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# Effects of Lactobacillus acidophilus on some intestinal physiological aspects in experimental colitis in rats

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***Abstract:***
***Background:*** *Effects of Lactobacillus acidophilus on some physiological and morphological aspects of intestinal mucosa in adult male rats with experimentally induced colitis were studied.*
***Material & methods:*** *Thirty six male rats were divided into equal four groups , 9 of each . The 1st control one( C) while the others were designated as T1,T2,T3, rats of C and T3 group received 1ml of D.W, while those of T1&T2 received Lactobacillus acidophilus at a dose of (5×108 CFU ) for two weeks ,as a pre colitis period, then acetic acid colitis induced for rats of T2 & T3and sham colitis for rats of C &T1, seven days later , at the end of post colitis period, D-xylose absorbance test, food intake, body weight, and FCR ratio were studied in addition to intestinal morphological changes.*
***Results:*** *revealed that L. acidophilus administration to AA colitis rats was useful in increase significantly ($p<0.05$) of body weight , FCR and food (%)of intestinal weight /B.W, and D-xylose absorbance( %)which affected by colitis in T1and T2 .The analysis of the photomicrograph of jejunum revealed the beneficial effects of Lactobacillus acidophilus on the intestinal mucosa by increasing the length of villi and the lesser colitis damage .*
***Conclusion:*** *L. acidophillus administration was efficient in improve the intestinal physiology and morphology in experimentally induced colitis.*
***Key words:*** *Lactobacillus acidophilus, D-xylose test, intestinal morphology, experimentally colitis*

## I. Introduction

Throughout the last decade the term probiotics attracted great interest, and is defined as a live microbial feed supplement which beneficially affects on the host . Probiotics produce their effects in gastrointestinal physiology and morphology due to their consumption can be direct or indirect [1][2]. The fate of probiotics in the gastrointestinal tract and consequent effects differ between strains. Some of them have a high survival capacity in the small intestine, and sometimes large intestine, while others are rapidly destroyed when they pass through the GIT,depends on their ability to adhere to epithelial cell lines while others do not[3]. Usually they do not colonize the intestinal mucosa for long periods of time, and are eliminated within few days after the subject stops ingesting them, [4]. In recent times the proven benefits of probiotics in treatment and prevention of several diseases of gastrointestinal tract have been reviewed extensively [5*]. Lactobacillus acidophilus*( LBA) is a probiotic strain available in conventional foods (milk, yogurt). Some *L. acidophilus* strains may be able to survive gastrointestinal transit, being resistant to bile, low pH, and digestive enzymes,they characterized in vitro, in animal studies, and in humans, may then be able to adhere to human epithelial cell lines [6]. Collitis, or irritable bowel disease (IBD), is a group of conditions characterized by gut-wall inflammation . Recent studies have also shown that some bacterial strains or mixtures may have the capacity to promote or reduce intestinal inflammation [7]. This evidence has led to an increased use of probiotic preparations in the therapy of IBD that usually contain many types of probiotics including; lactobacilli bifidobacteria, or Escherichia coli strains[8]. Probiotics promote the action within gastrointestinal tract by different mechanism, including ; acting antagonistically toward pathogenic bacteria that cause intestinal inflammation [9], colonization resistance ,the ability of normal flora to protect against the unwanted establishment of pathogen populations[10], production of antimicrobial substances[11], competition for nutrients and inhibits the adhesion of several enteric pathogens to intestinal cells[12]. A related activity is enzymatically modifying a toxin receptor [13].

Touched on previous research into the impact of preventive role of *Lactobacillus acidophilus* against colitis but devoid of reference to impact probiotics on intestinal absorptive function .The proposed study designed to investigate effects of probiotic (Lactobacillus acidophilus bacteria) on some physiological (absorptive) and morphological parameters related with small intestine in acetic acid induced colitis in male rats.

