Annexure-IX

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002

PERFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. Title of the Project**: "To study the antagonistic effect of symbiotic in Giardia intestinalis infected BALB/c mice"**
2. Name and Address of Principal Investigator: **Prof. Geeta Shukla, Department of Microbiology, Panjab University, Chandigarh.**
3. Name and Address of Institution: Department of Microbiology, Panjab University, Sec-25, Chandigarh
4. UGC approval Letter No. and Date: **F.No. 42-472/2013(SR) dated 30.06.2014**
5. Date of Implementation: **01.04.2013**
6. Tenure of the project: **From: 01.04.2013 to: 31.03.2017**
7. Total grant allocated: Rs. 12,61,700
8. Total grant received: Rs.11,79,560
9. Final Expenditure: Rs. 11,29,656
10. Title of the Project**: "To study the antagonistic effect of symbiotic in Giardia intestinalis infected BALB/c mice"**
11. Objectives of the project:

1. To study the effect of synbiotic supplementation on the outcome of murine giardiasis on the basis of stool examination, lactobacilli count, body mass and trophozoite count.
2. To assess the effect of synbiotic on the intestinal disaccharidases vis a vis histopathological alterations in intestinal tissue.
3. To investigate the underlying mechanisms of protection by the synbiotics in terms of
    a) Nitric oxide and secretory IgA antibody levels
    b) Gut T cell subset response (immunophenotyping)
    c) Oxidative and antioxidative properties

12. Wheather objectives were achieved: yes (below)

RESULTS **Annexure-VIII**

Giardia lamblia is a prevalent intestinal parasite and cause of diarrhoeal disease throughout the world. Giardiasis is often regarded as asymptomatic and can be characterised by gastrointestinal disorders, such as diarrhoea, nausea and vomiting, malabsorption, weight loss, and fatigue (Thompson et al., 2016). Several drugs can be used to treat *Giardia* infection like metronidazole, tinidazole, nitazoxanide, paromomycin, quinacrine, and furazolidone (Cimerman et al., 2007). Studies have shown that probiotic/prebiotic supplementation modulated the gut morphology and improved the immune status in malnourished-*Giardia*-infected mice (Shukla et al., 2008, 2010) but application of synbiotic in giardiasis is not available, thus the present study was generated to study the effect of synbiotic treatment *Giardia*-infected BALB/c mice.

BODY MASS

Table 1 and figure 1 show the pattern of body mass during entire period of experimentation. It was observed that there was a decrease in body mass of mice belonging to all the groups at each point of observation but decrease was maximum in *Giardia* infected mice. Surprisingly, it was observed that body mass dropped with administration of probiotic, prebiotic and synbiotic prior to the *Giardia* infection. The present observation is contradictory to earlier observation which is made in murine giardiasis (Shukla et al., 2008, 2010, 2013). This may be due to the fact that isolated probiotic may not have been able to colonise in gut, and led to reduction in body mass.

Table 1: Body mass (g) of mice belonging to different groups on various days of post inoculation

DAYS	Control	*Giardia*-infected	Probiotic	Prebiotic	Synbiotic	Probiotic-*Giardia* infected	Prebiotic-*Giardia* infected	Synbiotic-*Giardia* infected
DAY 1	20±0	20±0	25 ± 0	24.8 ± 0.64	25 ±0	22.2 ± 2.1*	32 ± 0.04*	24.75 ± 0.23*
DAY 3	20±0	20±0	21.5 ± 0.7	23 ± 0.64	24.5 ± 0	19.4 ± 2.1*	30.77 ± 0.04*	25.22 ± 0.23*
DAY 5	20±0	17.5±0.98	22 ± 0.76	23 ± 1.25	23.5 ± 0.73	20.9 ± 1.25*	31.6 ± 0.38*	24.9 ± 0.33*
DAY 7	22±1.21	17.5±0.98	22 ± 0.88	23 ± 1.25	23 ±0.73	19.4 ± 0*	34 ± 1.76*	24.85 ± 0.21*
DAY 9	22±1.21	17.5±0.98	22 ± 1.02	23.5 ± 1.09	23 ±0.87	17.5 ± 1.24*	32.5 ± 1.76*	24.17 ± 1.24*

Values are Mean ± SD, *p<0.05 v/s *Giardia*-infected.

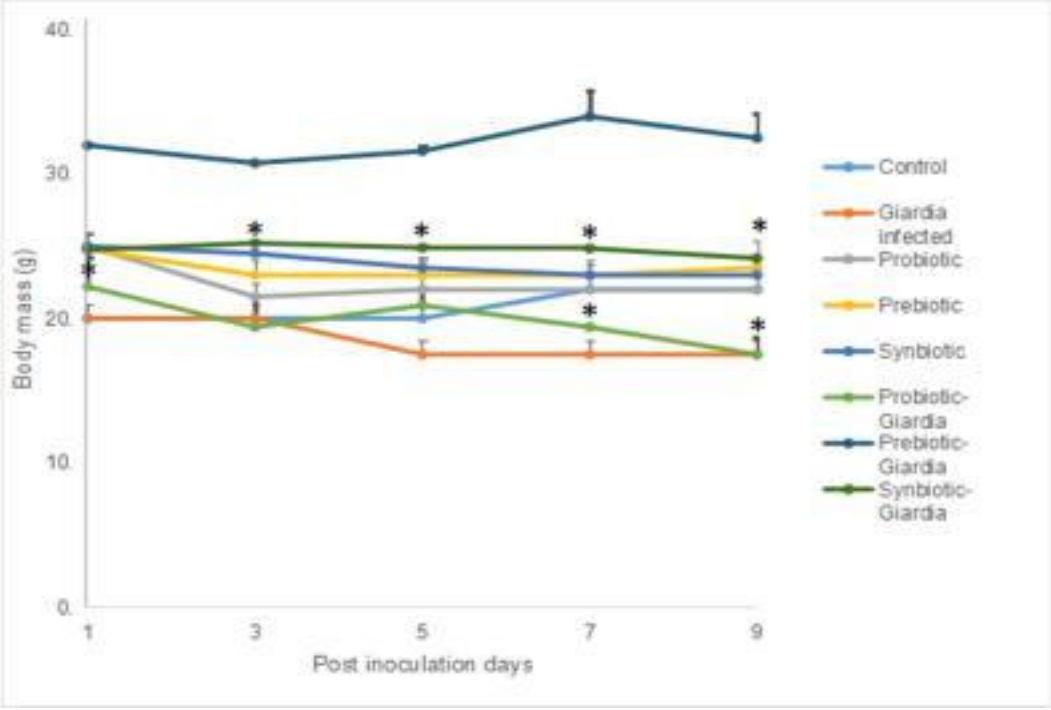


Fig 1 : Body mass (g) in different groups of mice. Values are Mean ± SD, *p<0.05 v/s *Giardia*-infected.

