Giardia* in the lumen of the gallbladder (Alhayali *et al*., 2020). Also, Buret and Cotton (2011) showed that the trophozoites colonize the lumen of the small intestine without invading host tissue or entering the blood stream. presence of the parasites manifested by chronic inflammatory response including slightly hyperemic blood vessels, lymphocytes, plasma cells and macrophages in filtration in mucosal and submucosal layer with degeneration in the epithelial layer.

Since there are few studies in Iraq and other countries in the world about the experimental infection of *Giardia* and study of haematological and histopathological changes in rabbits therefore the study was conducted due to the importance of *Giardia* sp. in human and rabbits.

Materials and Methods:

Experimental infection of rabbits

This study was conducted in the Faculty of Veterinary Medicine at the University of Diyala, in the animal house of department of internal medicine and preventive, after the adaptation period (2 weeks), from the period January to April / 2022. A total of 15 rabbits (aged 3-6 months from both sex) were divided into three groups:

1. **Control group**s: Normal feed and water without any treatment.
2. **Group 2:** Given orally 1 dose weekly (3 ml of solution contain 30 Giardia cyst) of Giardia cyst for 2 months.
3. **Group 3:** Given orally 2 dose weekly (3 ml of solution contain 30 Giardia cyst) of Giardia cyst for 2 months(Puebla *et al.*, 2017).

The cysts of Giardia are isolated from 100 stools taken from children complains of diarrhoea, abdominal discomfort, nausea and abdominal cramp.

Blood collection:

The blood samples were collected with EDTA tube used for hematological parameters monthly for 2 months, and the complete blood count (CBC) were collected blood samples by

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Vol. 1, NO. 1, March 2023

using Auto Hematology Analyzer including hemoglobin (Hb) concentration, white blood cells (WBCs) count (lymphocytes, monocytes and neutrophils), red blood cells (RBCs) count and Platelets count.

Histopathological Changes

Examination

Fifteen rabbits were employed, and they were all housed in the same way. Steel mesh cages were used to house the animals, who were kept at a constant temperature of 22–27 degrees Celsius with access to dry, absorbent bedding materials like wood shaving. Food and water were not restricted for any of the animals. A total of fourteen days were spent monitoring all of the animals prior to the start of the experiment, during which time any signs of odd behavior or illness were noted and eliminated. Both Xylene (0.01) and ketamine (0.09 ml/km of weight) were used to anesthesia. Intestine samples were collected, then fixed in 10% formalin, processed with standard histological techniques, and staining with Hematoxylin and Eosin (H & E). (Suvarna *et al.*, 2018).

Statistical analysis

Data were organized, tabulated, and statistically analyzed using SPSS version, 23.00. P values were calculated. Chisquare test ($\chi 2$) was used to compare the frequency data. P value < 0.05 indicates significant (S) values. P valu < 0.01 indicates highly significant (HS) values. P value > 0.05 Non significant (NS) and Experimental study using analysis of variance (ANOVA) (Leech et al., 2011).

Results

Haematological Parameters:

Hematological analysis revealed a statistically significant difference ($P<0.05$) in the mean of the following, as shown in table 1.

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Table (1): Haematological analysis between control group and treated groups at one and two months of experimental study.

	After 1 month			After 2 months		
parameters	First group/control	Second Group 1 dose weekly	Third Group 2 dose weekly	First group/control	Second Group 1 dose weekly	Third Group 2 dose weekly
HB	12.5±0.31a	10.1±0.39b	8.78±0.14c	12.76±0.36a	8.44±036b	5.7±0.52c
RBC1012/L	6.59±0.15a	5.39±0.14b	4.54±0.15b	6.73±0.19a	4.58±0.21b	3.2±0.28c
PCV	39.6±0.93a	32.4±0.87b	28.4±0.4c	40.4±1.08a	27.4±1.08b	19.2±1.56c
MCV	60.2±0.11a	60.06±0.04a	60.4±0.13a	60.26±0.12a	60.32±0.15a	60.38±0.25a
MCH	18.98±0.06a	18.74±0.05a	18.62±0.06a	19.05±0.04a	18.56±0.08b	17.862±0.18c
MCHC	31.52±0.07a	31.14±0.09a	30.88±0.07a	31.52±0.07a	30.766±0.12a	29.56±0.31b
Platelets 103 ul	237±0.9a	191±10b	198±10c	126±8a	179±11b	128±19c
WBC 109/L	3.84±0.07a	4.5±0.22b	5.44±0.19c	3.86±0.14a	6.04±0.34b	6.5±0.64b
Heterophiles	1587.8±52.78a	1562.2±77.72a	1779.8±47.30b	1487.4±71.24a	1915.2±136.34b	1388.8±164.9c
Lymphocytes	1244.6±42.04a	1953.2±103.13b	2661.8±151.96c	1427.2±60.03a	2723.8±195.21b	3745.4±383.06c
Monocytes	635.8±29.72a	671.2±62.05b	644.6±59.43c	575.2±43.28a	982.6±41.63b	1086.6±136.39c
Eosinophils	377.2±20.6a	334.2±37.3a	251.8±48.8b	387.4±29.8a	403.8±24.3a	279.2±34.2b

Data are expressed as the mean values ± SEM (n =5). Data in the same row with different superscript letters are significantly different in blood parameters ($p < 0.05$). Absence of a letter indicates that there were no significant differences ($p > 0.05$) between any of the time points.