## II. Material And Methods

The present study was conducted in the department of physiology and pharmacology in college of veterinary medicine /Baghdad university during the period from 1st Jan. to 10th of Feb. at 2013 , and designed according to the Research Ethic standards Thirty six male rats with age range 10-12 weeks, were kept under a suitable condition of (21-25°C)in an air conditioned room and photoperiod of 12 hours daily. they were fed freely with standard pellet diet( table-1 ). divided randomly into (4 groups ),9 animals per group, were placed into 3 replicate each of 3 animals and handled as follows ; control group (C) T1and T2 group received ( $5 \times 10^8$ CFU) of Lactobacillus acidophilus as probiotics for 2 weeks by oral gavages needle( ref-13), the 4th group considered as T3.C and T3 received (1m per animal) of distilled water for 2 weeks by oral gavages needle. After two weeks of administration acetic acid colitis was induced for T2&T3 groups and sham colitis for C&T1 groups. After 7 days of colitis at The end of the experiment blood sample ( 4-5 ml ) was collected from the rat obtained via cardiac puncture technique from each anesthetized animal using disposable syringe (5 ml) and blood was withdrawn into plastic test tubes ( gel tube ) for serum isolation for biochemical analysis.

**Table- 1 . Nutrient composition (%)of the diet(BESLER/Turkey)**

| nutrient composition | Percentage |
|---|---|
| Dry matter | 88% |
| Crude protein | 14-16% |
| Crude fat | 2.5-3.0% |
| Crude fiber | 18% |
| Crude Ash | 10% |
| Moisture | 10% |
| Calcium | 1-3% |
| Phosphorus | 1% |
| Nacl | 1% |
| Vitamin A | 5000 IU/KG |
| Vitamin D3 | 700IU/KG |
| Vitamin E | 30 mg/KG |
| Metabolizable Energy | 2400 Kcal/KG |

**Induction of colitis** Colitis was induced in rats by intra rectal (IR) inclusion of 1ml of 4%acetic acid (AA). Briefly after general anesthesia ,a soft 8Fpediatric catheter was introduced into the anus for 6 cm and AA solution was carefully administered .before taking the catheter out ,2ml air was applied in order to spread (AA)completely in the colon [14].

**Macroscopic scoring:** Assessment of colitis was assessed by clinical signs and macroscopic scoring. For each animal, the distal 10 cm portion of the colon was removed and cut longitudinally, and slightly cleaned in physiological saline to remove fecal residues. Macroscopic inflammation scores were assigned based on clinical features of the colon using an arbitrary scale ranging from0–5 as follows [15] .

**Table 2. Colitis assessment arbitrary scale ranging .**

| **Degree** | **Colitis scoring** |
|---|---|
| 0 | No macroscopic changes |
| 1 | Mucosal erythema only inflammation without ulcer |
| 2 | Mild mucosal oedema, slight bleeding or small erosions |
| 3 | Moderate oedema, slight bleeding ulcers with slight inflammation of one site |
| 4 | Two or more site of ulceration oedema and tissue necrosis |
| 5 | Two or more site of ulceration and inflammation and ulcer extending (7 cm) along the length of colon . |

**Physiological and morphological intestinal parameters.**

**D-xylose test .-**

After 7 days of colitis all experimental animals were food and water deprived over night. Orally D-Xylose solution( (100mg/ 1m / 100g B.W.), )was gavaged in adose of 100mg/ 1m / 100g B.W . Each animal was hold in a metabolic cage for 5 hours for urine collection. Blood and urine D-xylose concentration then measured.

