Histopathologic and Histochemic study of mice infected with Giardia

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Abstract

This research was conducted to investigate the histopathologic and histochemic changes of small intestine and hepatic tissues of mice infected with *Giardia lamblia*. Results illustrated variable changes of intestinel represented by blunting and shortening of villous in the mucosa associated with hyperplasia and hypertrophy of epithelium in addition to localization of different stages of parasite. In hepatic tissue lesions characterized by diffuse vacuolar degeneration in hepatocyte and bile duct epithelium, also apoptosis has been seen concomitant with presence of parasite. Histochemically infected intestine showing a strong positive reaction with both PAS and AB (pH2.5) stains denoting an increase in mucopolysaccherid . In heptocyte strong positive reaction with both PAS and best cermine Also observed.

The present study suggest that infection of mice with Giardia is (firstly time reported) communicated with alteration in tissue due to biochemical changes that produced from the interaction between parasite and cellular components.

الخلاصة

تضمن هذا البحث معرفة التغيرات المرضية النسيجية وكيمياء النسيج لنسيج كل من الكبد والأمعاء الصغيرة للفئران المخمجية بطفيلي *G. lamblia* . بينت النتائج وجود اختلافات متباينة في الأمعاء تمثلت بتسطح وتقعر الزغابات في المخاطية يرافقها فرط تنسج وضخامة الظهارة فضلاً عن تموضع مراحل مختلفة من تطور الطفيلي ، وفي نسيج الكبد تميزت الآفات بتنكس فجوى في خلايا الكبد وظهارة القنوات الصفراوية فضلاً عن استماتة الخلايا الكبدية يرافقها تواجد الطفيلي .

وفي كيمياء النسيج أظهرت الأمعاء المخمجة تفاعلاً موجباً شديداً مع كل من تقنية فوق حامض الايوديك والاليشيات الزرقاء عند أس ها(2.5) وهي تمثل زيادة في المواد المخاطية متعددة السكريدات ، في نسيج الكبد لوحظ التفاعل الموجب الشديد مع تقنية فوق حامض الايوديك .

تقترح هذه الدراسة بان خمج الفئران بالجياردیا (سجلت لأول مرة) يرافقها تغييرات في النسيج نتيجة لتغيرات كيموحيوية نتجت عن التداخلات بين الطفيل والمكونات الخلوية .

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1. Introduction

Giardiasis is a protozoal disease caused by many species of Giardia one of them is *Giardia lamblia* (Syn. *G. Duodenalis* or *G. Intestinalis*), is one of the most common causes. World wide of intestinal infection in human and domestic animals like dairy calves, dog and cats associated with substantial economic losses (Thompson, 2000).

Symptomatic infection is characterized by diarrhea, epigastric pain, nausea, vomiting and weight loss and it is the common cause of waterborne outbreaks of diarrhea diseases in developed countries (Stifko *et al*., 2000). Giardiasis is self-limiting in >85% of cases, indictating that effective host defenses exist although chronic cases occur occasionally in the absence of apparent immune difficencies.

The parasite has simple life cycle consisting of two forms, the infectious form (cyst) , which is resistant to environment factors and the transmission of parasites to new host (Adam, 1991). The trophozoite form , which colonize in intestinal host and responsible for pathogenesis with in the gastrointestinal tract (Berendsen *et al*., 1987; ARS, 2003). Infection with *G. lamblia* in human, animal or mice are remarkably devoid of typical histological characteristic, in many cases of Giardiasis there was no inflammation or grossly altered epithelium (Oberhuber *et al*., 1997). In other cases have mild doudenitis with infiltration of neutrophils and lymphocytes. In experimental infections a modest increase in mucosal cell numbers is observed (Hardin *et al*., 1997; Venkatesan *et al*., 1997). Also the infection reported in hepatic tissue it cause inflammatory lesions represent by chronic hepatitis (Sotto & Gra, 1985). The aim of this study were conducted to observed the ability of this strain isolated from children to induce histopathological and histochemical changes in mice.