In terms of red blood cell (RBC) count, there was a statistically significant (p 0.05) difference between of the first group and second group and third group at one months and two months. After one month the mean of RBC for first group was 6.59±0.15 and for second group was 5.39±0.14 and for third group was 4.54±0.15. While, after two month the mean of RBC for first group was

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6.73±0.19 and for second group was 4.58±0.21and for third group was 3.2±0.28.

Differences in Hb between the three groups were statistically significant ($p< 0.05$). (first group and second group and third group at the two time points (one months and two months Table 1). After one month the mean of HB for 1st group was 12.5±0.31 and for 2nd group was 10.1±0.39 and for 3rd group was 8.78±0.14. While, after two month the mean of HB for 1st group was 12.76±0.36 and for 2nd group was 8.44±036 and for 3rd group was 5.7±0.5.

Differences in PCV between the three groups were statistically significant ($p< 0.05$). (first group and second group and third group at the two time points (one months and two months Table 1). After one month the mean of PCV for 1st group was 39.6±0.93 and for 2nd group was 32.4±0.879 and for 3rd group was 28.4±0.4. While, after two month the mean of PCV for 1st group was 40.4±1.08 and for 2nd group was 27.4±1.086and and for 3rd group was 19.2±1.56.

Differences in MCH between the three groups were statistically significant ($p< 0.05$). (first group and second group and third group at the two time points (one months and two months Table 1). After one month the mean of MCH for 1st group was 12.5±0.31 and for 2nd was 10.1±0.39 and for 3rd group was 8.78±0.14. While, after two month the mean of MCH for 1st group was 12.76±0.36 and for 2nd was 8.44±036and and for 3rd group was 5.7±0.5.

After two months, only in the third group did we find a statistically significant ($p <0.05$) difference in MCHC compared to the first and second groups. After one month the mean of MCHC for 1st group was 31.52±0.07 and for 2nd group was 31.14±0.09 and for 3rd group was 30.88±0.07. While, after two month the mean of MCHC for 1st group was 31.52±0.07 and for 2nd group was30.766±0.12 and for 3rd group was 29.56±0.31.

The mean CV (MCV) was not significantly different ($p > 0.05$) between the first, second, and third groups, or between the two time points. (one months and two months Table 1). After one month the mean of MCV for 1st group was 60.2±0.12 and for 2nd group was 60.32±0.15 and for 3rd group was 60.4±0.13.While, after two month the mean of MCV for 1st group was 60.26±0.12 and for 2nd group was 60.32±0.15 and for 3rd group was 60.38±0.25.

Differences in platelets between the three groups were statistically significant ($p< 0.05$). (first group and second group and third group at the two time points (one months and two months Table 1). After one month the

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mean of Platelets for 1st group was 128±0.9 and for 2nd group was 191±109 and for 3rd group was 198±10 While, after two month the mean of Platelets for 1st group was126±8 6 and for 2nd group was 179±11and and for 3rd group was 237±19.

Differences in WBC between the three groups were statistically significant ($p< 0.05$). (first group and second group and third group at the two time points (one months and two months Table 1)after one month the mean of WBC for 1st group was 3.84±0.071 and for 2nd group was4.5±0.22 and for 3rd group B was 5.44±0.19. While, after two month the mean of WBC for 1st group was 3.86±0.14 and for 2nd group was6.04±0.34 and for 3rd group was 6.5±0.64.

There was a significant difference ($p < 0.05$) in the Heterophiles within the(first group and second group and third group at one month's Table 1). There was a significant difference ($p < 0.05$) in the Heterophiles within the (first group and second group and third group at two month's Table 4-12). After one month the mean of Heterophiles for 1st group was 1587.8±52.78 and for 2nd group was 1562.2±77.72 and for 3rd group B was 1779.8±47.30. While, after two month the mean of Heterophiles for 1st group was 1487.4±71.24 and for 2nd group was1915.2±136.34 and for 3rd group was1388.8±164.9.

There was a significant difference ($p < 0.05$) in the Lymphocytes in the 3 groups (first group and second group and third group) at the two time points (one months and two months Table 1). After one month the mean of Lymphocytes for 1st group was 1244.6±42.04 and for 2nd group was 1953.2±103.13 and for 3rd group was 2661.8±151.96. While, after two month the mean of Lymphocytes for 1st group was 1427.2±60.03 and for 2nd group was 2723.8±195.21 and and for 3rd group was 53745.4±383.06.

