# HISTOCHEMICAL CHANGES ON SMALL INTESTINE MUCOSA INFECTED WITH GIARDIASIS IN WISTER ALBINO RATS

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## ABSTRACT

Brush border enzymes activity were decreased in group infected with giardiasis comparing with the control group, probably represent a direct effect of this parasite on the brush border of the enterocytes. The present study indicated that mucosal enzymes (alkaline phosphatase and lipase) levels were altered in infected rats after (1-2) weeks post infection compared to control group. concentration of both enzymes were increased during infection and this phenomenon was taken as mucosal marker for malabsorption.

## INTRODUCTION

*Giardia lamblia* is the most binucleate flagellated parasite that infect the upper intestinal canal of human and many animal species[1].

Although [2] described the genus *Giardia* in greater detail , but the parasite as an important human pathogen has been recognized at the last 40 years [3]

*G.lamblia* has a simple life cycle consisting of an infective cyst and vegetative trophozoite . The trophozoites attach to the mucosa of the duodenum and jejiunum , where they multiply rapidly and responsible for symptomatic illness [4].

Cyst are infectious as passed in the host stool , where ingested by a potential host , excystation is stimulated by passage through the stomach and the motile trophozoite migrate to the small bowel to complete the life cycle [5].

The lining of the small intestine posses gross and microscopic devices for increasing the surface area available for digestive and absorptive activities [6].

[7] showed that the glycocalyx contains a number of enzymes ( brush border enzymes eg . lactase , peptase , sucrase , lipase and alkaline phosphatase ) which are important for digestion and transport .

Previous study performed by isolating the striated border by differential centrifugation showed that the striated border is the site of action of the enzymes which bound to micro- villi [8].

Brush border membrane forms digestive – absorptive surface and any changes that occurred in its structural and functional organization seem to provide a rational explanation of certain clinical conditions which impaired digestion and absorption [9].There for the aim of this worke is to examin the effects of giardiasis on the mucous layer of the small intestine , in order to clarify the histo- pathogenesis of *G.lamblia* by using histochemical stains.

## MATERIALS AND METHODS

**Samples collection and examination .**

Giardiasis samples were collected from patients aged 1-10 years old from Al-Sindibad center for children care .The specimens were brought directly to the laboratory by sterile prelabeled plastic vial°s on the same day of collection . *G.lamblia* trophozoites and cysts were searched and confirmed by direct fecal wet preparation method [10] .

**Experiment animals .**

( Wistar albino) rats aged 8-10 weeks was purchased from drug control center Baghdad .The rats which were used for breeding checked by three separated examinations to be sure that the animals were free from any intestinal parasites including giardial infection .The animals were maintained under controlled temperature ( 25±C°)

**Cysts purification**

*G.lamblia* cysts were obtained from freshly excreted feces .The cysts purification method of the present study was done by slightly modifying the procedure of [11,12 ].

The purified cysts suspensions were used freshly in the experimentes infection of rates or stored at 4C° in distilled water until needed .The density of cysts was quantitated by a hemacytometer.

**Inoculation of suckling rats**

Eighteen suckling rats (18-21) days old in delivered litters were separated in to 6 rats as a control group and 12 rats as experimental group.In all the experiments , $10^3$ cysts of freshly isolated cysts were administered in 0.5 ml of 0.85 % NaCl by gastic incubation .All rats were given a single feeding of *G.lamblia* cysts [13] .

**Naphthol phosphate method for alkaline phosphatase ( Gomori , 1952).**

Sample of duodenum , jejunum and ileum related to 6 infected rats and 2 control were removed after (1-2) weeks and immediately washed with cold normal saline .The content from each specimens were gently pressed out by cold 0.1 M phosphate buffer (pH 7.5) frozen sections were prepared by cryostate microtome and serial section of (8 µm ) thickness were processed as described by [14].

**Tween 80 method for lipase ( Mahadevan *et.al.*,1969)**

six infected rats were killed after (1- 2) weeks post – infection in addition to 4 control animals were used pieces of duodenum , jejunum and ileum ( 5 cm from the ileocecal valve ) were dissected out , washed immediately with cold 0.1 M phosphate buffer (pH 8) and serial frozen section of (10 µ) thickness were cut and processed according to [15].