CYST COUNT

Table 2 and figure 2 show *Giardia* cyst count in faeces of mice belonging to various *Giardia* infected groups. It was observed that cyst count was maximum in *Giardia* infected mice but prior administration of probiotic, prebiotic and synbiotic to infection, led to significantly decrease in cyst count initially. Further, it was observed that with progress of *Giardia* infection in synbiotic treated mice, the cyst count increased significantly compared to probiotic and prebiotic group. The present observation of reduced cyst count maybe either due to better survival of probiotic or effective adherence and colonisation in the gut by probiotic organisms and is in accordance with earlier studies (Goyal et al., 2011; Shukla et al., 2008). The reduced but increased cyst count in probiotics or synbiotic mice be due to species or strain variance in this probiotic culture or probiotic may be unable to utilise the prebiotic, thus unable to reduce the *Giardia* cycle in mice.

Table 2: Cyst count (#x 10^4 cysts/ml) in mice belonging to different groups on various days of post inoculation

DAYS	***Giardia*-infected**	**Probiotic-*Giardia* infected**	**Prebiotic-*Giardia* infected**	**Synbiotic-*Giardia* infected**
DAY 0	-	-	-	-
DAY 1	42.6 ± 1.26	3.5 ± 2.59*	4 ± 1.67*	3.5 ± 2.71*
DAY 3	51.8 ± 1.03	10.75 ± 1.29*	16 ± 0.36*	9 ± 0.78*
DAY 5	75.2 ± 0.98	15.7 ± 0.87*	38.25 ± 0.31*	18 ± 0.49*
DAY 7	93.3 ± 2.33	25.8 ± 0.45*	69 ± 0.05*	41.5 ± 0.37*
DAY 9	122.6 ± 1.89	8 ± 0.92*	17.5 ± 0.65*	58.7 ± 0.46*

Values are Mean ± SD, *p<0.01 v/s *Giardia*-infected

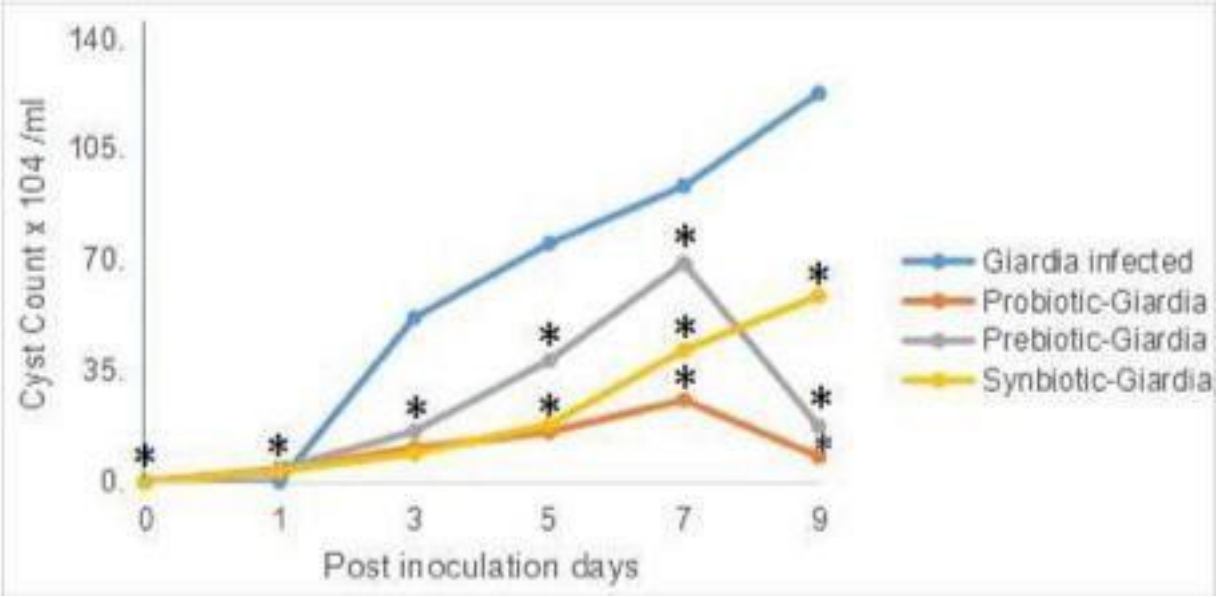


Fig 2 : Cyst count in different groups of mice. Values are Mean ± SD, *p<0.01 v/s *Giardia*-infected

ENUMERATION OF LACTOBACILLI COUNT

Table 3 and figure 3 show pattern of lactobacilli count in faeces of mice belonging to different groups. The ability of lactobacilli to persist in gastrointestinal tract and modulate infection was monitored by counting lactobacilli in faeces. It was observed that the lactobacilli count increased significantly ($p<0.05$) in probiotic-*Giardia,* ($p<0.05$), prebiotic-*Giardia* ($p<0.05$) and synbiotic-*Giardia* ($p<0.5$) compared with *Giardia*-infected mice. The *Giardia* infected mice had lower level of lactobacilli count which could be due to intestinal infection that may have eliminated the normal microflora. Interestingly, it was observed that synbiotic supplementation to *Giardia* infected mice through led to increased lactobacilli count but was less compared to prebiotic/ probiotic *Giardia* infected mice. This again supports the inability of prebiotic to modulate *Giardia* infection and metabolic prebiotic.

Table 3: Lactobacilli count (log10 CFU/ml) of mice belonging to different groups on various days of post inoculation

DAYS	Control	*Giardia*-infected	Probiotic	Prebiotic	Synbiotic	Probiotic-*Giardia* infected	Prebiotic-*Giardia* infected	Synbiotic-*Giardia* infected
DAY 1	6.31 ± 0.40	4.4 ± 0.94	6.61 ± 0.86	6.95 ± 0.57	5.78 ± 0.82	5.67 ± 0.56*	5.94 ± 0.81*	4.8 ± 0.63*
DAY 3	6.98 ± 0.37	5.09 ± 0.91	6.5 ± 0.80	6.98 ± 0.49	6.54 ± 079	5.69 ± 0.62*	5.98 ± 0.78*	5.04 ± 0.52*
DAY 5	7.7 ± 0.46	5.13 ± 0.82	6.32 ± 0.79	7.5 ± 0.42	6.39 ± 0.75	5.2 ± 0.44*	6.3 ± 0.69*	5.04 ± 0.47*
DAY 7	7.8 ± 0.35	5.18 ± 0.85	7.95 ± 0.73	8.61 ± 0.21	7.78 ± 0.71	6.9 ± 0.79*	7.4 ± 0.76*	6.6 ± 0.41*
DAY 9	8.51 ± 0.41	5.7 ± 0.60	8.161 ± 0.69	9.32 ± 0.21	8.04 ± 0.67	7.18 ± 0.11*	8.3 ± 0.49*	7.04 ± 0.54*