There was a significant difference ($p < 0.05$) in the Monocytes in the 3 groups (first group and second group and third group) at the two time points (one months and two months Table 1). After one month the mean of Monocytes for 1st group was 635.8±29.72 and for 2nd group was 671.2±62.05 and for 3rd group was 8.78±0.14. While, after two month the mean of Monocytes for 1st group was 575.2±43.28 and for 2nd group was 982.6±41.63 and for 3rd group was 1086.6±136.39.

There was a significant difference ($p < 0.05$) in the Eosinophils in (first group and second group) at the two time points (one months and two months Table 1). After one month the mean of Eosinophils for 1st group was 377.2±20 and for 2nd group was 334.2±37and for 3rd group was 251.8±48. While, after two month the

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University of Diyala, Iraq
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mean of Eosinophils for 1st group was 387.4±29 and for 2nd group was 403.8±24and for 3rd group was 279.2±34.2. On the other hand, abnormalities in erythrocytes morphology (Anisocytosis and Poikilocytosis).

Pathological examination:

There are non-pathological changes (macroscopic and microscopic) lesions observed in control group.

Macroscopic changes:

In 2nd group:

The intestine showed swelling and accumulation of food in large intestine, most layers congested and sometimes hemorrhagic with serosa thickening (fig. 1 & 2).

In 3rd group:

The intestine showed enlargement of intestine, swelling of mucosa with increase amount of mucus overlying (fig. 3).

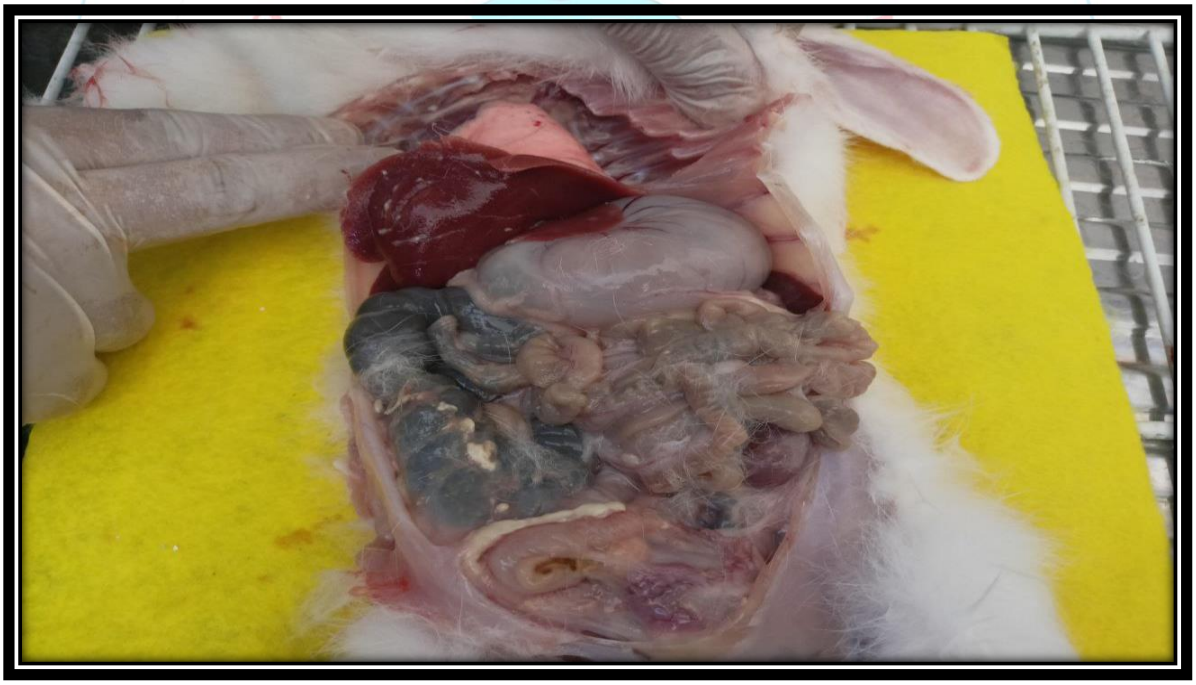

Fig. (1): Grossly appearance of intestine and liver in 2nd group shows accumulation of food in large intestine with enlargement and slightly congested of liver.