**Results and discussion**

The present study showed that the brush border enzymes ( alkaline phosphatase and lipase) activity were changed and altered in infected animals compared to control rats and this phenomenon detected by microscopic examination of duodenum , jejunum and ileum section .

Both enzymes were normally found in intestinal epithelium ( Fig. 1 A,B,C) and ( Fig2.A,B,C).these enzymes activity accomplished in the intestinal mucosa rather than in the intestinal lumen [16]..The activity of the brush border enzymes is not in the same levels but rises from low values near the crypts and reaching peak value at or near the villus tips [17].

Alkaline phosphatase activity was decreased during the primary infection , the concentration of enzymes was increased at 1-2 weeks post – infection ( Fig 3 A,B) , where the enzymes is present in strength , the precipitate is usually dark brown ands granular and some with yellowish brown ( Fig 4 A,B) .In this study we have examined the progression of changes in the activity of these two enzymes , which considered as a functional marker for physiological alteration in the mucosal layer .

[18] showed that the intestinal mucosa was modified during giardiasis and this lead to considerable decrease in the activities of its enzymes .

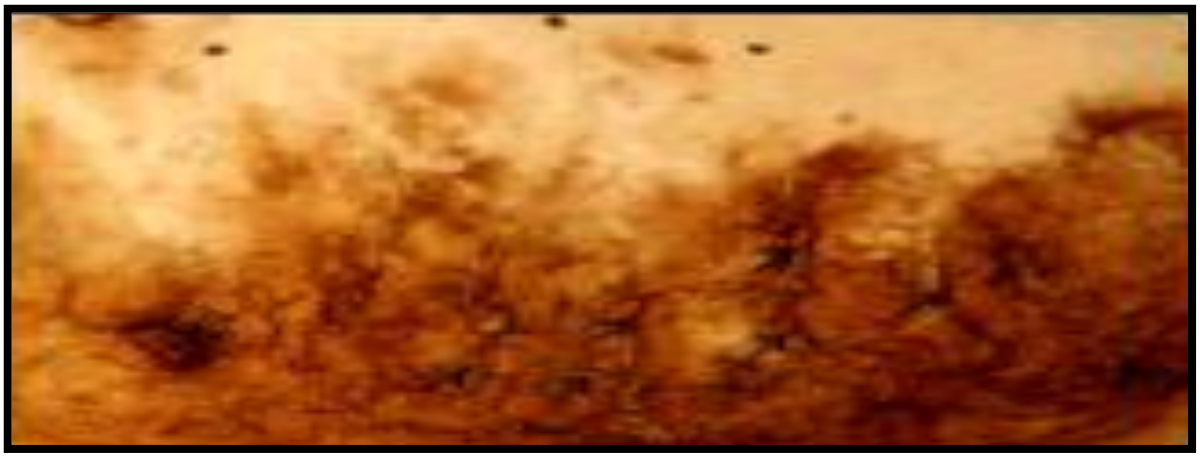
The enzymes activity of lipase was shown the same pattern of changes .The intensity of the staining was related to the enzyme concentration in different sites of tissue (Fig. 5 A,B,C).The dark brown or black sites of sections showed to contain high amount of lipase enzymes (Fig 6 A,B,C). The concentration of both enzymes were highly , this related to direct effect of giardiasis on structure changes along the border villi and crypts surface , and intense

staining clarified this suggestion which it is also explained the reduced enzymes absorption through the brush border .

