

Effect of probiotic *Saccharomyces boulardii* in weight losing and cytokines levels in mice before and after Infection by *Salmonella typhimurium*

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Abstract

This study was designed to evaluate some immunological and nutritional effects of three types of *Saccharomyces boulardii* yeasts, which was, Mutant Iraqi Isolate (Mutant *Saccharomyces boulardii*) MSb, commercial strain (Commercial *Saccharomyces boulardii*) CSb and Local Iraqi Isolate (Local *Saccharomyces boulardii*) LSb, in the weight and the level of both interleukin-10 (IL-10), interferon-gamma (IFN- γ) in the mice orally administered with yeasts inoculum for 30 days before and after infected with *Salmonella typhimurium*. The results shown significant losing in the weights and significant increase in the level of IL-10, but there are no significant difference in the levels of IFN- γ at (P<0.05) in mice administered with MSb, CSb and LSb yeasts before infected with *S. typhimurium*. The results also shown significant difference in the weights, significant increase in the level of IL-10 and significant decrease in the level of IFN- γ at (P<0.05) in mice administered with MSb, CSb and LSb yeasts after infected with *S. typhimurium*. Despite the effectiveness of three species of yeasts in these parameters before and after infection, but MSb was the most efficiency.

Keywords: Probiotics, *Saccharomyces boulardii*, *Salmonella typhimurium*, Loss Weight, Cytokines.

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تأثير المعزز الحيوي *Saccharomyces boulardii* في فقدان الوزن ومستويات الساييتوكينات

في الفئران قبل وبعد الإصابة ببكتريا *Salmonella typhimurium*

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الخلاصة

أجريت هذه الدراسة لتقييم بعض التأثيرات التغذوية والمناعية لثلاث أنواع من المعزز الحيوي *Saccharomyces boulardii* وهي كلاً من العزلة العراقية المحلية المطفرة MSb (Mutant *Saccharomyces boulardii*) والسلالة التجارية CSb (*Saccharomyces boulardii*) والعزلة العراقية المحلية LSb (*Local Saccharomyces boulardii*) في معدل الأوزان ومستوى الساييتوكينات IL-10 و IFN- γ للفئران المجرعة باللقاح اللبني للخمائر أعلاه لمدة ثلاثون يوماً قبل وبعد الإصابة بالخلايا الحية للبكتريا المرضية *Salmonella typhimurium*. أظهرت النتائج حدوث نقصان معنوي (P<0.05) في معدل أوزان الفئران وزيادة في مستوى الساييتوكين IL-10 في حين لم تسجل فروقات معنوية في مستوى الساييتوكين IFN- γ في الفئران المجرعة بخمائر MSb و CSb و LSb قبل الإصابة ببكتريا *S. typhimurium*. فيما أظهرت النتائج وجود فروقات معنوية (P<0.05) في معدل الأوزان وزيادة معنوية في مستوى IL-10 و حدوث نقصان معنوي في مستوى IFN- γ في الفئران المجرعة بخمائر MSb و CSb و LSb بعد

الإصابة ببكتريا *S. typhimurium*. رغم كفاءة الخمائر الثلاثة في المؤشرات التغذوية والمناعية المشار إليها قبل وبعد الإصابة ببكتريا *S. typhimurium*، إلا أن الخميرة المطفرة كانت الاكفا. الكلمات المفتاحية: المعزلات الحيوية، *Salmonella typhimurium*، *Saccharomyces boulardii*، فقدان الوزن، السابتوكينات.