2. Materials and Methods

Twenty female mice were divided into two groups (n=10) mice in group one inoculated with 1ml of normal saline orally by using stomach tube (St) which consider as control group two inoculated with 1ml of suspension containing 7×10^7 cysts (Roberts-Thomson *et al*., 1976) and kept in a separate Cag, fecal samples were examined daily until cyst started appearing in the faces. Cysts of *G. lamblia* were obtained from the feaces of children with acute symptomatic giardiasis. Cyst were isolated according to the methods by Shnawa (1995).

Samples were liquefied with distilled water (10 : 1 v:v) filtered through four layers of gause, then sieved through 125 and 90 mesh, respectively, 3ml of 0.85 µ sucrose was added each 5ml of the filter at, centrifuged at 2000 rpm for five minutes. Using Hittich bench centrifuge. This was repeated and the sediment was responded in normal saline (0.9% NaCl).

Densities of cysts were quantified in ahemocytometer (Craft, 1982). The final suspension was adjusted to 7×10^7 cysts/ml and kept in the refrigerator until uses. After 21 days post infection, mice which are positively infected with *Giardia lamblia* were anesthetisd and dissected. A gross lesion reported if observed, 1 cm segment of the duodenum, carefully oriented on a filter paper and fixed in 10% neutral buffered formalin, also biopsy from liver was fixed. After routine processing and staining with haematotylin-eosin (H&E), a 4-6 Mm thick sections were examined.

The histochemical techniques for intestine and liver sections were listed below (with aproperial controls), were undertaken according to pears (1985) and Culling (1985).

carbohydrate

A- Mucosubstances

1. Periodic acid shift (PAS).

B- Acid mucopolysacharid

1- Alician blue (AB) pH 2.5

2- AB pH 1.0

- **C- glycogen**
- **– Best carmine**
- **D- Lipids**
- **– Glycolipid**
- **– PAS**
- **– Neutral lipid**
- **– Sudun black B.**
- **E- Protein**
- **– Bromophenol blue**
- **F- Calcium**
- **– von Gossa**

Chemical Conversions

Acetylating (acetic anhydride/ pyridine) 1-5 hrs.

Saponification (1% koH in 70% ethyl alcohol) 20 min

Methylation 60 c. for 4 hrs.

3. Results

No cross lesions has been detected in infected mice after post mortem (21 days post infection).

Histopathologic study

1. Liver

Histopathological sections of hepatic tissues infected with *G. lamblia* showed many lesions represented by diffuse vacuolar degeneration, congestion and dilatation of blood vessels (central vein, portal artery & vein) and presence of a trophozoit in cytoplasm of hepatocytes in addition to, degenerative changes of bile duct (Fig 1).

Also apoptosis of hepatocyte was obsereved (Fig 2). Some sections showed metastatic calcification (Fig 3), and parasitic emboli in blood vessels of portal area appear as thrombus contain many trophozoites reach to hundred also observed (Figs 4&5).

2. Intestine

The histopathological changes of infected intestine with *G. lamblia*, showed several lesions include, blunting and shortening of villous in mucosa, hyperplasia and hypertrophy of epithelial

lining villi, also developmental stages of parasite have been seen in epithelial cells (like trophozoit), especially have adjacent to the penetrating parasite (Fig 6). There was lymphocytic inflammatory response noticed in crypte (cryptitis), degenerative changes of epithelial cell lining mucosa and submucosal gland was also noticed associated with localization of parasites and presence of Giradia cyst in epithelial of submucosal gland (Figs 7&8). Sever fatty changes in myocyte of muscles (muscular layers) was observed associated with localization of tropozoite.

Histochemical changes:

1. Liver

Table-1- illustrated the histochemical techniques. The liver tissues revealed in all infected mice positive reactions against PAS (magenta)(Fig 9), Best carmine compared with the non infected control group (Fig 10). Negative reaction have been seen with specimens stained with AB pH 0.1, sudan black B (for demonstration of lipid) (Fig 11).

2- Intestine

Histochemical techniques (includes carbohydrate lipid and proteins) are shown in table 2&3. The epithelial lining villi and submucosal glands revealed strong positive reaction with PAS (Fig 12), AB (pH2.5) (Fig 13).