Values are Mean ± SD, *p<0.05 v/s *Giardia*-infected

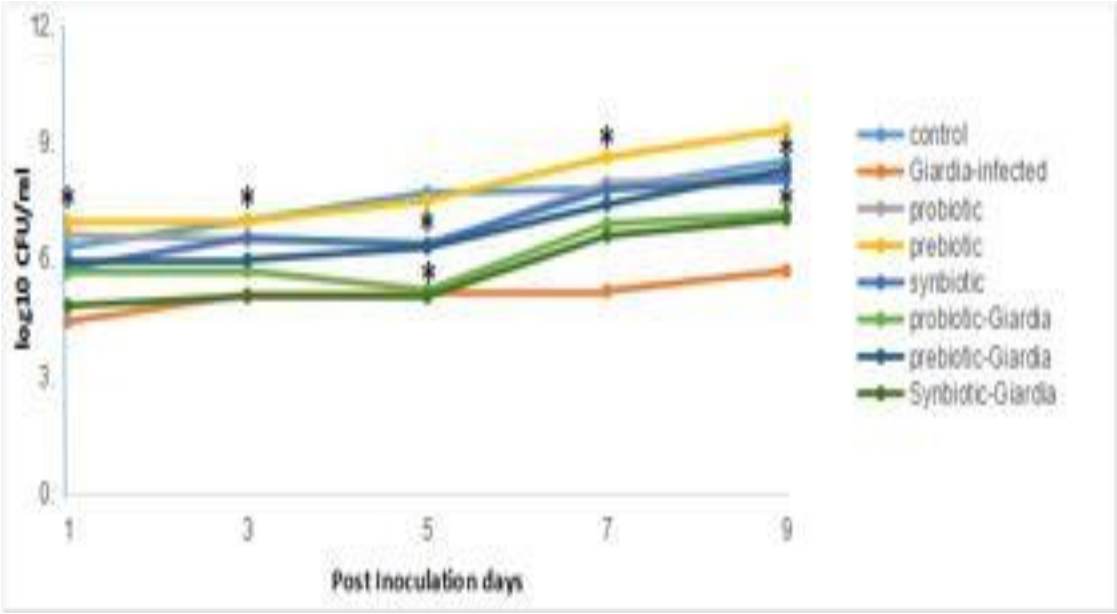


Fig 3 : Variation in lactobacilli count (log10 CFU/ml) in different groups of mice. Values are Mean ± SD, *p<0.05 v/s Giardia-infected.

Oxidant and Antioxidant levels

MDA levels

The levels of MDA was measured as an index of lipid peroxidation and was found that mice with synbiotic had higher level of MDA ($p<o.o5$) on 9 day PI compared with probiotic (0.22 ± 0.90 mmoles MDA/mg protein) and prebiotic (0.96 ± 0.83 mmoles MDA/mg protein)control mice.

Lipid peroxidation indicates tissue damage due to various redox agents or pathogens It was observed that oral administration of prebiotic with *Giardia* infection led to significant decrease ($p<0.05$) in MDA levels compared with *Giardia*-infected mice with mean concentration of 0.49 ± 0.67 mmoles/mg protein but surprisingly synbiotic administration led to significant increase in MDA levels compared with all treated and *Giardia* infected mice. The present observation showed increased MDA levels and also supports increased *Giardia* cyst in mice belonging to either probiotic/ prebiotic/synbiotic or supplemented *Giardia* infected mice.

Table 4: MDA levels in small intestine homogenate of mice belonging to different groups on day 9 of post inoculation

GROUPS	**MDA levels (mmoles MDA/mg protein)**
Control	0.09 ± 1.02
Giardia-infected	2.5 ± 1.01
Probiotic	0.22 ± 0.90
Prebiotic	0.96 ± 0.83
Synbiotic	2.0 ± 0.80
Probiotic-*Giardia* infected	2.0 ± 0.80*
Prebiotic-Giardia infected	0.49 ± 0.67*
Synbiotic-*Giardia* infected	9.58 ± 1.25*

