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## Effect of the probiotic yeast *Saccharomyces boulardii* on *Giardia lamblia* and electrolyte ratio in mice.

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### ABSTRACT

The principal objective for our study was to evaluate the efficacy of *Saccharomyces boulardii* against *Giardia lamblia* infection and investigated the mechanism of this anti diarrheal effect on electrolyte (sodium, potassium and chloride) changes compared with metronidazole by using mice models. A single oral dose of *S. boulardii* of up to  $5 \times 10^6$  cfu/0.1 of viable cells reduced parasite appear in feces and disappear completely at the 7<sup>th</sup> day compared with metronidazole the cysts parasite continued till 8<sup>th</sup> day. The yeast also caused increased potassium and sodium while decrease chloride level in serum. The histo morphological study findings showed in the group which was given *S. boulardii* the majority of intestinal villi return to normal shape. These finding indicated that *S. boulardii* may be effective in treating giardiasis also has specific effects on electrolyte secretary response.

**Keywords:** *Saccharomyces boulardii* ; *Giardia lamblia* ; probiotic

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## INTRODUCTION

*Giardia lamblia* is a protozoan parasite which replicated exclusively in the lumen of the small intestine of a wide variety of mammalian hosts. *Giardia* infection in young children are particularly common. In some cases, these infections result in acute or chronic symptoms, including malabsorption diarrhea, cramps, and nausea, although a symptomatic infections are common<sup>1</sup>.

In many regions of the world, giardiasis is endemic infection is practically universal by 2 years of age<sup>2</sup>. In developed countries, infections are more sporadic but have the less common whenever fecal contamination occurs, such as with contamination of water supplies or direct person – to – person spread in day care centers.

The courses of infections are highly variable among individuals some infections resolve quickly where as others can continue for years<sup>3</sup>.

A probiotic is a living microorganism administered to promote the health of the host by treating or preventing infection due to strains of pathogens<sup>4,5</sup> There are increasing experimental and clinical data to support probiotics use in the prevented and treatment to many gastrointestinal disorders including inflammatory bowel disease, infections and antibiotic related diarrhea & post surgical disorders<sup>6</sup>. The most commonly used probiotics are lactic acid bacteria, such as Lactobacilli, Bifidobacterium and yeast such as *Saccharomyces boulardii*<sup>7</sup>.

*S. boulardii* is a unique , non – pathogenic yeast supplement that has been utilized world: de as a probiotic to support gastrointestinal health it's does not colonize the human intestinal tract & is not one of the micro-organism that normally inhabit the intestines. *S. boulardii* does not attach to mucosal cell lining the intestinal lumen, but exerts its beneficial effects on the host as it moves through the gastro-intestinal tract<sup>8</sup>.

*S. boulardii* have been tested for clinical efficacy in served types of acute disease including antibiotic-associated diarrhea, *Closteridium difficile* infections *Helicobacter pylori*, ethereal nutrition related diarrhea and traveler's diarrhea<sup>9</sup>. Therapy with metronidazole is the recommended obtain in giardiasis. However , some clinical trial reports suggest the appearance of drug resistance to explain the rapeutic failure. Several investigations have been carried out on the effect of probiotic microorganisms for preventing or treating gastro- intestinal disease, but little is known about their efficacy of *S. boulardii* in children or mice with a cute diarrhea caused by *Giardia lamblia* therefore the aim of this study to compare the efficacy of the routine treatment by metronidazole with *S. boulardii* in the treatment of acute diarrhea resulting from *G. lamblia*.

## MATERIAL AND METHODS:

### Feces Samples

This study included the collection of 60 stool samples from people suffering from diarrhea and samples were collected from various areas of civil laboratories in Bagdad. Has slides were prepared by direct smear and after confirming the presence of *Giardia lamblia* at the samples Behaved. The refrigerated and transported to the laboratory at university of AL-Mustansiriya.

### Parasite purification

Robert and Thombson<sup>10</sup> method was used to isolate the Parasite then (cysts & Trophozoites) were suspended in phosphate buffer saline(PBS-7.2)and the final concentration was attended by rate  $1 \times 10^3$  cells/0.1 ml.

### Yeast cells preparation.

lyophilized *Saccharomyces boulardii* (bioflor-laboratoss –biocodex-franc) were obtained from the scientific offices then was reactivated by cultured on sabouraud dextrose broth [Difco] at pH(6) and incubation at mixing incubator for 48h at 30 °c Rigother *et al*<sup>11</sup> to the end of incubation the yeast were recovered by centrifugation for [10 minute] at 1000 rpm the deposit was diluted in 0.05 NaCl and counted under the microscope to obtained  $5 \times 10^7$  cells/0.1ml.