Best carmine in all infected mice, compared with non-infected control group give negative reaction. However moderate positive reaction have been seen with specimens stained with AB (pH 0.1) & negative reaction with Suden black B & promphenol blue.

Table -1- Histochemical reaction of hepatoytes in infected mice with *G. lamblia* and control

Techniques stains	Liver	
	Infected liver / hepatocytes	Control
Carbohydrate		
Periodic Acid Shift (PAS)	++ (magenta)	++++ (magenta)
Acetylating - PAS	–	–
Acetylating – Saponification PAS	++	++
Alcian (AB) pH 1.0	–	–
AB pH 2.5	++	–
Methylation - AB pH 2.5	–	–
Methylation – Saponification AB pH 2.5	++	–
Best carmine	++ (Red granule)	++
Lipid		
Suden Black B	++	–
Protein		
promophenol blue	–	+

+,++ staining intensity

Table -2- Histochemistry of normal intestinal villi and submucosal gland

Techniques stains	Intestinal villi	submucosal gland
PAS	+	+
Acetylating - PAS	–	–
Acetylating – Saponification	+	+
AB pH 2.5	+	+
Methylation - AB pH 2.5	–	–
Methylation – Saponification AB pH 2.5	+	+
AB pH 0.1	–	–
Best carmine	–	–
Lipid		
Sudan Black B	–	–
Protein		
promophenol blue	–	–

Table -3- Histochemistry of intestinal villi & submucosal gland of mice infected with *G. lamblia*

Techniques stains	Intestinal Villi			submucosal gland		
	Mild	Moderate	Sever	Mild	Moderate	Sever
PAS	+	++	+++	+	++	+++
Acetylation - PAS	–	–	–	–	–	–
Acetylation – Saponification	+	++	+++	+	++	+++
AB pH 2.5	+	+	++	+	+	+
Methylation - AB pH 2.5	–	–	–	–	–	–
Methylation – Saponification AB pH 2.5	+	+	++	+	+	+
AB pH 0.1	–	–	–	–	–	–
Best carmine	–	–	–	–	–	–
Lipid						
Sudan Black B	+	+	+	–	–	–
Protein						
promophenol blue	–	–	–	–	–	–

+,++,+++ staining intensity

4. Discussion

Infection with the ubiquitous intestinal parasite *G. lamblia* are a major public health problem world wide yet the interaction of the parasite with host are only poorly understood. Results of this study elucidate that mice is used to investigat the histopathological & histochemical of this strain of *G. lamblia,* although many reaserch reported that mice is not already infected only with *G. muris* and GSM-H7 strain (Byrd *et al*., 1994). Our findings of this strain of *G. lamblia* censes many changes histopathologically and histochemically in both duodenum and liver. In agreement with (Hill *et al*., 1983), who mentioned that, development factors are probably important in the natural resistance of mice to *G. lamblia* as in suckling mice (3 days old) are readily infected but clear infection spontaneously by 17-20 days of age. Such factors could encompass many different aspects of intestinal function and are poorly understood, it is possible that the intestinal microflora in normal mice might be interfere with infection, So this strain isolated from children may be very virulent, it may be similar to Gs SM-H7 strain (so it needed many future studies). Also this infections may be occur due to interaction between intestinal tract, flora with Giardia. which makes it a clinically relevant model pathogen. The reasons for the differences between *G. lamblia,* Gs/M-H7 and other *G. lamblia* strain is regarded to inflexibility of adult mice are not clear. Histopathological sections of intestine revealed variable changes characterized by hyperplasia and hypertrophy of the villous in agreement with Alkennany *et al*., 2003. all these changes that take place leads to malabosorption due to vitamins B12 and folic acid deficiencies (Guimaraes *et al*., 1999).

In this study tropozoit & cyst of *G. lamblia* will be demonstrate at brush border and in epithelium lining villi and submucosal gland. Under light microscope, this observation was disagreement with (Lars, 2003), Who elucidate that parasites don't invad the mucosa and causes little or no mucosal inflammation. Giardiasis is normally transient, indicating the existence of effective host defenses although infection can occur Andero (2007) who observed that epithelial barrier dysfunction.