**Measurement of D-xylose** :

Concentration of D-xylose was measured by using of the standard colorimetric method (Fowler and Cooke, 1960), which depends on measurement of density of the brown color produced from the reaction of D-xylose with O- toluidine in presence of acetic acid and heat, the brown compound was formed with maximum D-xylose absorbance .

$$\text{D-xylose concentration g\l} = \frac{\text{Mean absorbance of test}}{\text{mean absorbance of Standard )}} \text{ x Concentration of Standard}$$

**Absorbance D-xylose %**.

Absorbed D-xylose % was calculated according to (Guijarro et al ., 2007). by the division of the amount of D-xylose in (g) excreted in urine on the amount of ingested D-xylose in (g ) was measured by the following equation .

$$\text{Absorbance of D- xylose\%} = \frac{\text{Amount of urine excreted D-xylose (gm)}}{\text{Ingested d-xylose (gm)}} \text{ x100}$$

Amount of D-xylose excreted in urine (g)=( Concentration of D-xylose in urine(g/l)×urine volume (ml) )/1000.

**Histopathological changes.**

Immediately after animal sacrifice specimens were collected from colonic and jejunum and were fixed in 10% formalin in phosphate buffered saline, embedded in paraffin and cut into 4 µm sections. Paraffin sections were deparaffinized with xylene,hydrated and stained with hematoxylin and eosin for mucosal damage assessment.

**Villus height, crypt depth, and Villus height/crypt depth ratio .**

After staining ,the length of the villi and crypt depth were measured in the small intestine preparations at a low magnification (10X) using ocular micrometer (Germany). The villus height, crypt depth, and Villus height/crypt depth ratio measured in 30 well of each slid from small intestine, and were analyze

**Statistical Analysis.**

Complicated randomized design was applied (CRD) to study the effect of the transactions studied in different qualities and compared the moral differences between the averages by Duncan polynomial test, and used SAS(2010) program in the statistical analysis .

## III. Results

**Body and organ weight**

Results revealed that acetic acid induced colitis significantly ($p<0.05$) decreased in body weight in rats of T3 group (480.66∓11.2),as compared with other groups;C T1 andT2. Intestinal weight/ body weight ( % )was significantly($p<0.05$) increased in rats received probiotics T1and T2 as compared with C and T3.

**Table-3 .** The effects of probiotic on body weight(g) , and intestinal weight / Body weight(%) in rats ,mean ±SE n=6 animal

| Groups | Post colitis week<br>Body weight (g) | Intestine<br>W./B.W.(%) |
|---|---|---|
| C | 620.33∓45.63$^{A}$ | 5.08∓0.5$^{A}$ |
| T1 | 625.33∓19.67$^{A}$ | 5.43∓0.3$^{A}$ |
| T2 | 539.00∓ 27.59$^{A}$ | 6.52∓0.4$^{A}$ |
| T3 | 480.66∓ 38.15$^{B}$ | 5.30∓0.1$^{B}$ |
| LSD | 95 | 1.15 |

C received distill water and have sham colitis,T1 received probiotics in dose (5 × 108 CFU) and have sham colitis,T2 received probiotics in dose (5 × 108 CFU) and have acetic acid colitis,T3
received Distill water and have acetic acid colitis.The capital letter denote significant differences between groups(columns) .

**Intestinal absorption of D- xylose :**

The present results showen in table -4 revealed a significant($p<0.05$) increase in D-xylose excreted in the urine(g/l) and in serum (g/l) of rats received probiotics T1 in comparism with other experimental groups,(C,T2,T3) reflected the increase of D- xylose absorbance(%) as a result for the positive role of lactoacidophillus on the absorptive function of intestinal mucosa

**Table-4 .** The effects of probiotic in D-xylose in urine (g/l) ,D-xylose inserum (g/l), and D-xylose absorbance (%) in rats Mean ∓ SE, n=6.

| groups | D-xylose in urine (g/l) | D-xylose in serum (g/l) | Absorbance D- xylose(%) |
|---|---|---|---|
| **C** | $0.136 \mp 0.01^{A}$ | $0.040 \mp 0.004^{B}$ | $0.068 \mp 0.01^{AB}$ |
| **T1** | $0.148 \mp 0.004^{A}$ | $0.230 \mp 0.03^{A}$ | $0.091 \mp 0.008^{A}$ |
| **T2** | $0.042 \mp 0.008^{B}$ | $0.079 \mp 0.01^{B}$ | $0.080 \mp 0.01^{A}$ |
| **T3** | $0.054 \mp 0.01^{A}$ | $0.082 \mp 0.01^{B}$ | $0.042 \mp 0.007^{B}$ |
| **LSD** | 0.0034 | 0.063 | 0.039 |

C received distill water and have sham colitis,T1received probiotics in dose (5 × 108 CFU) and have sham colitis,T2 received probiotics in dose (5 × 108 CFU) and have acetic acid colitis ,T3 received Distill water and have acetic acid colitis. The capital letter denote significant differences between groups(columns)..