### Animals.

In this experimental used 40 male Swiss albino mouse about 3-4 weeks old, weighing 15-20g were obtained from the Animal house in college of medicine Baghdad university were housed under standard condition. Has feces of mice were examined to ensure that they are free from parasitic infection before starting the experiment.

### Experiment design.

Thirty mouse was inoculated orally with (0.1 ml) contain  $1 \times 10^3$  cell from *Giardia lamblia* , day after dosing each mice were examined prepare direct smear and after confirmation of infection get infected into four groups and each group of(10) mice was inoculated as follow:

**Group 1:**Given orally 0.1ml ( $5 \times 10^7$  cell) from *Saccharomyces boulardii* by using stomach tube once a day<sup>12</sup>

**Group 2 :**Given (0.1) ml of metronidazole (30mg/kg/ day) orally at asingle dose per day<sup>12</sup> .

**Group3:**Given only(0.1ml)of normal saline and promised as appositve control group.

**Group4:** This group intact mice and non infected with parasite and were given orally (0.1ml) of PBS pH 7.2 and promised as a negative control group .

**Enumeration of *Giardia lamblia*** [cysts & trophozoite ]in faces were enumerated as Shukla *et al*<sup>13</sup>.briefly mice feces were collected first three groups daily from each mouse, one gram of fecal sample was dissolved in10ml of normal saline ,homogenized then counted every day by suing hemocytometer.

#### **measuring changes of electrolyte**

To study changes by electrolyte during infection and treated it with *S. boulardii* and comparing with flagyl(metronidazole) drug , drawn blood of mice by capillary tube of the artery near the eye and place it in Eppendorf centrifuge tube not contain any heparin then separated serum using centrifuge(300rpm/min)for20minute. has been drawing blood periods different after [4,7 and 12 day] respectively and using the Humalyte Plus Human(Germany) by measuring the [Na<sup>+</sup>,K<sup>+</sup>, and Cl<sup>-</sup>].

#### **Histopathological study**

After the end of experimental period the mice in[G1;G2; and G3] were sacrificed and small intestine was removed fixed in 10% buffered formalin processed stained with hematoxylin and eosin for study histopathological changes

#### **Statistical analysis:**

Data are reported as mean± standard deviation and the inter group variation performed by t-test.

## **RESULTS AND DISCUSSION**

In this study we compare the efficacy of the *Saccharomyces boulardii* with metronidazole in mice with acute cause by *Giardia lamblia* the results in table 1 showed that the numbers of *G. lamblia* in G1(treated group)was reach to 2.66cell/gm at first day (like in positive control G3) while reach to 5.33 and 4 cell/gm at days 2 and 3 respectively but at third day the number of parasite was similar to first day and stand decrease in 5and6 days till reach to zero in 7<sup>th</sup> day compare with G3 that which reach to 15 cell/gm in 7 day and decrease tell to reach11 cell /gm in 14 day moreover when we compare the results inG1 withG2 we showed increase in number of parasite at (2,3,4,5) days and start decrease in( 6,7,8) days till reach to zero at day 3.

The yeast *Saccharomyces boulardii* also caused changes in level of electrolytes( Na<sup>+</sup>,K<sup>+</sup>, and Cl<sup>-</sup>) in serum of treated mice the results in table 2 showed that the *S. boulardii* in G1 caused increased Na<sup>+</sup> and K<sup>+</sup> level in serum form treated mice and reach to167.36 and 6.4 meq/L at 7<sup>th</sup>day respectively wherever 165.46 and 9.04 meq/L at 12<sup>th</sup> day respectively compared with group G2 and G3 that which reach to 158.66 and 154.46 meq/L at 7<sup>th</sup> day respectively and

153.53 and 159.7 meq/L at 12 day. while when we compare the level of Cl<sup>-</sup> in G1 with other group (G2 and G3) we showed in Cl<sup>-</sup> level in G1 from 4<sup>th</sup> day till 12<sup>th</sup> day which start 93.1 meq/L in 4<sup>th</sup> day and reach to 86.66 meq/L at 12<sup>th</sup> day compare with G2 and G3 which start 93.63 and 92.93 meq/L respectively at 4<sup>th</sup> day and reach to 87.6 and 90.33 meq/L at 12<sup>th</sup> day respectively, moreover the level of all electrolyte in G4 [negative control] not showed any changes.