Results of this study revealed ability of *G. lamblia* trophozoites to reach the hepatic tissue and produce variable lesions, it may be due to that Giradia known to contain and / or release a variety of potentially toxic substances, such as proteinases and these proteinases activate host proteins receptors (Scott, 2000). and have ability to localization hepatic tissue throw portal area to produce many changes such as vacuolar degeneration, fatty change and apoptosis via the mucosal injury after attachment of large numbers to brush order, or may be due to decreased in activity of enzymes that localized in brush border (Scott *et al*., 2000; Nain *et al*., 1991).

Tropozoit and dormant cyst that serves in the transmission of parasite and it's responsible for pathogenesis with in the gastrointestinal tract. Increased proliferation of intestinal lymphocyte has been seen associated with giardiasis in a number of report (Gillon *et al*., 1982; Oberhuber *et al*., 1996). Histopathologic results of liver section in infected groups revealed many changes particularly, degeneration and apoptosis in addition to presence of trophozoid & cyst in hepatocytes and in luman of blood vessels. Firstly which appeared as parasitic emboli (Figs 4,5) these changes was reported, In patient with chronic giardiasis, enterocyte apoptosis

showed by Andero (2007). In hepatic tissues it may strain dependent activation hepatocyte apoptosis as well as degeneration, it may occur in absens of any other type of cells. Apoptosis is plays crucial roles in the interaction between the host and the parasite, this includes in rate and adaptive defense mechanisms to restrict, intracellulare parasite replication, in addition to, regulatory functions to module the hosts immune responses. Not surprisingly, Giardia also extensively modifies apoptosis of its own host cells or of uninfected by stander cells. Since *G. lamblia* resides and undergoes critical phases of its life cycle within the intestinal epithelium and hepatic tissue, it may be has developed strategies to alter epithelial and hepatic apoptosis that may enhance its survival within that environment. Moreover, apoptosis in hepetocyte infected with *G. lamblia* may be occurs due to oxidative stress management is believed to be accomplished by a thioredoxin reducatase class of disulphide reducatase, which uses cystine as primary electron acceptor (Brown *et al*., 1996b). Recent finally have revealed that the cytoplasmic enzyme NADH menadione oxidoreductase also play a role in oxidative stress management & can significantly enhance growth upon over expression (Sanchez *et al*., 2001; Lei *et al*., 2006).

Histochemically, *G. lamblia* infected intestine and hepatic tissues showed positive reaction with PAS, AB pH 2.5, Sudan black B, Best carmine. These results indicated that infection with Giardia cause increase secretion particularly from goblet cell in epithelial lining villous of mucopolysaccharides, which facilitating attachment and adhesion of the parasite to intestinal villi. Moreover , mucopolysaccharide, play important role in defense mechanism against Giardia due to their content of IgA & IgM (Hick *et al*., 2000; Farthing and Coke, 1987).

The present study revealed positive reaction of cyst or trophozoit with shift's reagent it was suggested that the tropozoit, filaments, cyst are composed of carbohydrate (polysaccharide)-protein complex (glycoprotein), With respect to the carbohydrate component and the major monosaccharide consistent is N-acetyl (Jarrol and Paget, 1995).

Galactosamine (gal NAC), which synthesized de nova from endogenous glucose through pathway of inducible enzymes during encystment (Macechko *et al*., 1992), and incorporated into an insoluble material resembling Giardia cyst wall filaments. Additionally, glucose is required for trophozoite growth for energy storage, so these results elucidate that trophozoit uses glycogen & other types of succharide such as mucopolysaccharide as an energy reserve for growing & metabolism in liver & intestines . Negative results for the reaction of promphenol blue with hepatocyte in infected mice give evidence for depletion of protein. It may occurs due to that trophozoite uses protein for synthesis cell wall protein and transport to the periphery during encytation. So because all these changes in Giardia infection diarrehasis causative combination of leak flux, malabsorption and malsecretion, down regulation of light junction protein elaudin 1, and epithelial apoptosis causes failure of sodium- dependent glucose absorption, which results in active chloride ion secretion. Consequently, water enters the human, eliciting diarrhea (Troegers, 2007).