Values are mean ± SD, *p<0.05 v/s Giardia-infected

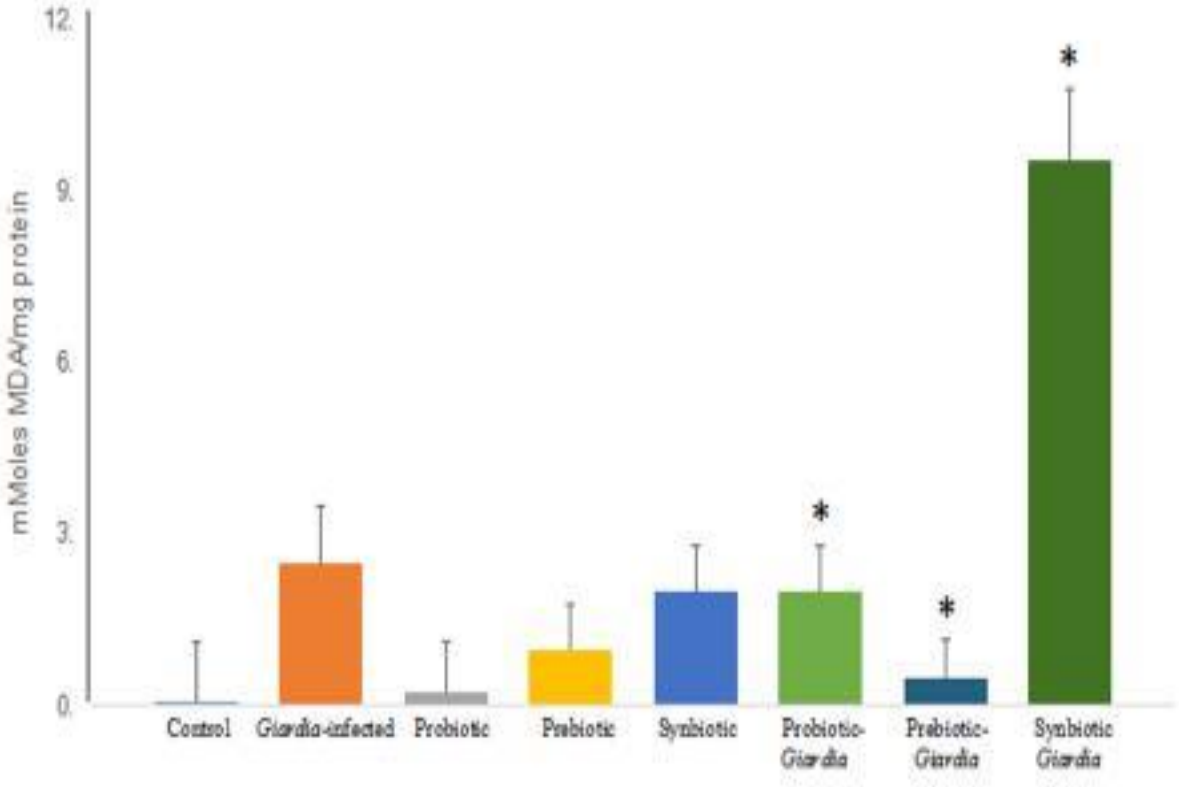


Fig 4 : MDA levels in small intestine of mice belonging to different groups. Values are mean ± SD, *p<0.05 v/s Giardia-infected.

Superoxide Dismutase (SOD) levels

Superoxide is produced as a by-product of oxygen metabolism and, if not regulated, causes many types of cell damage. Superoxide dismutase is an enzyme that alternately catalyses the dismutation (or partitioning) of the superoxide ($O2^{-}$) radical into either ordinary molecular oxygen (O2) or hydrogen peroxide (H2O2). Fig. 5 shows SOD levels in different groups of mice. A significant decrease was seen in *Giardia*-infected mice compared with probiotic ($p<0.005$), prebiotic($p<0.005$) and synbiotic($p<0.05$) mice with mean SOD levels of 130.15 ± 0.03 units/mg protein, 165.6 ± 3.79 units/mg protein and 46 ± 0.01 units/mg protein respectively. Surprisingly, oral administration of synbiotic prior to *Giardia* infection led to significant decrease ($p<0.05$) in SOD compared with prebiotc-*Giardia* infected mice. The result again showed that this isolated probiotic culture doesn't have anti-giardial potential as it led to increase in SOD level too.

Table 5: SOD level in mice belonging to different groups on day 9 post inoculation

GROUPS	SOD levels in small intestine (units/mg protein)
Giardia-infected	16 ± 0.03
Probiotic-*Giardia* infected	130.15 ± 0.03*
Prebiotic-Giardia infected	165.6 ± 3.79*
Synbiotic-*Giardia* infected	46 ± 0.01*

Values are Mean ± SD, *p<0.01 v/s *Giardia*-infected

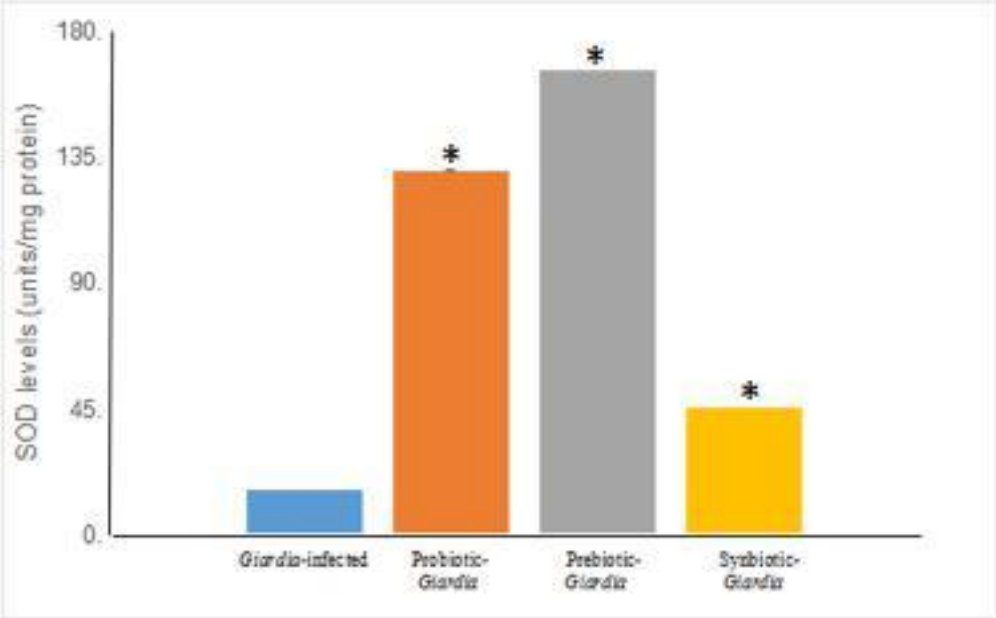


Fig 5: SOD levels in the small intestine of mice belonging to different groups. Values are Mean ± SD, *p<0.01 v/s *Giardia*-infected.

Glutathionine levels

Glutathione is an important antioxidant which is capable of preventing damage to important cellular components caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides, and heavy metals. Activities of GSH in mice infected with *Giardia* was significantly decreased ($p<0.05$) compared with probiotic-*Giardia,* prebiotic-*Giardia.* and synbiotic-*Giardia* because *Giardia intestinalis*, is highly vulnerable to both O2 and reactive oxygen species (ROS), due to the lack of conventional ROS-scavenging enzymes, such as catalase, superoxide dismutase, and glutathione (GSH) peroxidase (Mastronicola et al., 2011). Though, synbiotic administration prior to *Giardia* infection led to significant increase in levels of GSH compared with *Giardia* infected mice but was not able to kill the *Giardial* trophozoites probably due to low level of GSH.

Table 6: GSH levels in small intestine of mice belonging to different groups on day 9 of post inoculation

GROUPS	**GSH levels in small intestine (mmoles GSH/mg protein)**
Control	20.23 ± 1.321
***Giardia*-infected**	0.10 ± 0.240
Prebiotic	3.315 ± 0.01
Prebiotic	1.56 ± 0.058
Synbiotic	15.35 ± 0.007
Probiotic-*Giardia* infected	0.36 ± 0.002*
Prebiotic-*Giardia* infected	0.79 ± 0.004*
Synbiotic-*Giardia* infected	3.3 ± 0.005*

Values are Mean ± SD, *p<0.05 v/*s Giardia*-infected.