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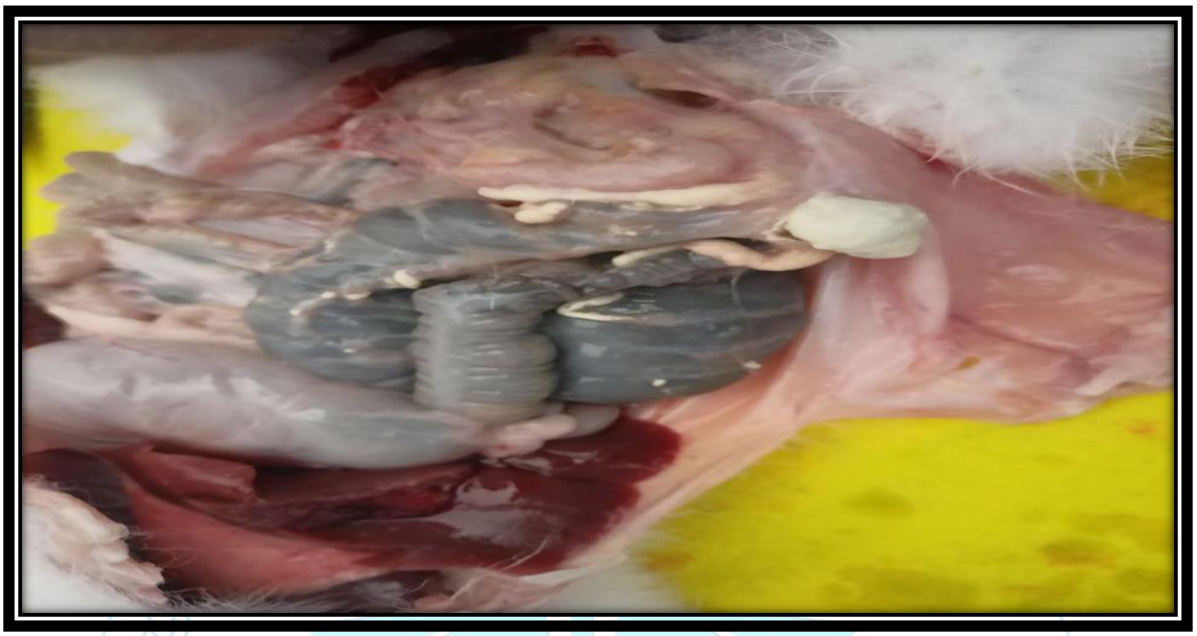

Fig. (2): Grossly appearance of intestine in 2nd group shows swelling and accumulation of food in large intestine.

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E-ISSN:2958-6178
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Fig. (3): Grossly appearance of intestine in 3rd group shows enlargement of intestine with filled by gas.

Microscopic Examination:

In 2nd group: Showed heavy infiltration of mucosa and submucosa by mononuclear cells and polymorphic cells mostly eosinophilic, atrophy and necrotic of intestinal gland (fig. 4), also heavy increase in no. and size of crypts of intestinal gland (fig. 5).

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University of Diyala, Iraq
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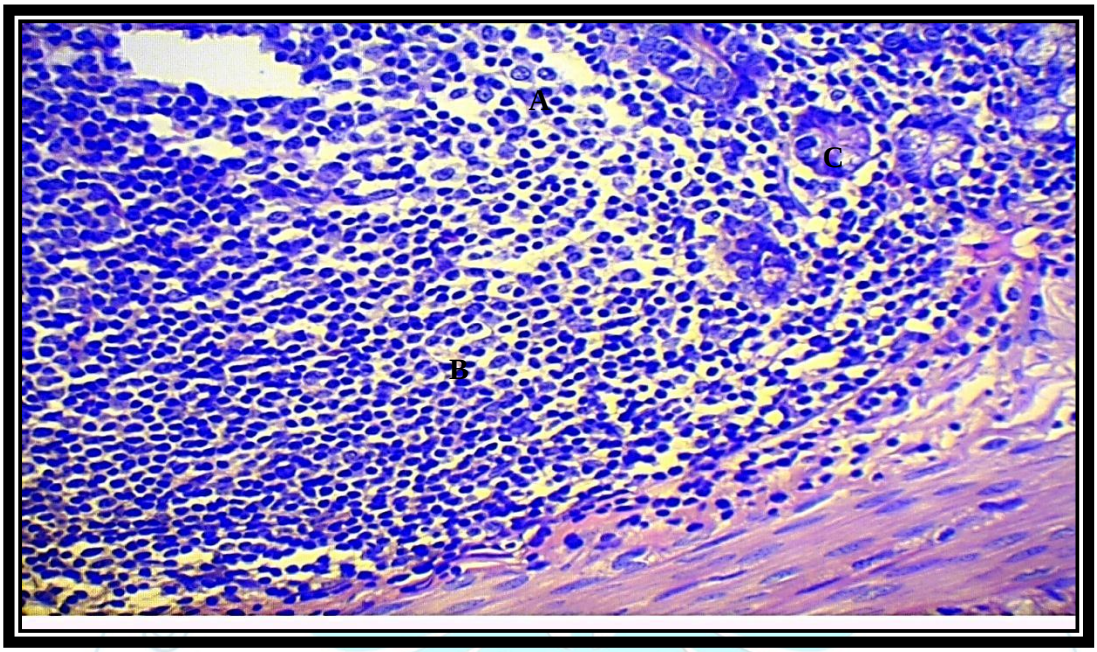

Fig. (4): Histopathological section of intestine in 2nd group shows: **a:** Eosinophils **b:** heavy infiltration of mucosa and submucosa by mononuclear cells and polymorphic cells mostly eosinophilic **c:** atrophy and necrotic of intestinal gland (X40; H&E stain).