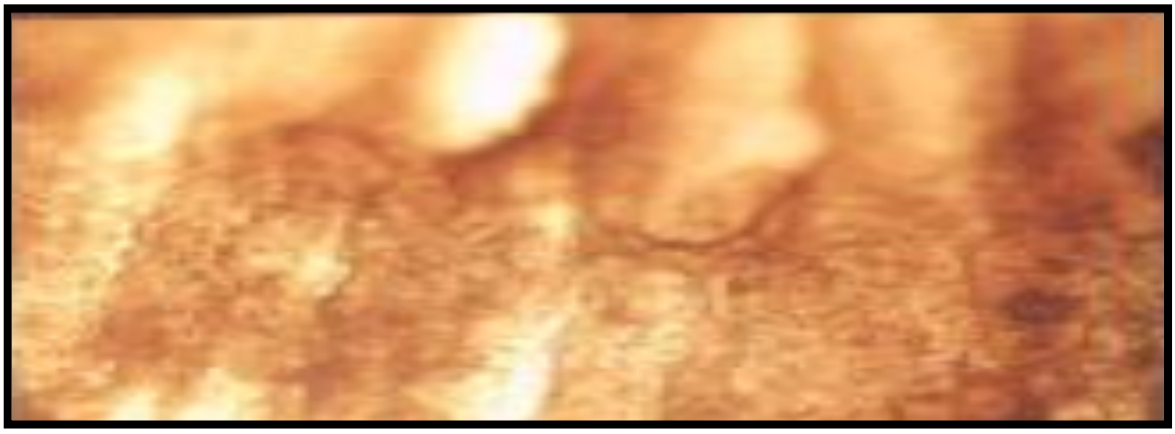
These results agreed with findings of [19] who showed that the alteration in glycocalyx structure and any modification in mucosal surface showed lead to alteration in the level enzyme activities.

[20]and [21]showed that decrease of enzymes activities to pancreatic inflammation or pancreatic duct blockage because of large number of *G.lamblia* trophozoites migration .

**Fig 1 :Histochemical preparation of control small intestine showing the distribution of alkaline phosphatase**
**A-section of duodenum showing the alkaline phosphatase enzyme (thick arrows) . Gomory method. X300**



**B-section of jejunum showing the alkaline phosphatase enzyme (arrows) . Gomory method. X300**



**C-section of ileum showing the same distribution of phosphatase enzyme. Gomory method. X300**

**Fig 2 :Histochemical preparation of control small intestine showing the distribution of lipase enzyme.**
**A-section of duodenum showing the lipase enzyme (arrows) . Tween method. X800**