Introduction

Salmonella SPP. a bacterium that was discovered more than 100 years ago and specifically in 1881, contains more than 2.610 species. Affects in humans and animals and responsible for many cases of food poisoning for human and animal, most important serotype *Salmonella typhimurium* (1). Hurley *et al* (2) referred to the pathogenic action of *Salmonella SPP.* by binding to receptors on the surface of epithelial cells and induce secretion of cytokines that express primary inflammation and inhibit the production of anti-inflammatory cytokines, as well as necrosis, ulceration of the intestine, destruction of the mucous layer reached to the liver and spleen. In recent years, probiotics have gained interesting value in the medical and commercial fields, with a new global trend emerging as a substitutional antibiotic therapy for multiple disease cases (3, 4). As well as insert it in many food industries as therapeutic and functional foods numbers not less than 10^7 c.f.u/ g or ml food in order to get the required effects Probiotics defined as Non-pathogenic living microorganisms (yeast or bacteria) that restore the balance of intestinal flora of the human digestive tract to give health benefits (6). *Saccharomyces boulardii* yeast is one of the most important probiotics and the only species of *Saccharomyces* genus authorized to use for human consumption (7). It was discovered by the French scientist Henri Pollard in 1923 when he noticed that the chewing of the crustaceans of lychee and mangestin fruits by the local population of Indonesia during the outbreak of the cholera epidemic increased their resistance and did not develop the symptoms of the disease. Pollard isolated a kind of yeast from these fruits were known as his name (*S. boulardii*) (8). *S. boulardii* yeast has a higher resistance to extreme acidic conditions due to the strength and robustness of its cell wall compared with bacterial probiotics (9), as well as the possibility of concurrent use with antibiotics to treat many diseases without any symptoms or negative effects compared to bacterial probiotics which can't be used with some antibiotics (10). *S. boulardii* has many features and affirmative characteristics that have enabled it to prove the medical importance in the prevention and treatment many diseases such diarrhea associated with antibiotics (11), diarrhea associated with HIV and to reduce the infection of bacterial pathogens like *shigella flexneri* and *Salmonella typhimurium* by inhibiting inflammation through increasing levels of anti-inflammatory cytokines and lowering inflammatory cytokines levels and regulating immune functions by enhancing their IgA production (12, 13). Martins *et al* (14) pointed out to the protective effect of *Saccharomyces boulardii* on *Salmonella typhimurium* infection by inhibiting Rac-1, which stimulates primary inflammation and reduces bacterial penetration of liver.

Materials and Methods

- **Source of isolates:** Local isolate of *S. boulardii*, was supplied as a lyophilized powder, which was isolated by (15) from mangastin tropical fruits. It was mutated chemically by exposed to different concentration of Ethidium bromide (5, 10, 15, 20 and 15 µg/ ml) under sterile conditions by the same researcher (16) and considered as a mutant isolate, commercial strain of *S. boulardii* was supplied by Sarrow Fomalas Company, Los Angelos- USA, as a lyophilized powder. *S. typhimurium* was provided by Biotechnology Department/ Al-Nahrain-Uni, which was isolated from adult patient with diarrhea resulting from food poisoning.

- **Preparation of *S. typhimurium* suspension:** *S. typhimurium* bacterial suspension was prepared according to (17) at a concentration of (1×10^8 cfu/ ml). The mice were orally challenged with this bacterial suspension by gavage needle with 200 μ l.
- **Yeast inoculum preparation:** Three yeasts of *S. boulardii* were activated by transferring a small amount of lyophilized powder of each, by a spatula under sterile conditions to the liquid yeast extract dextrose pepton broth medium and were incubated at 37 °C for 48 hours. (The activation repeated three times). The inoculum was prepared by inoculating 100 ml of reconstituted skim milk 12% (weight/ volume) containing 1% of sucrose with 2% of the liquid culture of each yeasts, and incubated at 37 °C until coagulation (repeated three times). and was used for mice oral administration.
- **Experimental Design:** Forty Swiss albino male mice 12-14 weeks of age and 35-38 g of weight were randomly divided into four groups of (10 mice) each group as follows in Table (1):

Table (1) Groups of mice

Group	Administration
First group (control)	fed on a standard feed only which consist of (23 g of cazine, 10 ml of Sunflower oil, 6 g of milled wheat bran, 5 g of Mineral mixture + vitamins, 51 g of Corn Starch, 5 g of table sugar)
Second group (MSb)	administrated with mutant <i>S. boulardii</i>
Three group (CSb)	administrated with commercial <i>S. boulardii</i>
Four group (LSb)	administrated with local <i>S. boulardii</i>