diarrhea is a common health problem worldwide. Antibiotic, nosocomial infections and microorganism are the common factors responsible for diarrhea. Moreover imbalance in intestinal microbial flora also leads to diarrhea. Probiotics is a living microorganism administered to promote the health of the host by treating or preventing infections, which are effective against diarrhea and act by various mechanisms like colonization resistance production of antimicrobial substances competitive inhibition for bacterial adhesion sites, anti secretory effect and inhibition of toxin binding tropic effects of intestine mucosa. *Saccharomyces boulardii* is very useful against different types of diarrhea<sup>14</sup>. although the mechanism of action of *S. boulardii*, in the clearance of *G. lamblia* from the gut have not been clarified, the effects may well be involved; tropical effects on the small intestine. increasing intestinal disaccharides levels, enhancing stimulating of the gut immune system, alteration of enzymatic activation in gut and capability of assembling host defense against potential pathogenesis<sup>15,16,17,18</sup> although Szajewska *etal*<sup>19</sup> found that *S. boulardii* is moderately effective in preventing antibiotic associated diarrhea in children and adults treated with antibiotics for any reason, moreover Guillot *et al*<sup>20</sup> evaluated the effects *S. boulardii* in children 6-36 months old and management of chronic diarrhea due to *G. lamblia*.

*S. boulardii* may be effective in treating giardiasis when combined with metronidazole therapy through 10 days<sup>21</sup>. Dinleyici *et al*<sup>22</sup> reported that *S. boulardii* significantly reduced the duration of diarrhea approximately 24hr and that of hospitalization approximately 20h. *S. boulardii* shortened the initial phase of watery stools mean number of stools to decrease at day 2, moreover, a significant reduction was reported at days 3 and 4. Therefore with *S. boulardii* the shortened duration of diarrhea and the reduction in hospital stay result in social and economic benefits

The data reported in this study confirmed the findings in mice with *Giardia lamblia* :cysts, trophozoite in stool and histological lesion disappeared more rapidly<sup>23</sup>. metronidazole does not treat cysts as such. On the other hand, the *S. boulardii* few reduction of Cl<sup>-</sup> during 7 and 12 days while increased Na<sup>+</sup> and K<sup>+</sup> since 4<sup>th</sup> day this effect could be explained by the *Saccharomyces*

causes reduction of Na during 8 days of treatment of mucosa cells while net fluxrates of  $CL^-$  were not affected by *S. boulardii*<sup>24</sup>. Also Givard *et al*<sup>25</sup> found that *S. boulardii* possesses potent anti secretory properties versus water and electrolyte secretion induced by castro oil in the rat colon. The mechanism by which the trophozoite of *Giardia laumbli* impairs absorption have not clarified and damage to the intestinal mucosa with or without invasion<sup>26</sup>. The morphological alterations of the jejunal mucosa range from absence of lesions to severe atrophy of the intestinal villi, also been reported and chronic inflammatory infiltration with destruction of the epithelial cells<sup>27,28</sup>.

The use of *S. boulardii* in addition to specific treatment can provide solution to the effects of parasitic damage of the intestinal mucosa in diarrhea caused by *Giardia lamblia* whether acute or chronic. *S. boulardii* can play an important in combating the harmful effects of giardiasis, as the parasite responsive for the condition is capable of upsetting the balance of the intestinal ecosystem<sup>29,30</sup>.

*S.boulardii* has been shown to decrease the number and severity of lesions caused by another protozoan parasites *Entamoeba histolytica*<sup>31</sup>. This probiotic can inhibit the attachment of *E.histolotica* trophozoites to erythrocytes *in vitro*<sup>32</sup>, probably the ability to adhere to gastro intestinal mucosa is an important prier quest for *G.lamblia* to induce sustainable infection<sup>33,34</sup>.