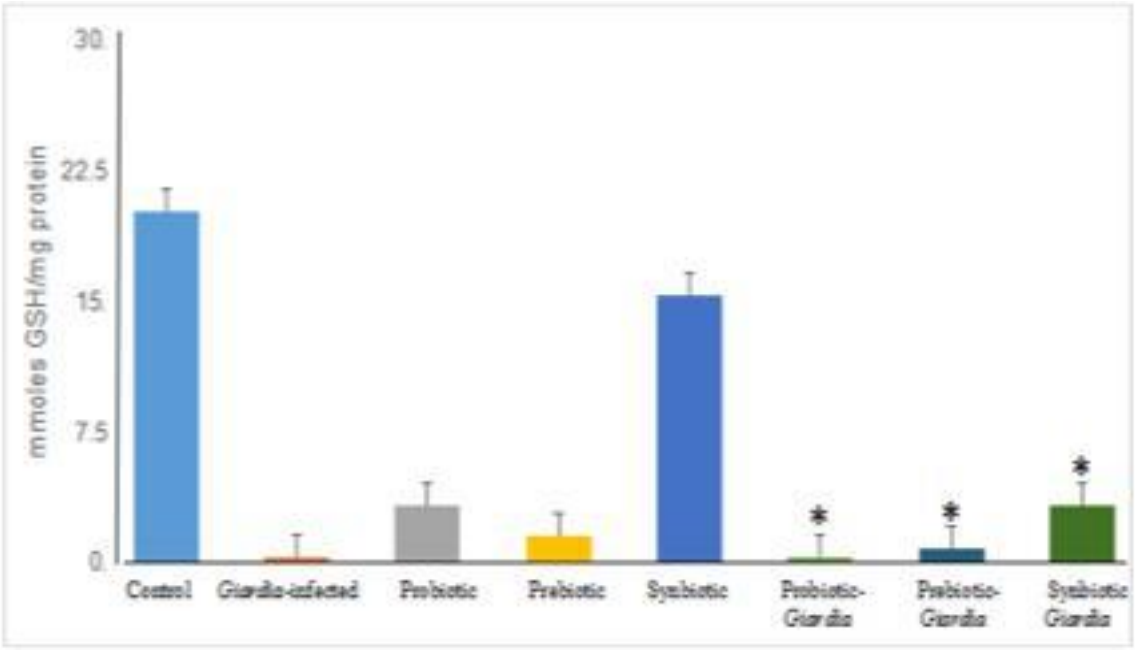


Fig 6: GSH levels in the small intestine of mice belonging to different groups. Values are Mean ± SD, *p<0.05 v/*s Giardia*-infected.

IgA ANTIBODY LEVELS IN SERUM AND INTESTINAL FLUID

Table 7 and figure 7 show the IgA antibody levels in the serum and intestinal fluid of the mice belonging to various groups on day 9 post inoculation. It was observed that anti-*Giardia* antibody was maximum in intestinal fluid of mice belonging to prebiotic-*Giardia* group of mice with mean optical density of 0.351 ± 0.003 and was least in synbiotic-*Giardia* group with mean optical density of 0.312 ± 0.010 compared to *Giardia* infected group of mice with mean optical density 0.085 ± 0.009. However, much difference was observed in IgA antibody level in serum and intestinal fluid of *Giardia*-infected mice and similar observation was seen in other groups and anti-giardial IgA antibody levels were more in intestinal fluid compared to serum in mice belonging to different groups that is in concordance with Okamoti et al., 2007. Here also we, saw that probiotic, prebiotic and synbiotic increased anti-giardial IgA level in serum and intestinal fluid but in *Giardia* infected mice al such treatments led to decreased IgA levels.

Table 7: Secretory IgA levels of mice belonging to different groups on day 9 of post inoculation

GROUPS	Optical density (serum)	Optical density (intestinal flu-id)
Giardia	0.083 ± 0.001	0.085 ± 0.009
Prebiotic	0.689 ± 0.003	0.517 ± 0.007
Prebiotic	0.636 ± 0.009	0.810 ± 0.004
Synbiotic	0.697 ± 0.007	0.865 ± 0.003
Probiotic-*Giardia* infected	0.062 ± 0.001*	0.348 ± 0.005*
Prebiotic-*Giardia* infected	0.084 ± 0.009*	0.351 ± 0.003*
Synbiotic-*Giardia* infected	0.059 ± 0.006*	0.312 ± 0.010*

Values are Mean ± SD, *p<0.01 v/s *Giardia*-infected.