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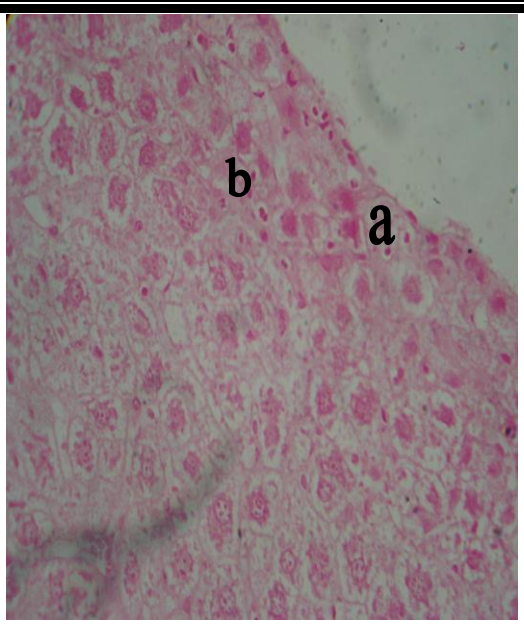

Fig 2: Histological section of liver mice infected with *G.lamblia* (21days p.f), showing apoptotic hepatocyte (a) in addition to presence of parasite in cytoplasma of hepatocyte (b) H & E. 190x.

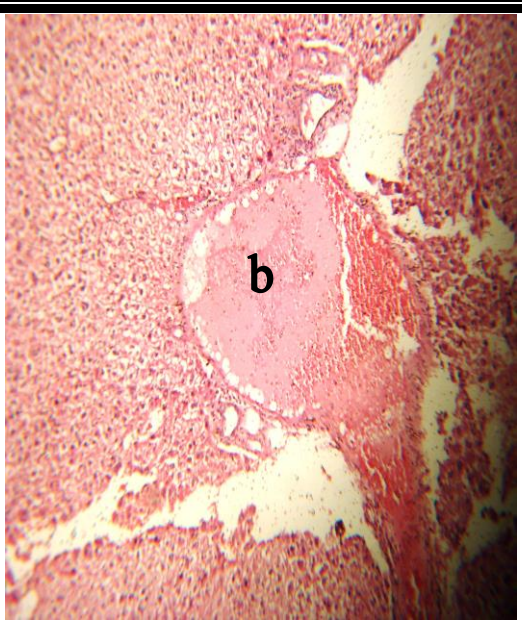

Fig 1: Histological section of liver mice infected with *G. lamblia* (21 days p.I), showing diffuse vacuolar degeneration (a) associated with presence of parasite and sever congestion (b) H & E. 460x.

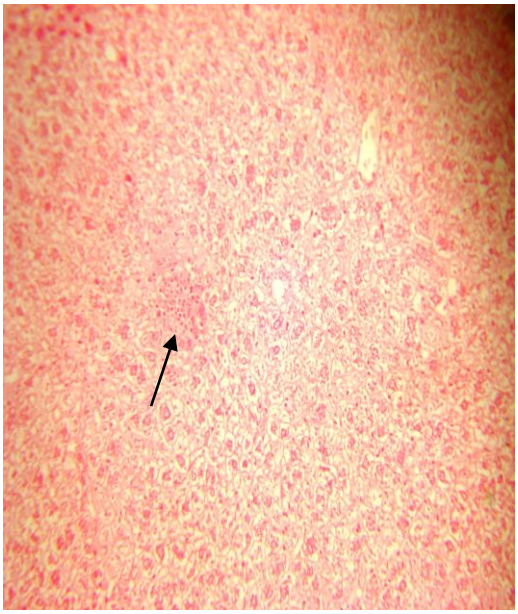

Fig 4: Histological section of liver mice infected with *G. lamblia* (21 days p.f), showing parasitic emboli in lumen of central blood vesseles H & E. 140x