**Colitis scoring**

Colitis scoring assessed by taking into account the mucosal lesions of hyperemia, swelling, ulceration (number and extent of ulcers). In this study the colitis scoring value of treated groupT1 and C , were significantly lower($p>0.05$) than those of the colitis groups either treated group T2 or untreated group T3, (table-5) which indicated the effect of Lactoacidophillus alone in promoting mucosal healing to normal mucosa at 2 weeks post reception of . On the other hand , probiotics improve mucosal healing of inflamed mucosa in the colitis treated group ,T2 than colitis untreated groupT3. The present results also revealed the that acetic acid induced colitis caused sever intestinal mucosa damage represented by the highest scoring value in table -5

**Table-5:** The ameliorative effects of probiotic on villus height(um),crypt depth(um) ,villus/ crypt ratio(um),in rats mean ∓SE .n=30well.

| Groups | Villus height | Crypt depth | Villus/crypt ratio | Colitisscoring |
|---|---|---|---|---|
| **C** | $108.5 \mp 3.96^{C}$ | $39.33 \mp 1.73^{A}$ | $2.82 \mp 0.12^{B}$ | $0.11 \mp 0.03^{C}$ |
| **T1** | $188.75 \mp 4.25^{A}$ | $33.66 \mp 1.57^{B}$ | $5.80 \mp 0.25^{A}$ | $0.38 \mp 0.13^{C}$ |
| **T2** | $178.50 \mp 2.30^{B}$ | $33.5 \mp 0.81^{B}$ | $5.43 \mp 0.16^{A}$ | $1.90 \mp 0.20^{B}$ |
| **T3** | $101.66 \mp 2.4^{C}$ | $35.08 \mp 1.1^{B}$ | $3.01 \mp 0.11^{B}$ | $3.35 \mp 0.33^{A}$ |
| **LSD** | 9.41 | 3.82 | 0.47 | 0.6 |

C group received distill water and have sham colitis,T1received probiotics in dose (5 × 108 CFU) and have sham colitis,T2 received probiotics in dose (5 × 108 CFU) and have acetic acid colitis ,T3 received Distill water and have acetic acid colitis. The capital letter denote significant differences between groups(columns).

**Intestinal histomorphological changes**

In the present study the light microscopic examination showed that lacto acid producing probiotics recipient caused significant differences of gastrointestinal morphology in adult male rats represented in ansignificantly increase of villus height, villus/crypt ratio with decreased crypt depth ( table-5 )of T1 and T2 when compare with C and T3 groups. Further more, results of the microscopic analysis of wall (jejunum) tissues section in the present study revealed the ameliorative effects of probiotic on negative and deleterious effects of acetic acid in the colitis treated groupT2 when compared with the untreated colitis group T3. Intestinal wall sections of the C group showed normal structure (fig-1 ) resembled by no alteration in goblet cells , mucus production, normal lymphoid tissue with no evidence for hyperplasia, and congestion. Examination of jejunum wall tissue section of rats in T1 group (probiotic), showed normal architecture with long twisting villi and sub mucosal cellular aggregation (fig-2) In untreated colitis group,T3, histopathologic feature ( fig-3) revealed intra rectal acetic acid induced colonic inflammatory reaction includes mucosal and sub

mucosal moderate to sever ulceration associated with infiltration by polymorphnuclear (PMNLs) leukocytes, macrophages, lymphocytes, connective tissue mast cells, and fibroblasts , in addition other sections showed crypt distortion, or abscess, sub mucosal edema and granulomatosis, and thickening of intestinal wall. Histological examination of T2 (probiotics and colitis )group showed milled changes characterized by mucosal slight sleighing with necrotic regress as well as increase in macrophages and lymphocytes aggregation in sub mucosa, with no evidence for necrosis was observed in crypts ( fig-4).