**B-section of jejunum showing the lipase enzyme (arrows heads) . Tween method. X800**

**C-section of ileum showing the lipase enzyme (thick arrows) . Tween method. X800**

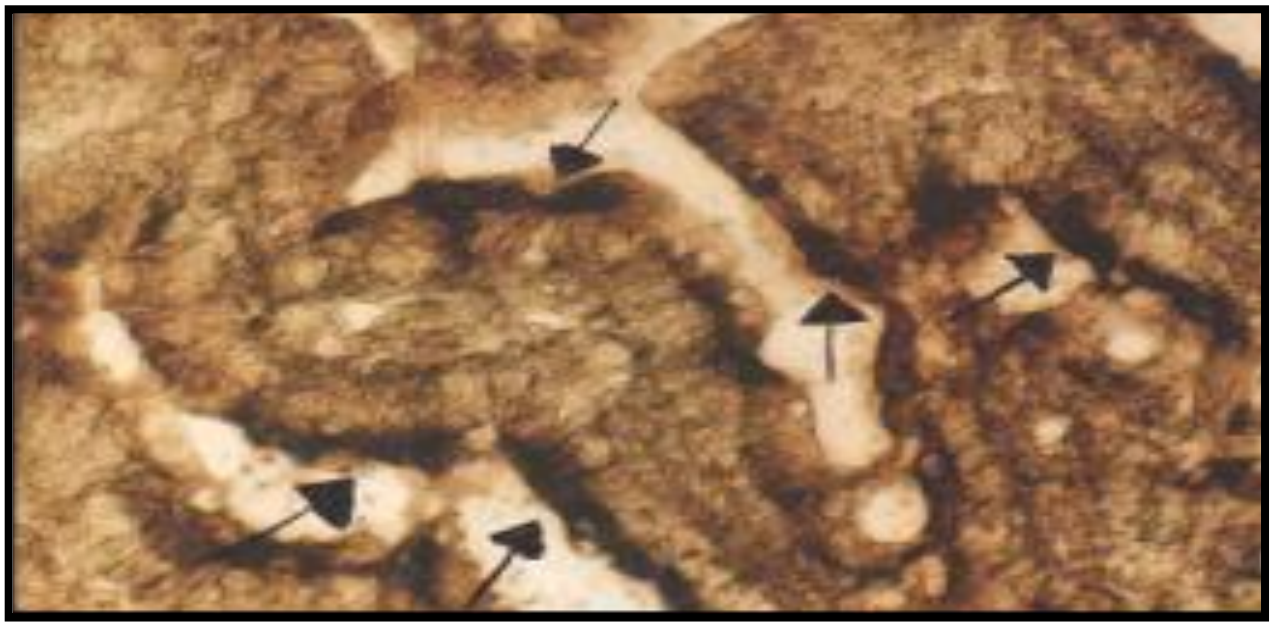


**Fig 3 :Histochemical preparation of infected rat small intestine two weeks post infection**

**A-infected of duodenum showing the alkaline phosphatase precipitate strongly (thick arrows) . Gomory method. X600**

**B-infected of jejunum showing the alkaline phosphatase (thin arrows) . Gomory method. X300**

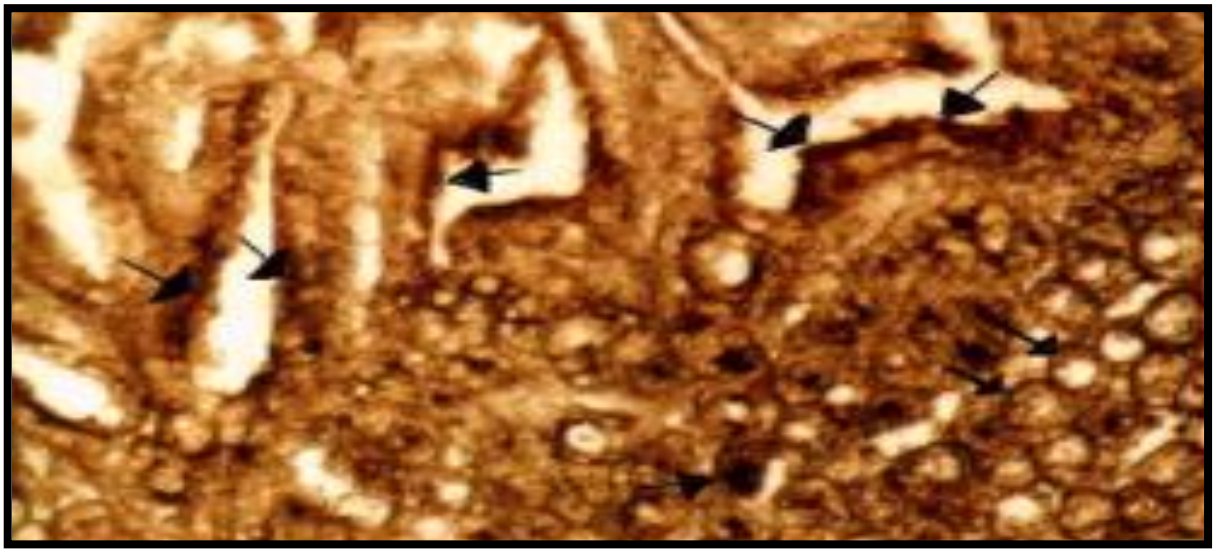
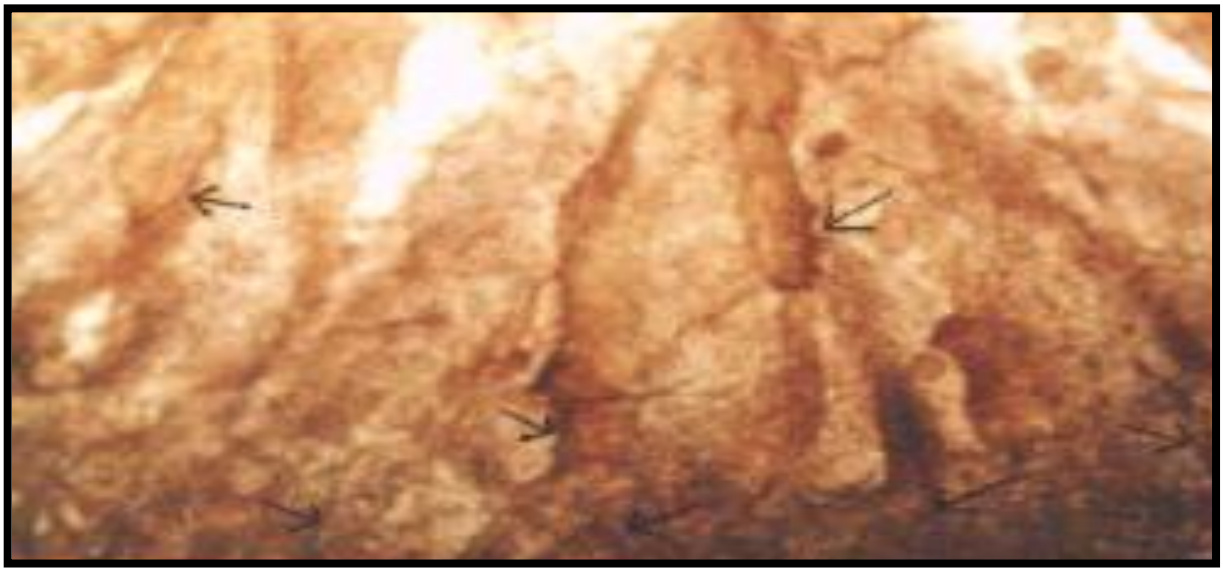