All groups administrated orally with 200 μ l/ day of yeast inoculum for 30 days except control group. In day 31, five mice of each group were anesthetized with chloroform and the blood samples were collected from heart directly by cardiac puncture and placed in clean abandrof tubes and centrifuged at 5000 rpm for 10 minutes at 4°C to get the serums. The rest mice of each group were infected with 200 μ l of *S. typhimurium* suspension at concentration 1×10^8 c.f.u/ml for 8 days, and sub-group have been derived, which is: St+ control, St+MSb, St+CSb, St+LSb, the serums samples were collected to estimate the level of immunological cytokines interferon-gamma and interleukin-10.

- **Estimation the levels of interleukin-10 and IFN- γ :** The levels of immune cytokine interleukin-10 and IFN- γ was determined in the samples of serums according to the instructions of the American company production (Ray Bio) by using Mouse IL-10 and Mouse IFN-gamma ELISA kits, which are consist of (pre-coated 96-well strip microplate, wash buffer, stop solution, assay diluent D, assay diluent B, lyophilized standard protein, biotinylated detection antibody, streptavidin- conjugated HRP, TMB one-step substrate).
- **Statistical Analysis:** Statistical Analysis System program (SAS) (2010) was used to study the effect of different coefficients on the studied traits, the mean differences between the averages was compared by a Least Significant Difference test (LSD).

Results and Discussion

- **Effect of *S. boulardii* in mice weights before and after infected by *S. typhimurium* bacteria:** Table (2) showed no significant differences at ($P < 0.05$) in primary mice weights average between the groups, while the results showed a statistically significant decrease at ($P < 0.05$) in the final mice weights average for MSb, CSb and LSb, which recorded 32.27 and 35.24 and 34.05 g, respectively. Where the highest rate of weight loss after 30 days was recorded by treatment MSb, which was -5.4 g, as a variation ratio (-14.3%), followed by the effect of CSb and LSb groups with a rate of weight loss about -3.3 and - 3.1 g, and as a variation ratio

(-8.5 and -8.3%) respectively, compared with control group which recorded increase in the weights average 41.11 g, with a rate of weight increase about +5.7 and expressed by a variance of +16.0%, as shown in figure (1).

Table (2) Average weight of mice administrated with *S. boulardii* yeast after 30 days

Group	primary weight rate (gm)	finally weight rate (gm)	weight Variation (gm) after 28 day
control	35.41 a	41.11 a	+5.7 a
CSb	38.54 a	35.24 b	3.3 b-
MSb	37.67 a	32.27 d	5.4 c-
LSb	37.15 a	34.05 c	3.1 b-
L.S.D value P<0.05	3.40	1.10	2.0

The averages with different letters within the same column significantly between them.

Everard *et al* (18) found a very close result when the mice administration with *S.boulardii* probiotic, He attributed this to existence β -glucan in the cell wall of *S.boulardii* which is not digested by the host because of absence of enzymes necessary for its metabolism, where acts as an important prebiotic for the naturally probiotics in the gut, specifically for the species of Bacteroides phylum, Which leads to increased its numbers that positively affects the levels of some immunological and hormonal indicators (19), reduction of body fat mass through physiological changes in the host (20, 21, 22) and decline in the number of bacterial strains responsible for obesity that contribute to increased body mass.