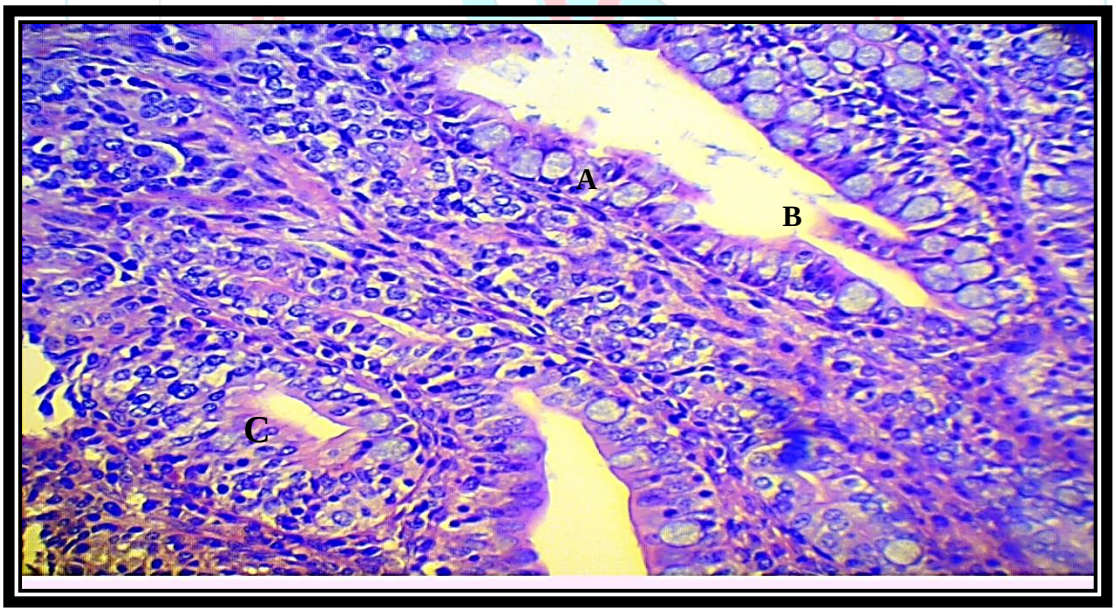

Fig. (5): Histopathological section of intestine in 2nd group shows: **a:** heavy increase in no. and size of mucosa goblet cells with basophilic mucine **b:** sloughing of mucosa **c:** hyperplastic of crypts of intestinal gland (X40; H&E stain).

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Vol. 1, NO. 1, March 2023

In 3rd group: showed thick pseudomembrane covered the mucosa, hyperplasia in colonic glands, infiltration of mononuclear cells, present of eosinophilic materials and damaged and destroyed of glands (fig. 6), also showed multiple areas of necrosis in mucosa and submucosa with dead neutrophils, increase tissue thickening in fibromuscular and congested of blood vessels (fig. 7). In other section showed increase in no. and size of goblet cells in mucosa layer, the lumen dilated contains several parasites, congested of blood vessels, infiltration of lymphocytic cells in submucosal layer, and few basophilic lymphocytes infiltration (fig. 8), the mucosa and submucosa appear heavily infiltrated by large no. of ,mononuclear cells most of them lymphocytes, present edema in submucosa layer, few infiltration of lymphatic cells in submucosa layer (fig. 9), also showed hyperplasia of mucosa, apoptotic cells, parasites aggregation in the mucosa, some lymphocytes infiltrated in submucosa (fig. 10).