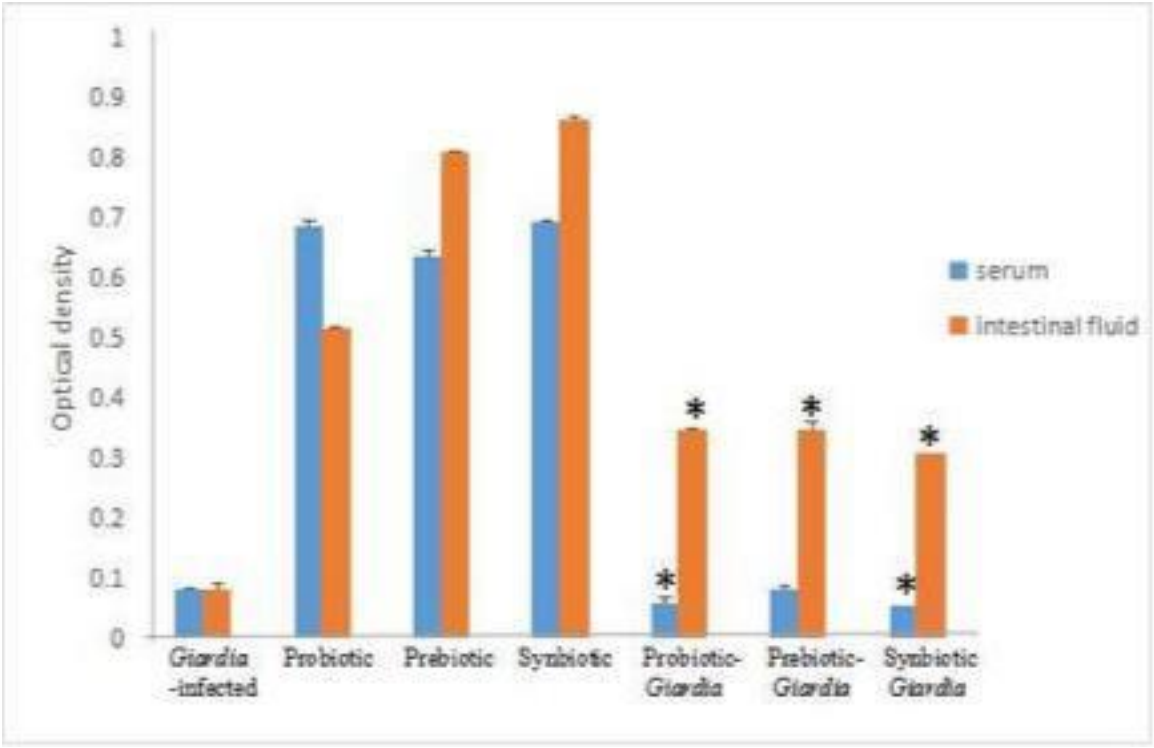
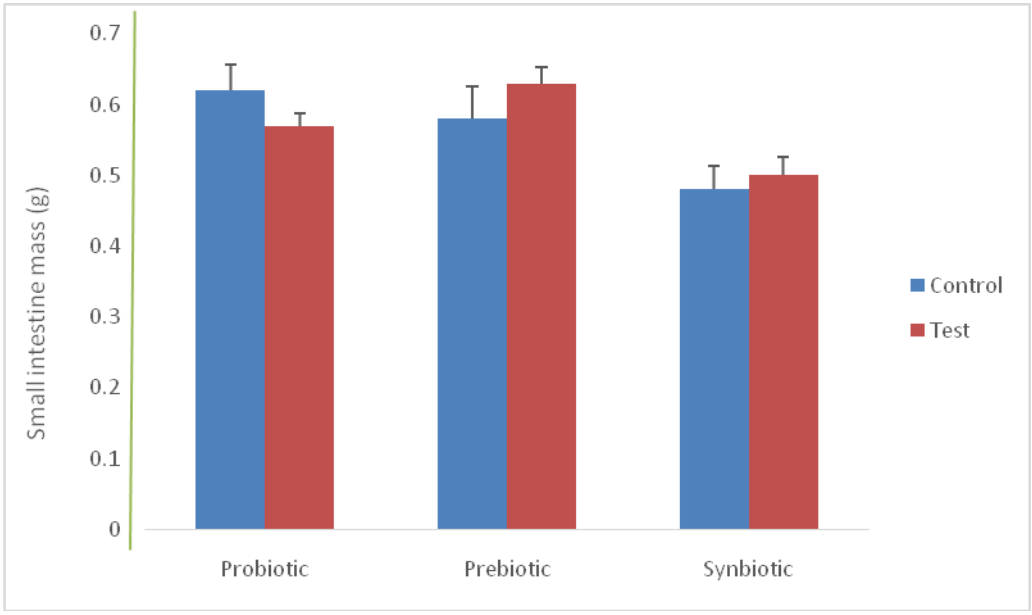


Fig 7 : IgA levels in the serum and small intestine of mice belonging to different groups. Values are Mean ± SD, *p<0.01 v/s *Giardia*-infected.

Determination of intestinal mass



HISTOPATHOLOGY

The morphological and cellular alterations in the proximal part of the small intestine following *Giardia* infection with probiotic, prebiotic and synbiotic supplementation was assessed by histological examination in the acute phase of infection i.e Day 9 PI. Histologically, it was found that *Giardia* infected mice had damaged microvilli and mild ileitis (Fig. 11) compared with normal basal crypts and villi of control mice (Fig. 10). Interestingly, small intestine of mice belonging to probiotic, prebiotic and synbiotic showed well formed, thickened mucosal epithelial lining along with increased number of inflammatory cells, increased villi length compared with damaged microvilli, crypts (Fig. 12,14,16) in probiotic-*Giardia*, prebiotic-*Giardia*, synbiotic-*Giardia* (Fig. 13,15,17). More specifically, probiotic-*Giardia*, prebiotic-*Giardia* mice had somewhat increased villi, crypts and ileitis than synbiotic-*Giardia* mice (Fig. 17) indicating that synbiotic was not effective in eliminating *Giardia* infection compared with probiotic and prebiotic.

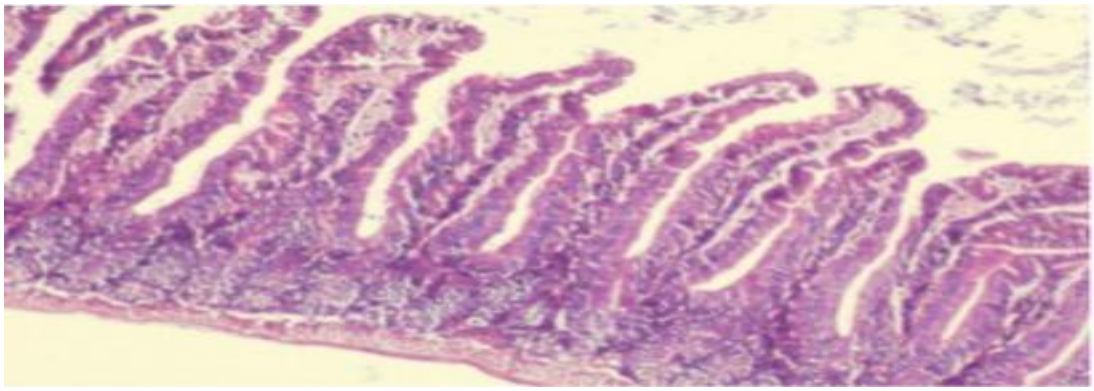


Fig 10: Photomicrograph of the small intestine of control mice on day 9 showing intact mucosal epithelium lining, basal crypts and normal villi (H & E stain, 400x)

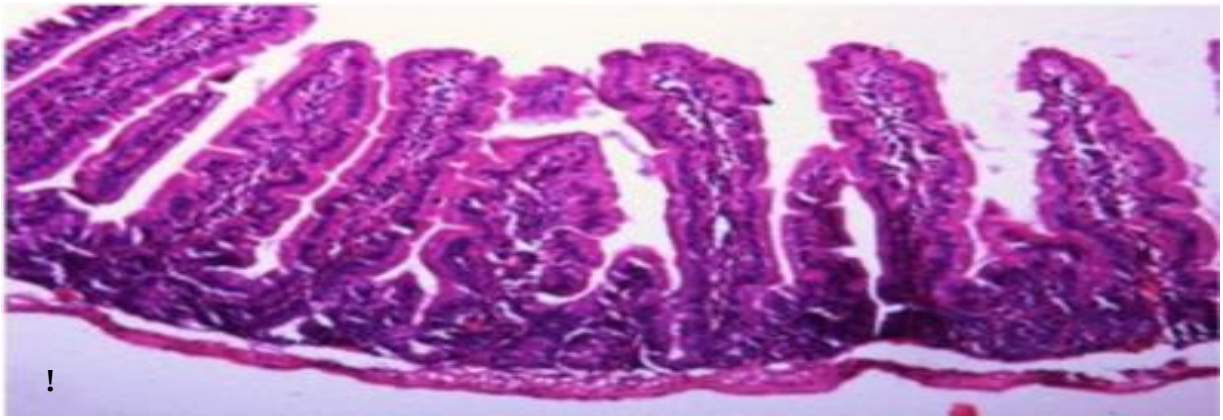


Fig 11: Photomicrograph of the small intestine of *Giardia*-infected mice on day 9 PI showing damaged micro villi, mild ileitis (H & E, 400x)