**Table (1) :- Numbers of *Giardia lamblia* parasites in treatment & control groups**

Days after treatment	Numbers of <i>Giardia lamblia</i> means±Stander deviation x10 <sup>2</sup>		
	G1 ( <i>Saccharomyces boulardii</i> )	G2 (metronidazole)	G3 Positive control
1	2.66±0.57	3±1	2.66±0.57
2	5.33±0.57	5±1	6±0
3	4 ±1	5.33±0.57	9±1
4	2.66±0.57	5.33±2.08	11.33±1.15
5	1.66 ±0.57	5±1	12.33±1.15
6	0.66±0.57	3.66±0.57	14.66±0.57
7		2.77±0.57	15±1
8		1.33±0.57	13.33±0.57
9		0	11.66±0.57
10			10.33±0.57
11			9.33±0.57
12			11±1
13			11.33±0.57
14			11±1

$P < 0.005$

**Table 2:- Electrolyte changes of experimental mice during *Saccharomyces boulardii* and Metronidazole trials .**

Electrolytes	Group	4 <sup>th</sup> day	7 <sup>th</sup> day	12 <sup>th</sup> day
Na <sup>+</sup> (meq/L)	G1 ( <i>Saccharomyces boulardii</i> )	168.6±1.1	167.36±1.42	165.46±1.85
	G2 (metronidazole)	153.36±1.1	15.8.66±1.87	153.53±2.85
	G3(positive control)	154.7±2.16	154.46±1.06	159.7±2.16
	G4(negative control )	160.43±6.10	160.43±6.10	160.43±6.10
K <sup>+</sup> (meq/L)	G1	9.43±0.20	9.93±0.32	9.06±0.68
	G2	6.46±0.20	6.76±0.05	6.43±0.32
	G3	6.26±0.05	6.4±0.1	5.56±0.55
	G4	6.3±1	6.3±1	6.3±1
Cl <sup>-</sup> (meq/L)	G1.	93.1±0.52	86.5±0.7	86.66±0.4
	G2	93.63±0.20	86.9±0.65	87.6±0.60
	G3	0.32±0.32	90.2±0.50	90.33±0.65
	G4	93.4±0.72	93.4±0.72	93.4±0.72

Results expressed as mean ± SD.

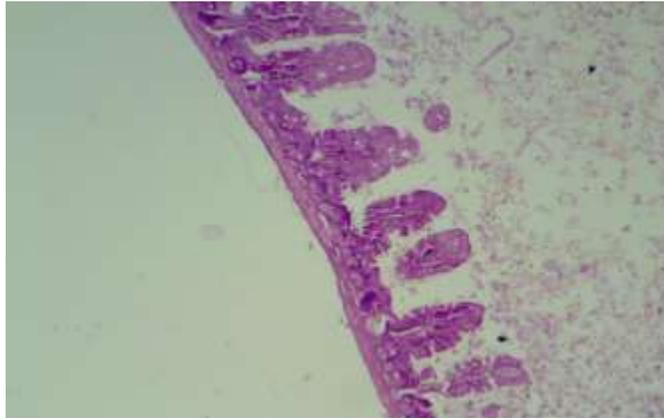
\* 4<sup>th</sup> day after administration of *Saccharomyces* / metronidazole

\*7<sup>th</sup> day after administration of *Saccharomyces* / metronidazole

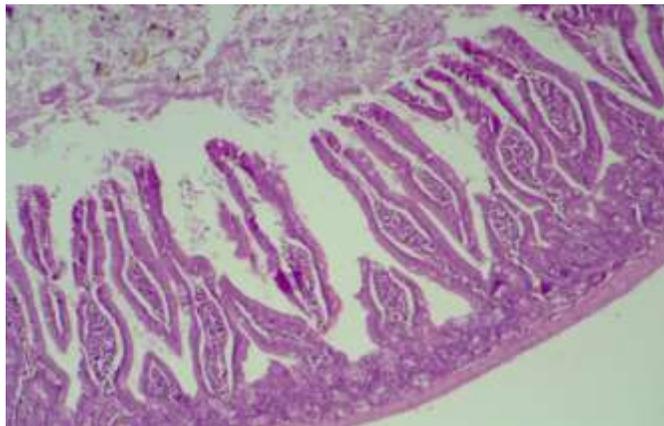
\*12<sup>th</sup> day after administration of *Saccharomyces* / metronidazole

*S. boulardii* might interfere with this adhesion based on the present results , resuggest that use of the *S. boulardii* for treatment of *G. lamblia* significantly decreases the duration of gastrointestinal clearance of *Giardia* cysts as compared with metronidazole also, the electrolyte value retain to normal value. This helps to reduce the likelihood of getting drought and by the electrolyte imbalance in the body in addition to that the reform of yeast bowel tissue and maintain normal compared with metronidazole.