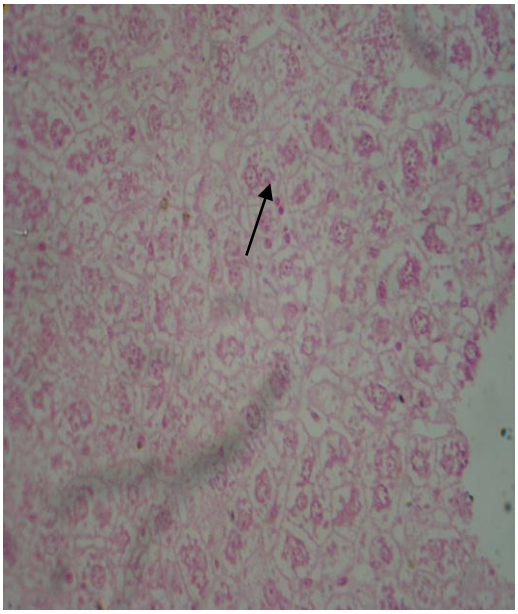

Fig 3: Histological section of liver mice infected with *G. lamblia* (21 days p.f), showing black granules (a) in hepatocyte and arround blood vesseles (b) Vone Gossa. 320x

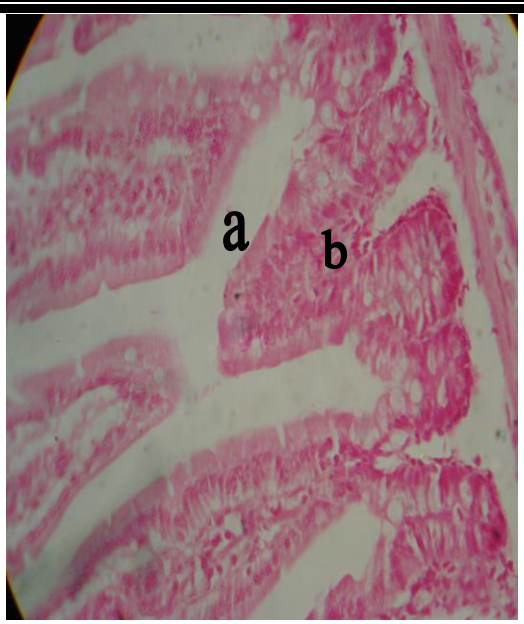

Fig 6: Histological Section of intestinal mice, showing blunting and shortening of villous in mucosa (a) in addition to cryptitis(b)H & E.

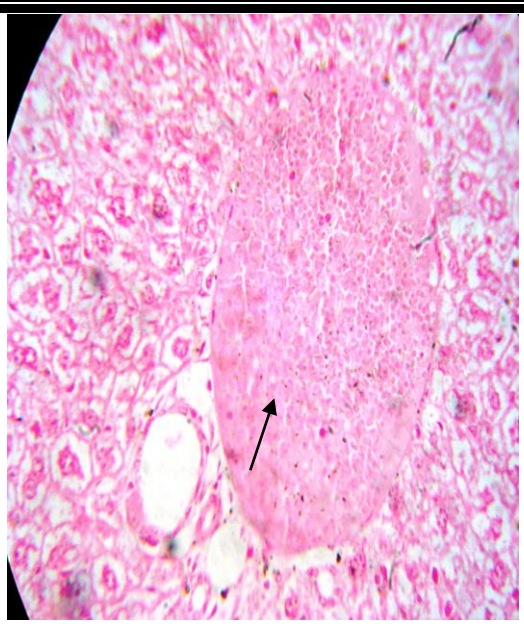

Fig 5 : High magnification of fig 4, showing emboli containing many trophozoit (arrow) H & E.