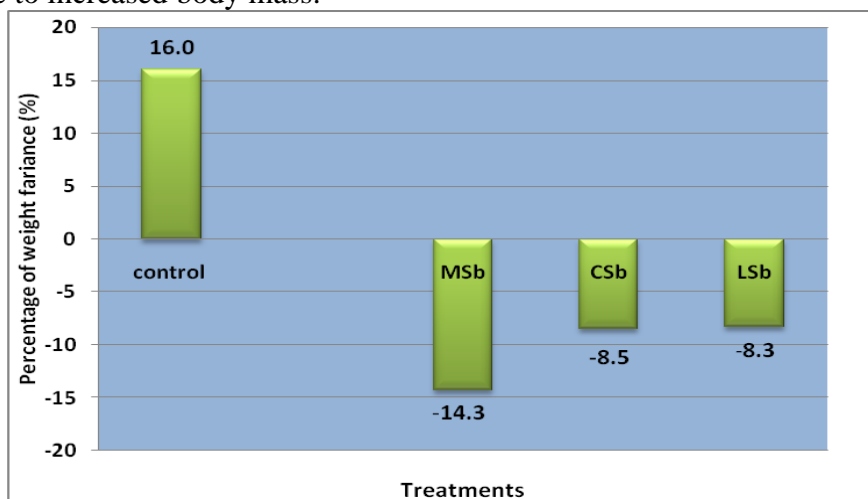


Fig. (1) Percentage of variance in the weights of treatments after 30 days of administrated with *S. boulardii*

While, after 8 days of infection with *S. typhimurium*, the results in Table (3) showed significant differences at (P<0.05) in mice weights average for groups MSb, CSb and LSb, which recorded 31.01, 32.73, 30.95g, with a mean of weight loss about -2.51,-1.25, -3.1 g and as a variation ratio -3.8, -7.1, -9.1% respectively, compared to the weights average of mice in group St + control, which recorded 34.95 g, with a mean of weight loss -6.76 and expressed by a variance -16.2%, this may be due to anorexia and diarrhea caused by Infection with live bacterial cells, as shown in Figure(2).

Table (3) average weight of mice after 8 days of infection with *S.typhimurium*

Group	The average of weight before infection (gm)	The average of weight after infection (gm)	weight Variation after 8 day (gm)
Control+St	41.11 a	34.95a	6.76a-
CSb+St	35.24 b	32.73 b	-2.51c
MSb+St	32.27 d	31.01 c	1.25 d -
LSb+St	34.05 c	30.95 d	3.1 b-
L.S.D value(P<0.05)	1.10	0.60	0.95

*The averages with different letters within the same column significantly between them

From the results, we can observed that the mean decrease in mice weights for MSb+ St, CSb+ St and LSb+ St compared to the high mean decrease of St+ control group caused by *S. typhimurium* infection, indicating the role of yeasts MSb, CSb and LSb. In enhancing host immunity and reduce the complications of disease caused by living cells of bacteria, through several mechanisms, but the decrease in the group of MSb+ St was less than in CSb+ St and LSb+ St, which indicates the efficacy of yeast MSb in preventing the occurrence of a high reduction in weights, that may be attributed to its ability to reduce the damage caused by bacteria in the host, which may caused by give it many advantages after mutagenesis process, where Ethidium bromide acted as a mutagen material and was distorts the DNA because it inserts itself between the DNA strands, and deformed double stranded DNA, this affect could DNA biological processes (16).

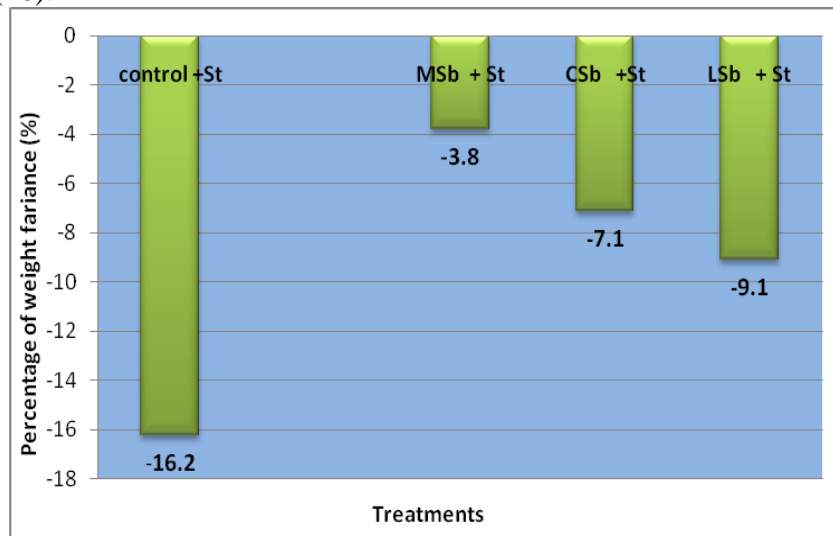


Fig. (2) Percentage of variance in the weights of groups after 8 days of infection with *S.typhimurium*