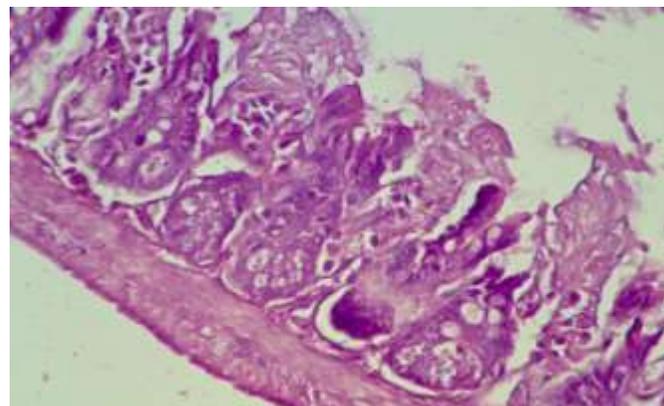
The histomorphological study showed that the intestinal mice infected with *Giardia* (G3) had showing shortening and atrophy of intestinal mucosal villi with goblet cells depletion figure 1. In the group(1) which was given *Saccharomyces boulardii* showed the majority of intestinal villi return to normal shape with few villi showing still goblet cells in few in numbers figure 2 and intestinal villi gradually to increase in its elongation and presence of goblet cells Figure 3. while after the treatment with metronidazole (G2) still there was atrophy in villi but with increase in goblet cells Figure4.



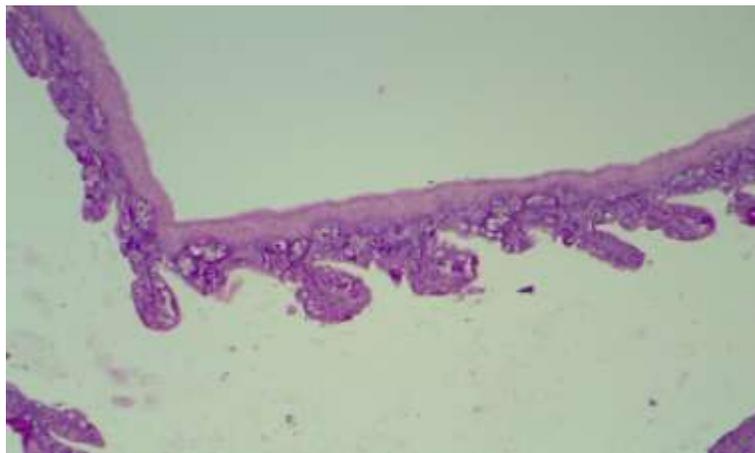
**Figure1:Section of control infected showing shortening and atrophy of intestinal mucosal villi with goblet cells depletion X250.**



**Figure[2:Group which was given *Saccharomyces boulardii* showed the majority of intestinal villi return to normal shape with few villi showing still goblet cells in few in numbers X400.**



**Figure 3:Group which was given *Saccharomyces boulardii* intestinal villi gradually to increase in its elongation and presence of goblet cells X400.**



**Figure 4: Treatment with metronidazole still there was atrophy in villi but with increase in goblet cells X250.**

The histomorphological study findings showed in the group(1) which was given *Saccharomyces boulardii* the majority of intestinal villi return to normal shape with few villi showing still goblet cells in few in numbers. Marshall *et al*<sup>35</sup> showed that intestinal mice tissue infected with *Giardia* became atrophy of villi infiltration in crypts and in lamina propria. the ratio between villi /crypts in the mice infected with *Giardia* lead to causes malabsorption who plays an important role in pathogenicity leading to the occurrence of diarrhea<sup>36</sup>

The atrophic effect of in the *Saccharomyces boulardii* intestine can be explained by the release of polyamines in the intraluminal space during intestinal transit of the yeast *Saccharomyces boulardii*<sup>37</sup>. The mechanisms by which the trophozoite of *Giardia* impairs absorption have not been clarified<sup>38,40</sup> the morphological alterations of the jejunal mucosa range from an absence of lesions to soccer atrophy of the intestinal villi with changes in their height configuration and increased depth of the crypts other modifications of intestine structure have also been reported, including acute and chronic inflammation infiltration with destruction of the epithelial cells<sup>39</sup>.

## CONCLUSION

*Saccharomyces boulardii* effective in treating giardiasis and without side effect compared with metronidazole also has specific effects on electrolyte secretory response. Therefore, we recommend to use individually or concrescence with other drug for the treatment of intestinal parasites

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