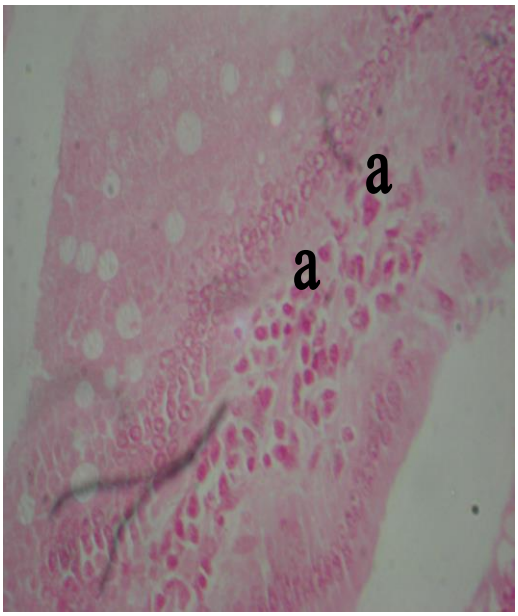

Fig 8: High magnification of Fig 7, showing hyperplasia of epithelial cells lining villia (a) and localization of different stages of parsite in crypt (b) H & E. 460x

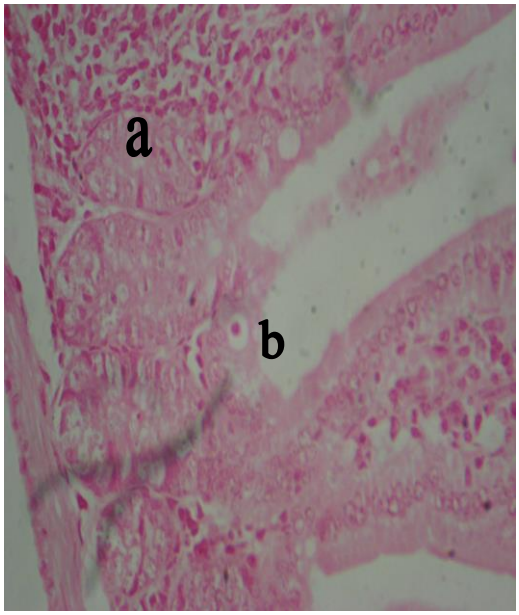

Fig 7: Histological Section of intestinal mice, showing sever proliferantion of inflammatory cells paraticularly lymphocytes (a) and presence of the cyst with other stages in epithalial cells of villous and submucosal gland (b) H & E. 390x

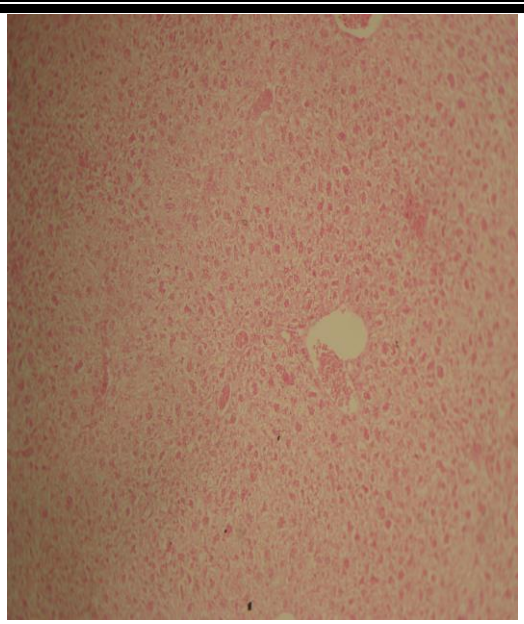

Fig 10: Histological section of liver mice infected with *G. lamblia* (21 days p.I), showing positive reaction with best carmine (→). 390x

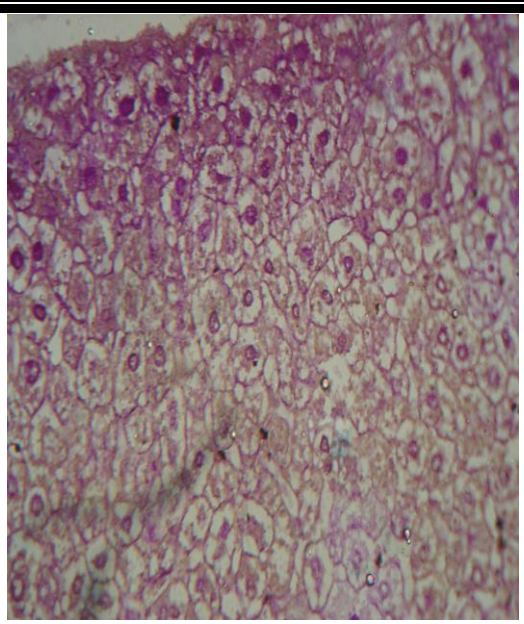

Fig 9: Histological section of liver mice infected with *G. lamblia* (21 days p.I), showing strong positive reaction with PAS staine (→). 460x