**Fig 4 : Histochemical preparation of infected rat duodenum and jejunum A-section of duodenum , three weeks post - infection showing the alkaline phosphatase (thick arrows) . Gomory method. X250**



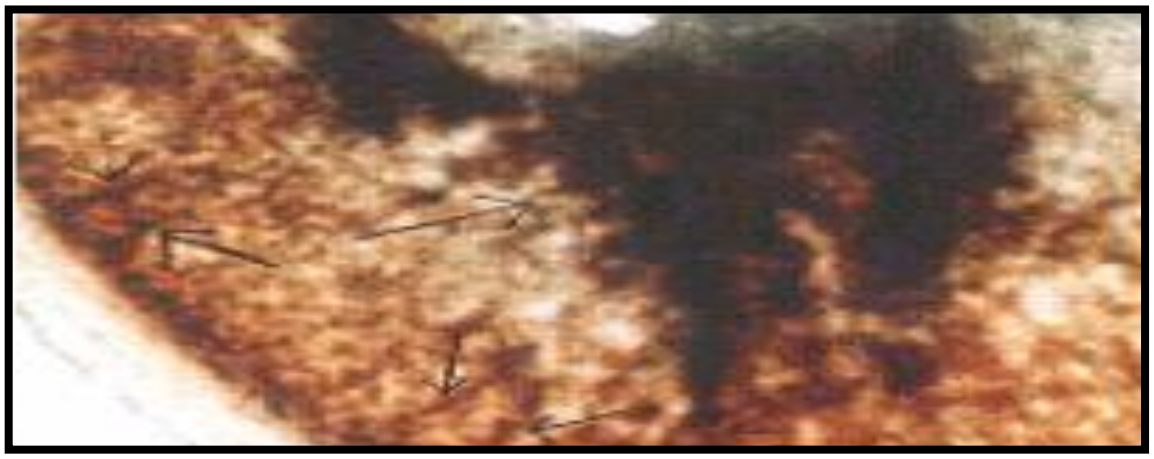
**B-section from infected jejunum , three weeks post - infection showing the alkaline phosphatase (arrows) . Gomory method. X250**

**Fig 5 : Histochemical preparation of infected rat small intestine one week post infection , showing the distribution of lipase enzyme**
**A- Duodenum section showing the distribution of the lipase ( thick arrows) . Tween method. X800**

**B- Jejunum section showing the distribution of the lipase ( thin arrows) . Tween method. X400**

**C- Ileum section illustrate the lipase localization ( thin arrows) . Tween method. X400**

**Fig 6 : Histochemical preparation of rat small intestine two weeks post infection**
**A-infected of duodenum showing the lipase (thick arrows) . Tween method. X600**



**B-infected of jejunum showing the lipase (thin arrows) . Tween method. X400**

**C-infected ileum showing the lipase localization (thin arrows) . Tween method. X400**

## التغيرات الكيميائية النسيجية في الأمعاء الدقيقة المصابة بداء الجيارديات في الجرذان البيضاء

مها خليل الملاك ؛ ضياء قاسم سكر ؛ بشرى حسين شناوة
قسم علوم الحياة - كلية العلوم - جامعة البصرة – البصرة – العراق

**الخلاصة**

سجلت النتائج حصول نقصان في الفعالية الانزيمية لانزيمات الحافة الحرة في الحيوانات المصابة بداء الجيارديات مقارنة مع مجموعة السيطرة.

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