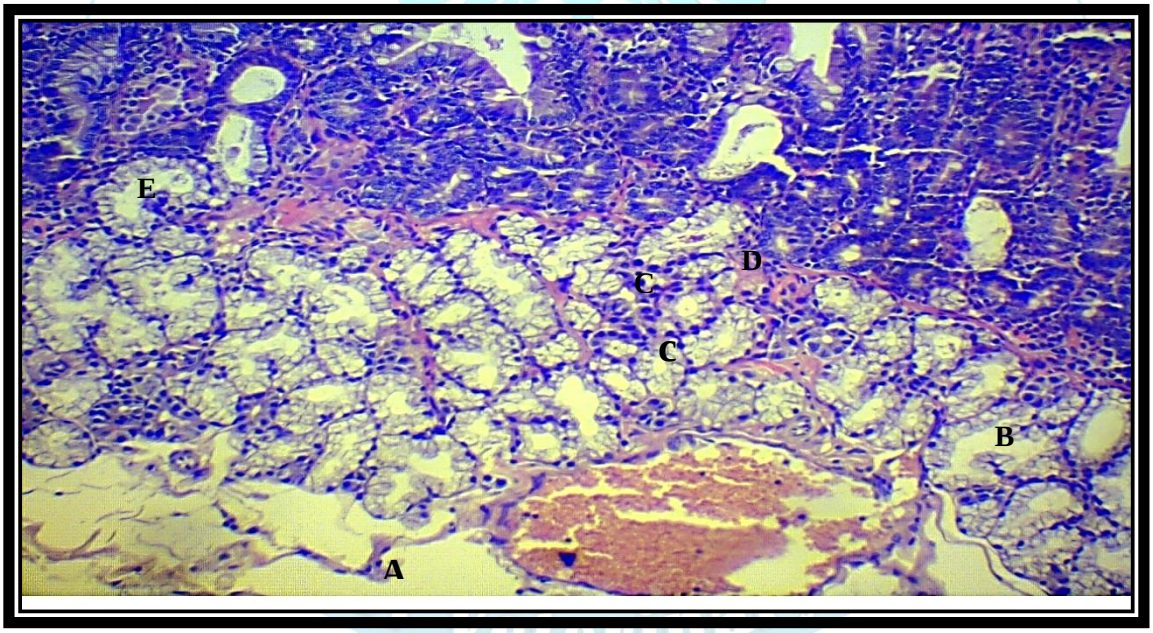

Fig. (6): Histopathological section of intestine in 3rd group shows: **a:** thick pseudomembrane covered the mucosa **b:** hyperplasia in colonic glands **c:** mononuclear cells infiltration **d:** eosinophilic materials **e:** damaged and destroyed gland (X20; H&E stain).

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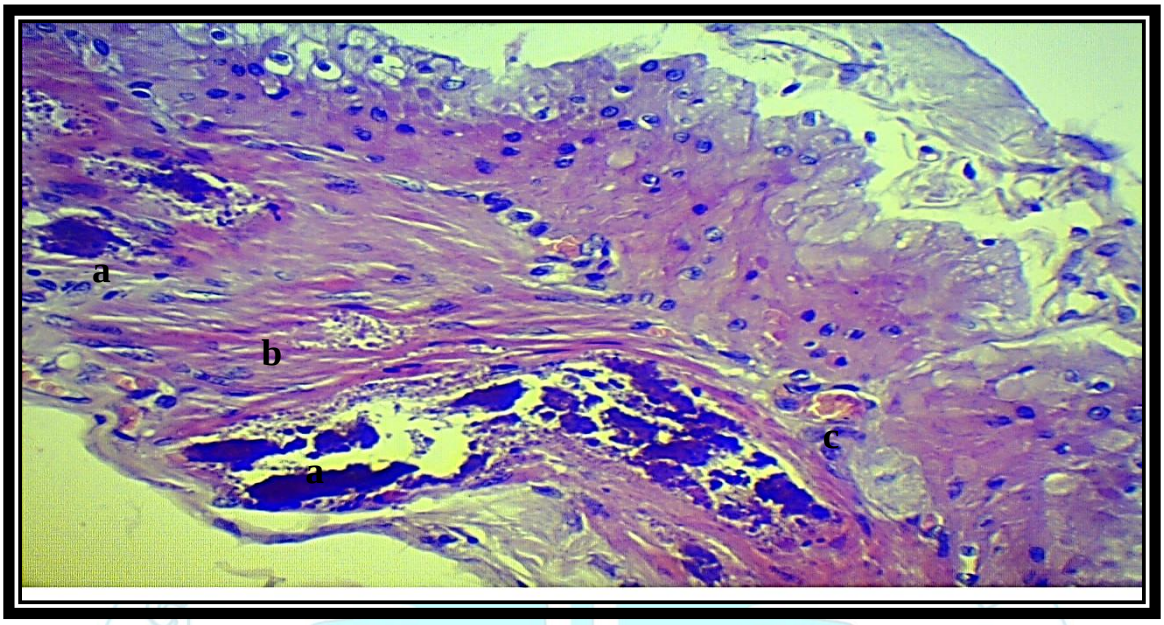
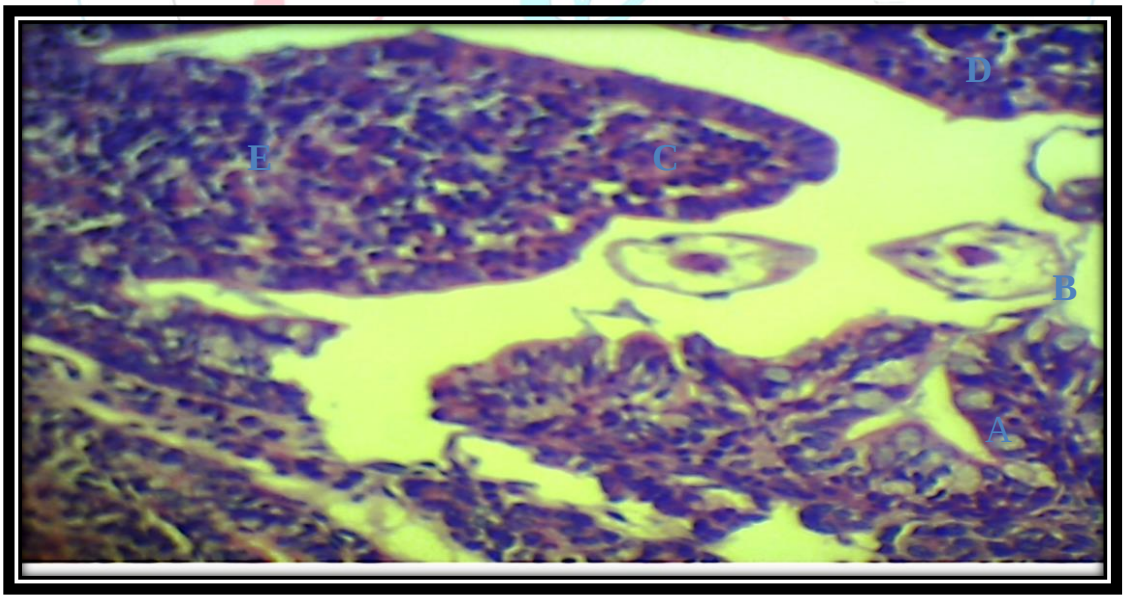