## IV. Discussion

**Body and organ weight:** The decreased BW of a 7 days after acetic acid induced colitis resulted from produced acute inflammation decreased intestinal nutrient uptake and consequently decrease B.W. Animals with IBD have an affected quality of life with altered metabolic status [19]. This decrease was improved by the probiotics recipient in rats of group T2, scoring the ameliorative role of probiotics in rat;s health benefits, this result is agreed with results [20] who suggested that caloric density, rather than probiotics per se, is a main determinant of body weight. Interestingly, the weight of small intestine relative to B.W. showed a slight increase in the experimental rats in comprise with control which may reveal the histological changes of intestinal wall [21].

**Intestinal absorption of D- xylose :**D- xylose is a pentose not normally present in significant amounts in blood. When it was given orally , approximately 60% is passively absorbed in the proximal small intestine, and most of it is subsequently excreted by the kidneys . The amount of D- xylose recovered in the urine or blood in specified time interval after administration of a measured dose used to evaluate intestinal mucosal absorption [22]. The potency of LBA on absorptive function was clearly represented by the increase in D-xylose absorption % denoted in our study , which could be attributed to the increased absorptive surface length determined by villus length scored in the present study, since absorption of D-xylose depends on the length of the absorptive and on the other hand, the ability of probiotics to increase the efficiency of the intestinal barrier function, [17][23] , D-xylose absorption reduced by inflammation [24].

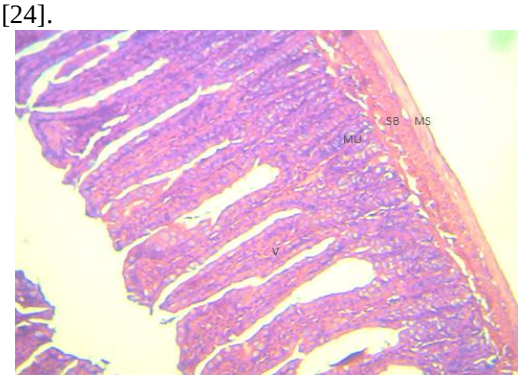



Figure -1: Photomicrograph of a section of the wall of the small intestine of the control group ,showing normal submucosa with brunner's glands , mucosa and tall cylindrical villi with goblet cells.layer mu=mucosal Layer, sb= sub mucosa ,m= muscular , v=villi . X40, H&E stain

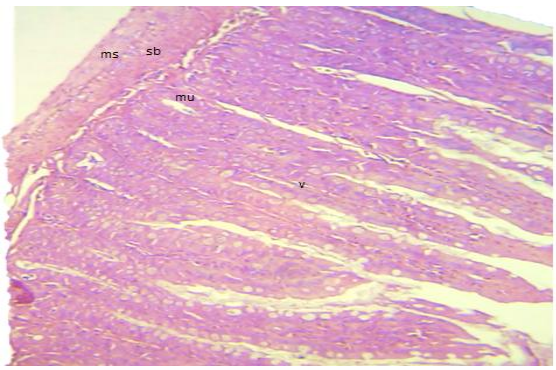



Figure-2 :Photomicrograph of a section of the wall of the small intestine of the probiotic's group , showing Long villi twisting villi (elongated ),enterocyte ,sub mucosal cellular aggregation ,intestinal layer mu=mucosal Layer, sb= sub mucosa ,ms= muscular ,v= villus heightX40, H&E stain.