Martins *et al* (14) explained that *S.boulevardii* was able to prevent a significant decrease in the weight of the mice orally administrated with *S.boulevardii* then infected with *S.typhimurium* bacteria compared with infected mice that were not given *S.boulevardii* which showed a sharp decrease in their weights. Lu *et al* (23) found the same result when he observed a decrease in the weights of mice infected with the living cells of *S.typhimurium* compared to non-infected control weights.

- **Effect the *S.boulevardii* in the levels of IL-10 and IFN- γ :**

- **Before infection with *S. typhimurium*:** The results of the statistical analysis in figure (3) showed significant increase at ($P < 0.05$) in the level of interleukin-10 in groups administration with (MSb), (CSb) and (LSb), compared to the control group. The highest value was recorded for MSb at 267 pg/ ml, followed by CSb at 237pg/ ml and LSb at 230 pg/ ml, compared with 150 pg/ ml for control group. The results indicated the ability of living cells of mutant *S.boulevardii* (MSb) to promote the level of IL-10 more than commercial (CSb) and local (LSb) *S.boulevardii*. Some factors derived from *S.boulevardii* cells walls have contributed to the enhancement and increase of the host's immune response and the balance between Th2/ Th1, which has promoted increased IL-10 production (24, 25). Besides β -glucan, which is derived from the walls of the dead cells of *S.boulevardii* yeast in intestinal tract, has a strong effect in stimulating and increasing the immune response by binding to special receptors, especially in the gastrointestinal tract such as (TLR-2), Toll Like Receptor, Dectin-1 receptor and other receptors, stimulating immune cells to produce anti-inflammatory cytokines IL-10 (26). On the other side, the effect of β -glucan can either be directly in stimulation the immunity through its association with the future of Dectin-1 without the need for TLR-2, which ultimately leads to enhanced production of IL-10 (27).

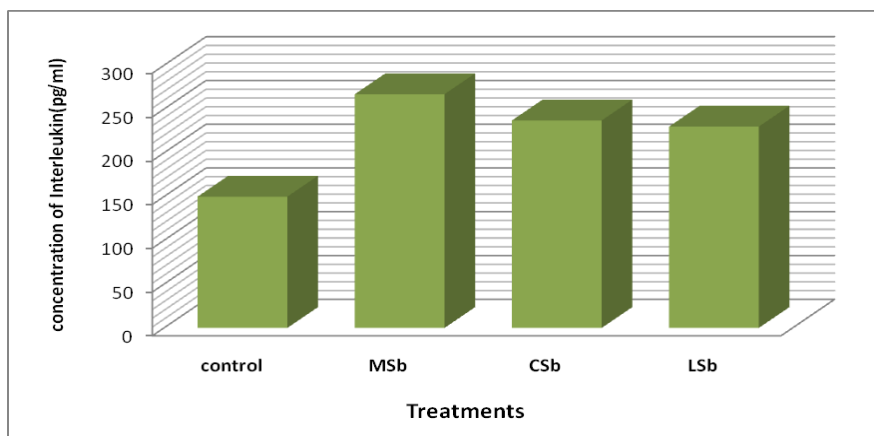


Fig. (3) level of interleukin-10 in groups administrated with *S. boulardii* for 30 days

Also, the results of using the ELISA method in figure (4) showed no significant differences in the level of IFN- γ between (MSb), (CSb) and (LSb) groups that recorded 192, 189 and 188 pg/ ml respectively and control group 187 pg/ ml. These results agreed with the findings of (28), which showed no significant differences in the concentration of IFN- γ between the chicken group fed on *S.boulardii* and the control group. Collier *et al* (29) noticed the high level of IFN- γ in the initial stages of the dosage of the pig group fed with *S.boulardii* probiotic and then returned to its normal level. Rodrigues *et al* (30) indicated that intravenous injection of mice with *S.boulardii* promoted the production of cytokine IFN- γ in the early stages of injection and subsequently reached a close concentration level with the control mice serums.