Fig. (7): Histopathological section of intestine in 3rd group shows: **a:** multiple areas of necrosis in mucosa and submucosa with dead neutrophils **b:** increase tissue thickening in fibromuscular **c:** congested of blood vessels (X40; H&E stain).



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Vol. 1, NO. 1, March 2023

Fig. (8): Histopathological section of intestine in 3rd group shows: **a:** increase in no. and size of goblet cells in mucosa layer **b:** lumen dilated contains several parasites **c:** congested of blood vessels **d:** lymphocytic cells infiltration in submucosal layer **e:** few basophilic lymphocytes infiltration (X40; H&E stain).

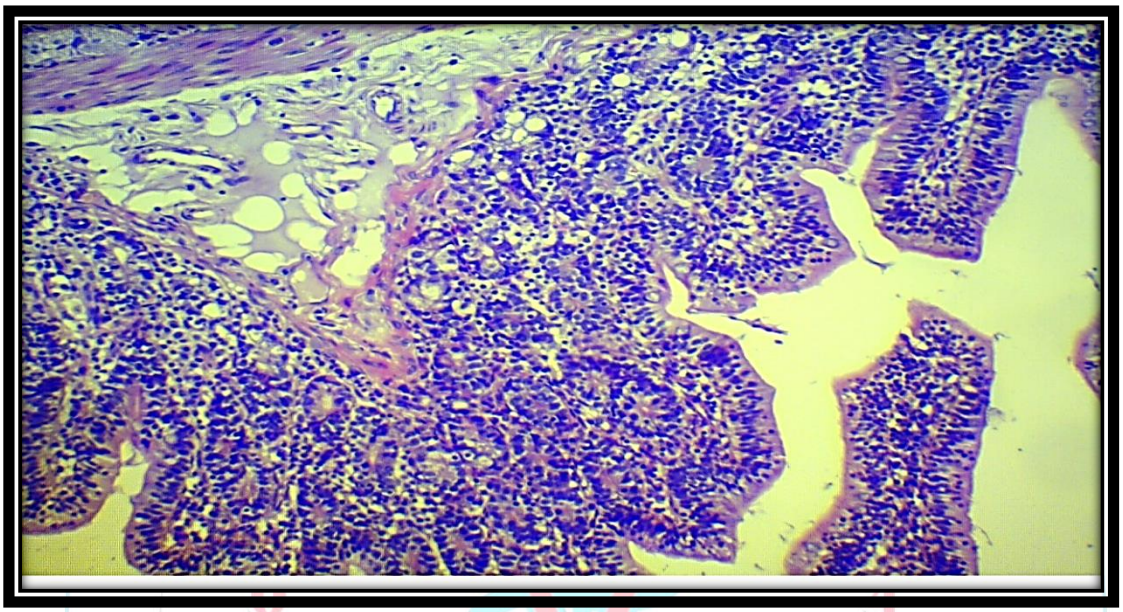

Fig. (9): Histopathological section of intestine in 3rd group shows: **a:** mucosa and submucosa heavily infiltrated by large number of mononuclear cells most of them lymphocytes **b:** edema in submucosa layer **c:** lymphatic cells infiltration in submucosa layer (X20; H&E stain).