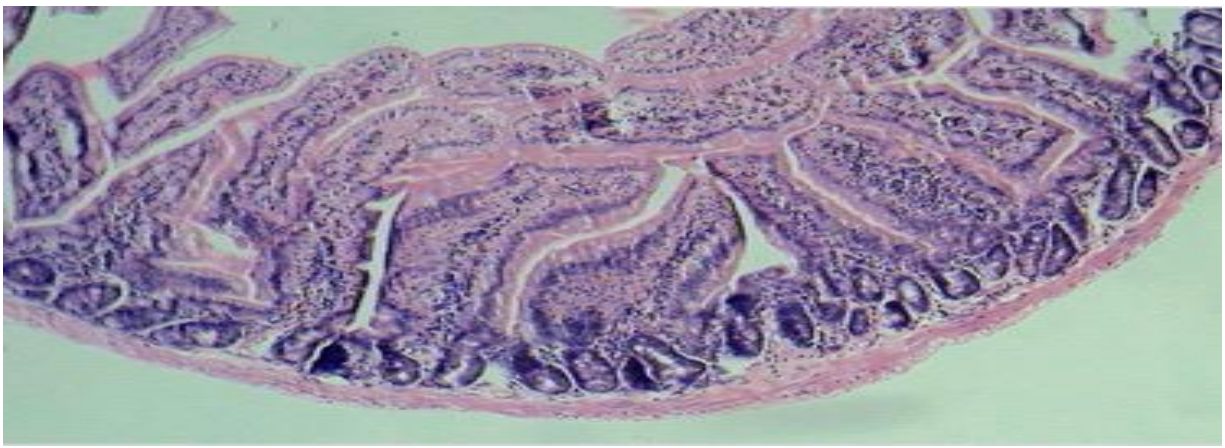


Fig 12: Photomicrograph of the small intestine of control mice belonging to probiotic showing intact mucosal epithelial lining, basal crypts and normal villi (H and E stain, 100x

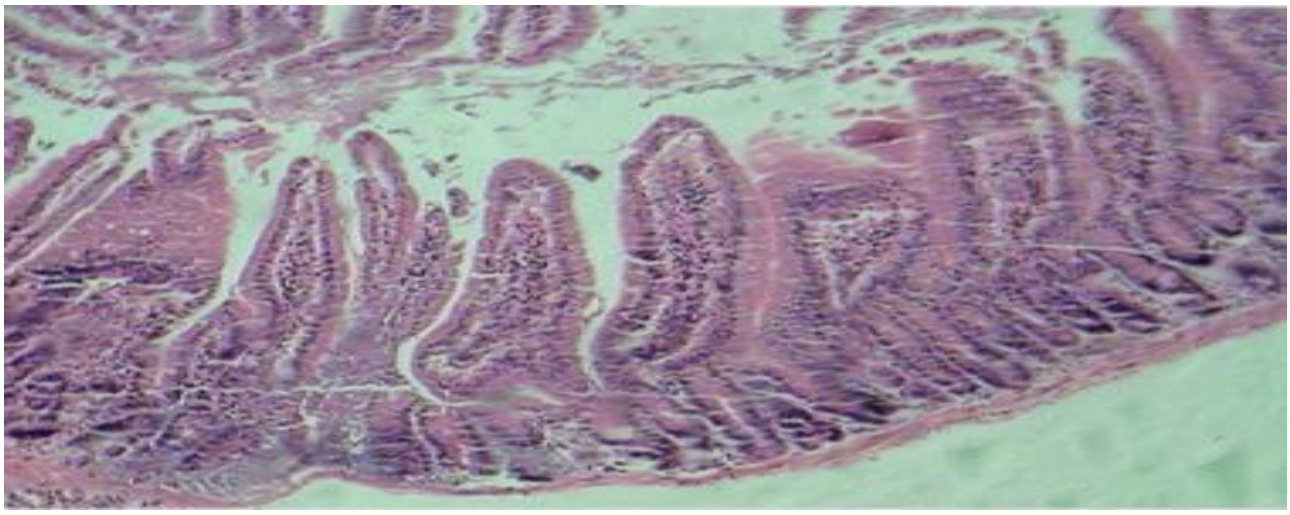


Fig 13: Photomicrograph of small intestine of mice fed with probiotic-*Giardia* showing increased villi size and crypts but ileitis (H and E stain, 100x)

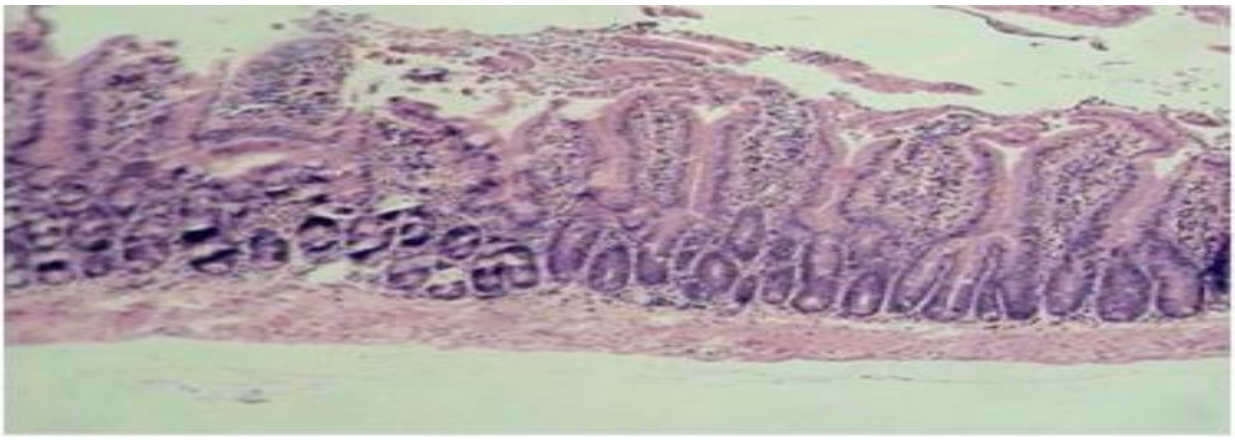


Fig 14: Photomicrograph of small intestine of control mice belonging to prebiotic showing intact mucosal epithe-lial lining, basal crypts and normal villi (H and E stain, 100x)