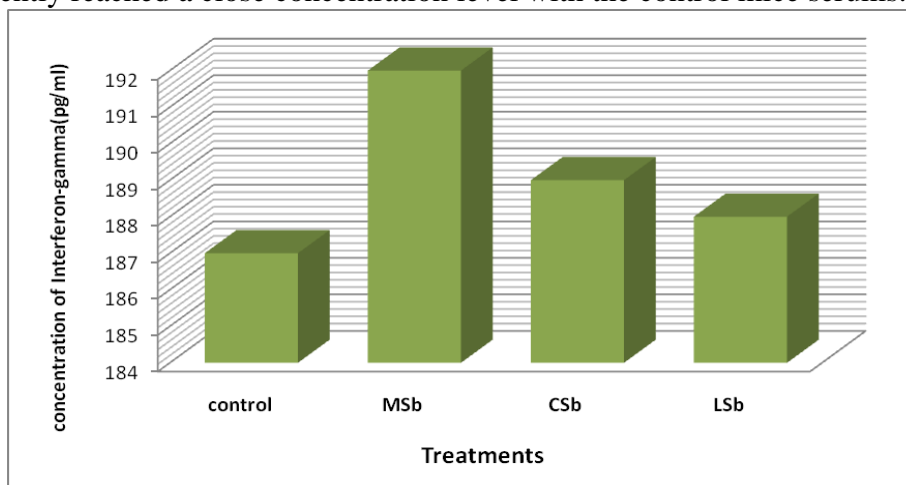


Fig. (4) level of interferon- γ in groups administrated with *S. boulardii* for 30 days

- **After 8 days of infection with *S. typhimurium*:** Besides, the results also showed significant difference in the level of interleukin-10 after infection with *S.typhimurium*, where MSb + St group recorded highest value 219 pg/ ml, followed by groups CSb+St and LSb+St that recorded 186, 176 pg/ ml respectively, compared with St+ control that was recorded 85 pg/ ml as shown in figure (5). This study is in line with (31) who found that *S.boulardii* enhances the production of cytokine IL-10 by modifying the function of dendritic cells (DCs) and inhibiting the body's induction of inflammatory response to the pathogen LPS. The results of our current study are consistent with the findings of (32) who refer to the increase the level of cytokine IL-10 in *S.boulardii*-treated mice after infection with *S.typhimurium* compared with control group.

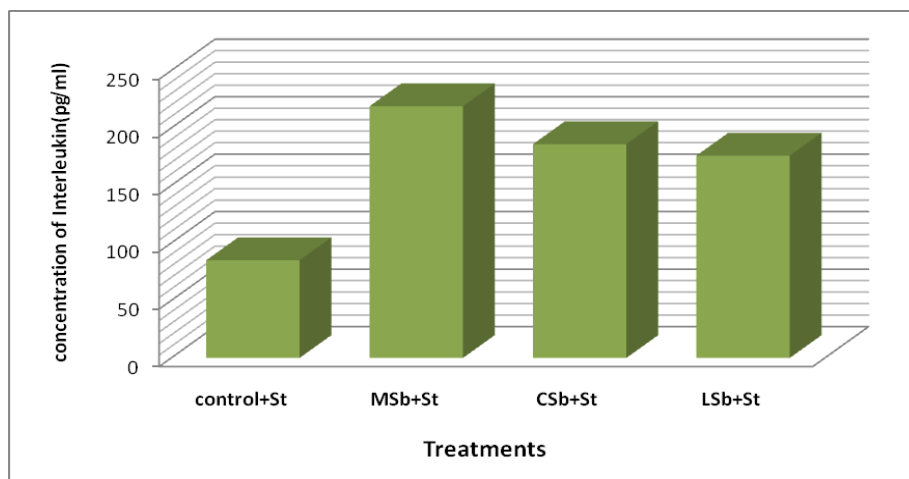


Fig. (5) level of interleukin-10 in groups after 8 days of infection with *S.typhimurium*