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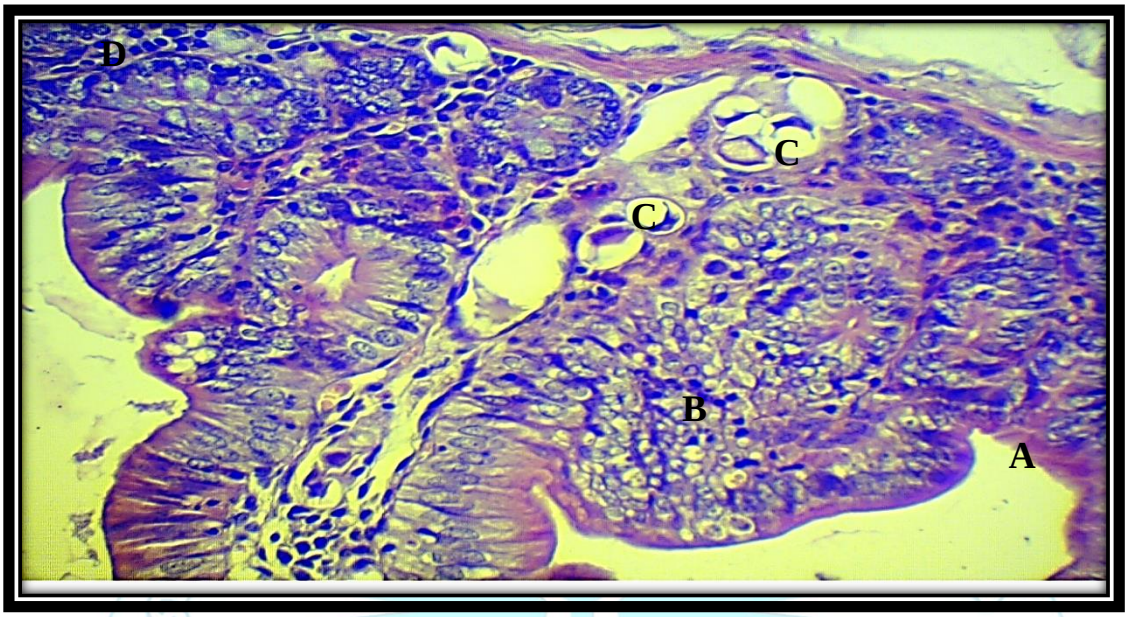

Fig. (10): Histopathological section of intestine in 3rd group shows: **a:** hyperplasia of mucosa **b:** apoptotic cells **c:** Parasite aggregation present in the mucosa **d:** some lymphocyte infiltrated in submucosa (X40; H&E stain).

Discussion:

In different regions of the world, the intestinal protozoan Giardia duodenal is frequently seen in cases of diarrheal sickness harming humans and other mammalian species. (Caccio and Ryan 2013) Due to the nature of the reason that results in digestive problems, the majority of the clinical symptoms found in the patients in the current study were documented in patients from inside the nation and in adjacent countries (Julio et al., 2012; Hasan *et al.*, 2020).

The The study's CBC data showed that accumulator rabbits infected with Giardia had modest to moderate selective leukopenia etc and lower absolute monocyte, neutrophil, and neutrophil counts. Bloodwork results obtained eight weeks after infection show that the chronic infection appears to be partially disintegrating over time. Intriguingly, and maybe counterintuitively given the results from other parts of this study, the total MCV counts are almost completely unaffected by infection (Khana *et al*., 2017).

Together, the hemoglobin properties suggest that Giardia infection only results in little erythropoiesis. There is evidence of increased erythrocyte production at 4 and 8 weeks following

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E-ISSN:2958-6178
Vol. 1, NO. 1, March 2023

infection, which is characterized by a boost in the number of large, immature red blood cells that cause macrocytic hypochromic anemia. These findings are consistent (Obaid, 2014;Khana *et al*., 2017).

The effects on the blood, monocytes, neutrophils, and eosinophils in this study have a source that is unclear, although it may be related to a variety of different factors. First, the parasite may have been the source of the selective leukopenia and erythropoiesis seen here. Second, given that lymphocyte function appears to be impacted by Giardia infection, it is conceivable that the selective leukopenia and erythropoiesis seen here are being mediated by alterations. Finally, because we lack access to colony samples from untreated, Giardia-infected animals, it is still feasible that the selective leukopenia and erythropoiesis are directly related to the Giardia infection itself (Varga, 2014) The inability to compare our findings to those found in this study and the lack of data from the Giardia-infected group of Rabbits are limitations of our investigation.

Giardia can cause the villi's enterocyte production to decline which lowers the number and height of these structures (Pires *et al*., 2013), the villous atrophy is a very common symptom of diarrhea, The strong association between villous atrophy and intestinal flaccidity shows that a thin-walled, atonic gut is a rough mirror of reduced mucosal thickness. epithelial lesions in the stomach, also looked to be often associated with diarrhea in our analysis, but given their modest occurrence, these lesions do not seem to be relevant to include in a case definition.

The immune suppression plays a significant role in the severity of injury with the Giardia in duodenum and liver cells.

Conclusions:

1. Laboratory animals experimentally infected with Giardia showed similar clinical signs to infected children in terms of bloody diarrhea, abdominal pain and flatulence.
2. Experimentally infection of *Giardia duodnalis* in rabbits have haematological changes

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represented by decrease in RBC, Hb, PCV, erythrocytes indices and variation in differential WBCs and thrombocytopenia. In addition abnormalities in erythrocytes morphology (Anisocytosis and Poikilocytosis) with macrocytic hypochromic anaemia.

3. Experimentally infection of *Giardia duodnalis* have sever histopathological changes in intestine in 3rd group.

Recommendations:

1. More research epidemiological study of infection in other pets animal like dogs and cats.
2. Control measure programs are often recommended to help reduce disease spread.
3. 3. Studies on the pest treatment for infected animals' trails must be applied.

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