Figure-3 :Photomicrograph of a section of the wall of the small intestine of the colitis group , showing Sever ulceration of mucosal layer with sever congestion sever distraction of villus mucosa resulting in fragmentation of villi mucosa , sever distraction of villi mucosa ,sever degeneration change of crept some of them suffer form narrowing g lumen ,necrosis of crept and villi atrophy ,ul=ulceration ,sb=sub mucosa.X40, H&E stain

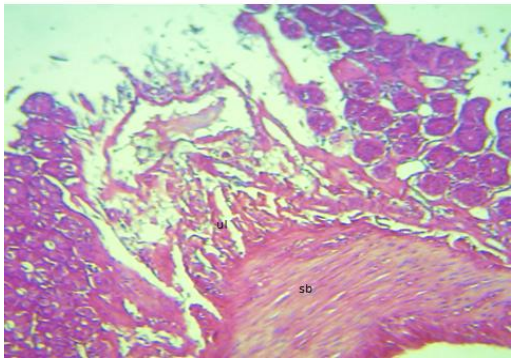
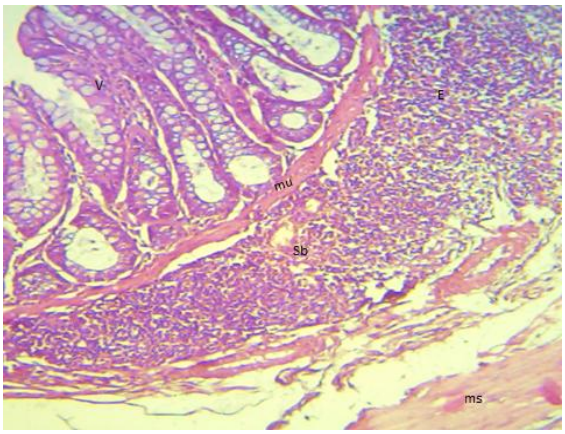



Figure-4 :Photomicrograph of a section of the wall of the small intestine of the probiotic's & colitis group , showing Long villi tortuous villi (elongated ),enterocyte ,sub mucosal cellular aggregation ,intestinal layer mu=mucosal Layer, sb= sub mucosa ,ms= muscular ,v= villus height → = cellular aggregation.X40, H&E stain




**Intestinal morphological changes:** The morphological parameters of intestinal section wall (jejunum) such as villus height, depth of crypt, and villus/crypt depth ratio proportion studied in the present study are used to investigate the effects of Lactobacillus acidophilus on intestinal morphology and function[25].In the present study, supplementation rats with lactobacillus acidophilus increased the villus height and villus height/crypt depth ratio and decrease crypt depth to promote renewal of the villus as needed in jejunum, suggesting an increased an epithelial cell turnover. .Feeding of probiotics has been shown to induce gut epithelial cell proliferation in rats , increasing villi length and vilus/crypt ratio [26][27][28].The crypt depth changes differ according to the dosage and duration of different types of probiotics administration , furthermore, at different parts of intestine[1][21].

The histopathological analysis of intestinal mucosa in the present study confirmed the protective effect of the probiotics against the sever inflammation of acetic acid colitis by decreasing the colitis hitological scoring in T2 group. This potential effects of Lactobacillus acidophilus in intestinal epithelial cells may be occurred by various mechanisms, these mechanisms caused an increasing of epithelial cells regeneration , regulation of tight junction proteins expression and prevention of attachments of pathogens to mucosa, and the regulation of mucus secretion [23][29][30] and as a result of an increase the resistant of intestinal epithelium to the pathogen bacteria .

## V. Conclusion

The present findings thus, indicated that using Lactobacillus acidophilus bacteria as probiotics gives a good results in reduction of colitis severity induced experimentally by acetic acid. . further more ,uses of *Lactobacillus acidophilus* as probiotics exert a development in intestinal histomorphological characteristic were the reason for development of absorpative functions by increase in absorbance D-xylose ( %) as a result for increase of the absorptive area. the villus height ,crept depth and villus :crept ratio

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