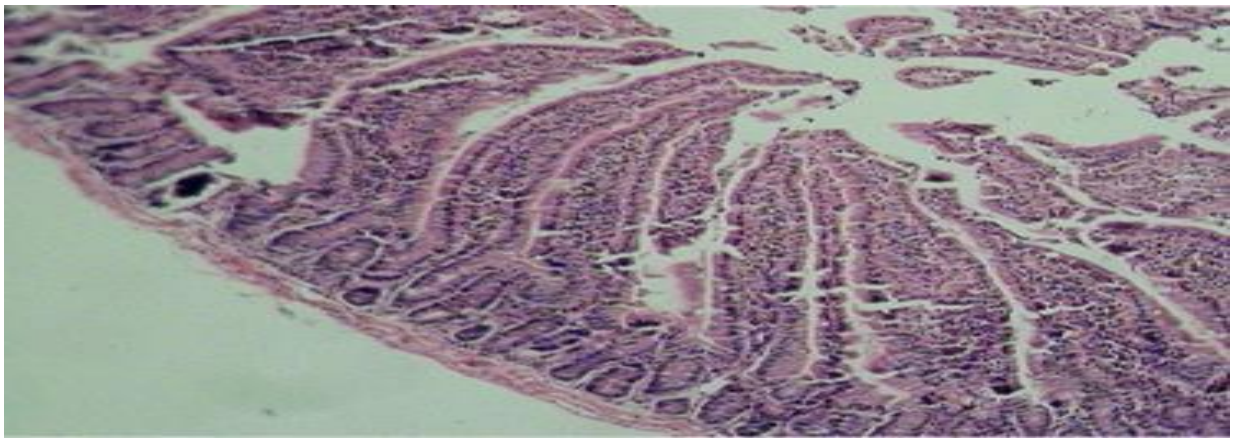


Fig 15: Photomicrograph of small intestine of mice fed with prebiotic-*Giardia* showing increased villi filled with inflammatory cells (H and E stain, 100x).

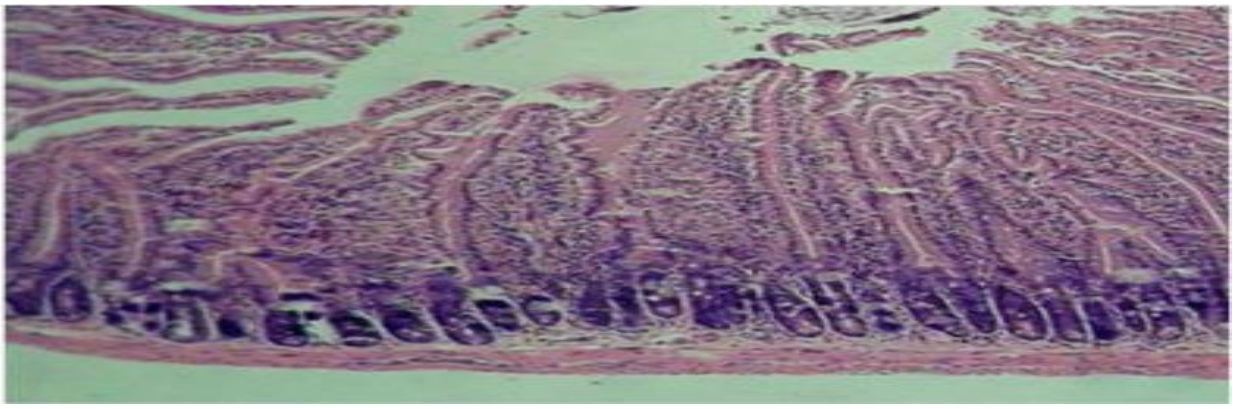


Fig 16: Photomicrograph of small intestine of control mice belonging to synbiotic showing intact mucosal epithe-lial lining, basal crypts and normal villi (H and E stain, 100x).

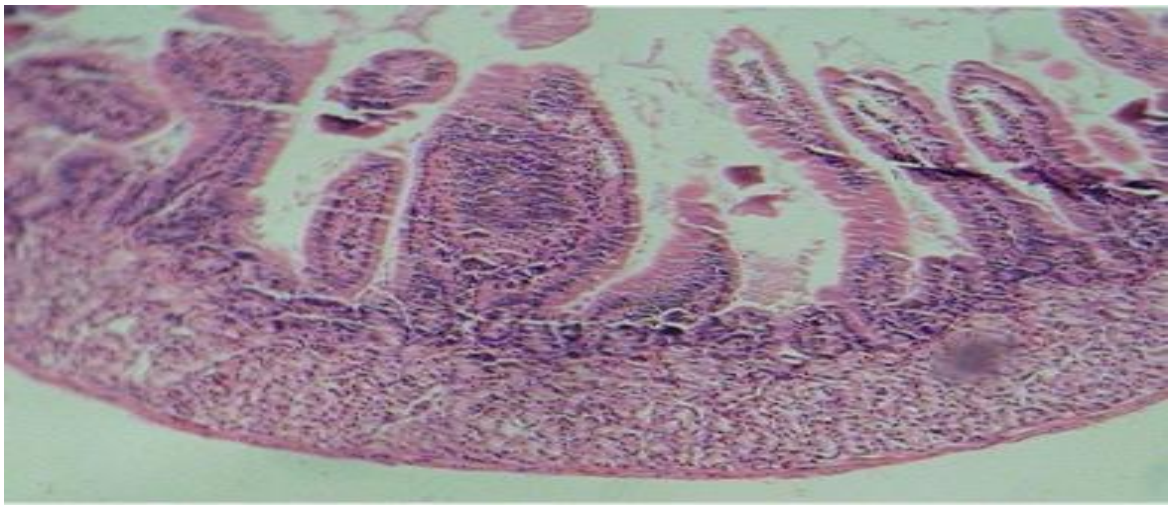


Fig 17: Photomicrograph of small intestine of mice fed with synbiotic- *Giardia* showing somewhat thickened mu-cosal epithelial lining with damaged crypts and thinning of villi (H and E stain, 100x)

results, attempts can be made to validate such study in school going children by clinicians only.

16. WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT……No

17. NO. OF PUBLICATIONS OUT OF THE PROJECT : Nil

(PRINCIPAL INVESTIGATOR)

(REGISTRAR/PRINCIPAL)

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24/7/2018

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