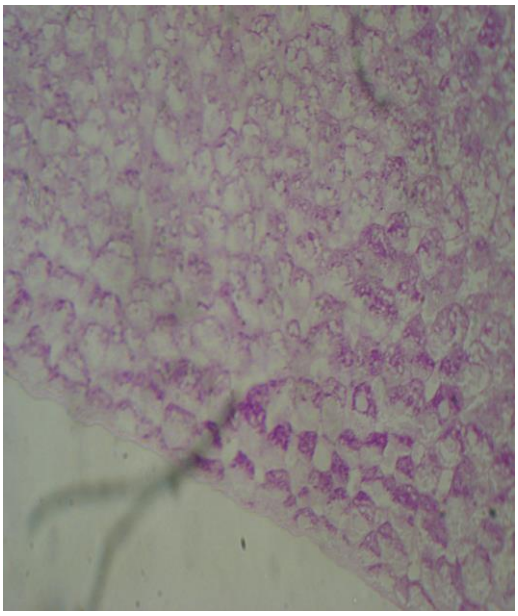

Fig 12: Histological Section of intestinal mice, showing strong positive reaction with PAS staine (→). 320x

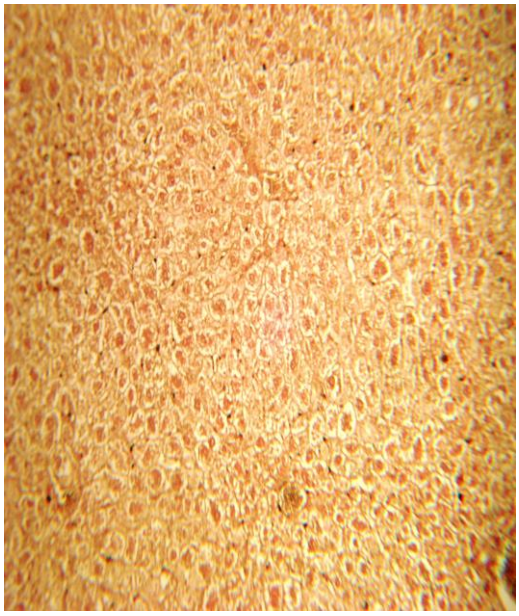

Fig 11: Histological section of mice liver infected with *G. lamblia* (21 days p.I), showing negative reaction with Sudan black B.

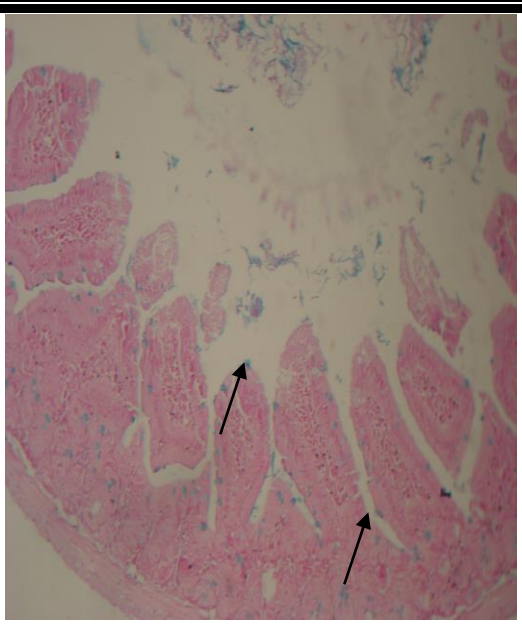

Fig 13: Histological Section of intestinal mice, showing positive reaction with Alcian blue pH(2.5) (→). 320x