On the other hand, the results in figure (6) showed significant differences between MSb + St, CSb + St LSb+St groups and control +St group in the level of IFN- γ after infection with *S.typhimurium*. Where (LSb+St) gave the highest concentration 218 pg/ml followed by (CSb+St) at a concentration 210 pg/ml and 201 pg/ml for MSb+St, compared with control +St, which was recorded a statistically high level 360 pg/ml. Therefore, MSb + St is the best group, to give lowest concentration of interferon- γ This refers to the high efficiency of MSb yeast in stimulating high immune response which contributed to the activation of phagocytic cells, that promoted the killing and elimination of the living cells of *S.typhimurium* bacteria before their proliferation within the phagocytic cells (16). Our resulting is agreed with the findings of (33) who found that oral administration of *S.bouardii* probiotic to the *Shigella*-infected mice contributed to lowering the levels of pro-inflammatory cytokines IL-8, IFN- γ , IL-6 and IL-1 β as a result of their interference and inhibition of inflammatory pathways caused by bacteria infection.

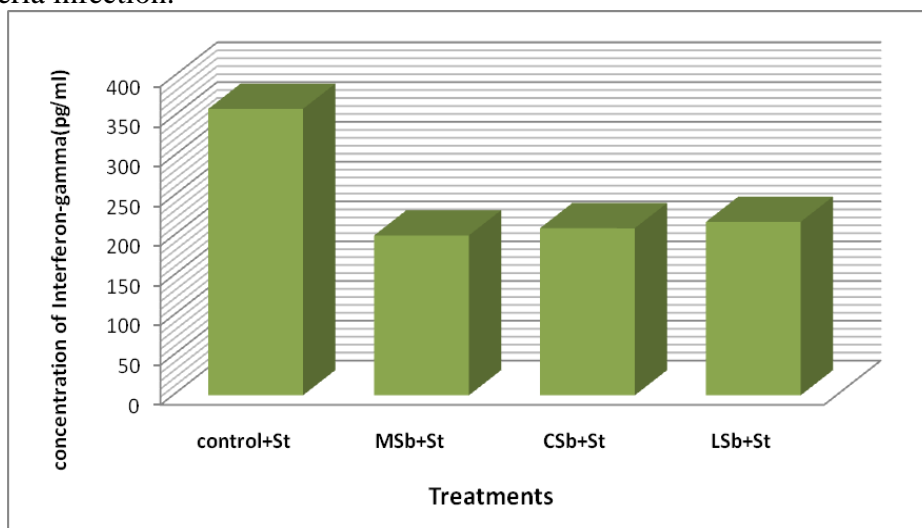


Fig. (6) level of interferon- γ in groups after 8 days of infection with *S.typhimurium*

The result showed that control +St recorded statistically high level of IFN- γ and low in the level of IL-10, this indicates that bacterial infection of tissue and phagocytic cells stimulated the cellular immune response, which contributed to an increased in the level of IFN- γ concentration as an actual result of the inflammatory response to eliminate these bacteria within the infected cells (34). Martins *et al* (35) found that dosed mice with *S.typhimurium* bacteria contributed to increased the level of pro-

inflammatory cytokines, due to the activation of MAPKS and NF-KB. Also (36) showed that mice infected with *S.typhimurium* bacteria induced increased expression to produce high levels of IFN- γ . **Conclusions**, The three species of *S.bouardii* (MSb, CSb and LSb) modulate the immune response in a murine model before and after infection with *Salmonella serovar enterica typhimurium*, but mutant isolate (MSb) was the best, this may due to enhance some therapeutic properties caused by mutagenesis.

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