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The role of *Lactobacillus casei* on some physiological and biochemical parameters in male laboratory rats infection with salmonellosis

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Abstract---This research was conducted to isolation and diagnosis of *Salmonella typhimurium* that caused child diarrhea infections, whom attend in Salah-Adin Teaching Hospital in Tikrit. and determine the susceptibility of isolates against some antibiotics, also, determine the *Lactobacillus casei* as probiotics which, isolation and identification from fermented dairy samples collected from different local in Salah-Adin governorate markets, then assay the effect of orally dosage of probiotics on some physiological and biological parameters in rats that infected with *Salmonella typhimurium* isolate. *Salmonella typhimurium* were appeared at 15 isolates from child diarrhea infections samples and the *Lb. casei* isolates from fermented dairy products, then identified according to morphological, microscopic, cultural and biochemical characterizes, then selective the probiotics isolate from *Lb. casei* which depended according to their ability to grow in pH 2 and able to tolerance growth at 0.3% bile salts, furthermore their ability to adhesion with intestine mucus surface at 43.8%. The roles of orally dosage of *Lb. casei* isolate on rats infected with diarrhea induced with *Salmonella typhimurium* on some physiological, serum biochemical and the microbial intestine balance were appeared that caused to significantly modulate to enhance the negative effects by salmonellosis infection and the parameters values becomes nearly it's in control group. The orally dosage of Ciprofloxacin antibiotics were caused a significantly increased the negative effects of salmonellosis infection However, it showed to decrease the bacterial

counts of intestine from coliform and lactic acid bacteria compared with its counts in control group.

Keywords---probiotics, lactobacillus casei, Salmonella Typhimurium, diarrhea.

Introduction

Infections occurs when a microbe enters through our bodies and start to reproduction and giving symptoms. The invasive microbe was directly damage cells, or effects on the immune system and cause symptoms such as fever, as it tries to rid your body of the invader (Nguyen et al.2021) All infections caused by the microbes were fall into three major groups, acute, chronic and latent infections (Ehuwa et al., 2021) Some species of organism's are found within the patient's as normal flora, from environment around the patients, and from healthcare personal. Exogenous microbes, from the environment of hospital are mostly more resistant to antimicrobial than endogenous organisms (Kurtz et al. 2017).Worldwide, salmonellosis is one of the most frequently reported foodborne diseases, and capable to resistant most of the antibiotics. Many risk factors such as, cancer, Diabetes, human immunodeficiency, age and malnutrition, Crippled or other transition factors and Hospitalization, were the most increases risk for infection by microbes that are resistant to antibiotics (Salih et al., 2016)

Several of Lactobacillus species are markedly as essential used in fermented food production or as starter cultures or food preservative factors. Additionally, confident strains of human origins are being utilize as probiotics or vaccine carriers. The Lactic acid bacteria (LAB) often it's most commonly used in medical applications (Jung et al., 2009) specially the species of Lactobacilli bacteria (sidira et al., 2015). The increase of LAB importance in recent years were for its ability to produce a healthy material such as lactic acid, H₂O₂, bacteriocins, acetone and the diacetyl and some specific materials that acts on decreased the effects of pathogenic microorganisms, which cause different illness (Bang et al., 2018). Some *Lb. casei* strains were lends general health benefits through acting as probiotics. They are capable to decrease the incidences of gastrointestinal disorders caused by pathogenic bacteria (Tang and Lu, 2019), modulate immunity and reduce the risk of bladder cancer in the host (Aguilar-Toalá et al., 2018 ; Aryana and Olson , 2017), According to this mention, the aimed of this research was conducted to determine the role of orally dosage of probiotics as *Lb. casei* isolated from dairy products in treatment of diarrhea infections induced with *Salmonella typhimurium* on laboratory rats.

Materials and Methods

Salmonella isolation and identification: The colony of bacterial isolates which appeared on XLD agar after enumeration of stool samples were obtained then subculture on tetra-thionate broth after shaking the broth, it was incubated at 41.5 °C for 24 hours. The loop full from the bacterial growth was separated on each of XLD and SS agar plates and incubated at 37°C for 4 hours. The pure colonies of bacterial isolates were determining the shapes, color, diameter, In

addition, the gram stain type, the bacterial cells shapes and colors after observed under light microscope. After these were examine the bacterial isolates according to (Baker et al., 1985), to determine the oxidase, catalase, IMViC tests, then assurance the diagnosis of bacterial isolates used the Vetik-2 Compact system. The bacterial isolates were stored in a medium containing 15% glycerol, and stored at -20 °C.

Lactic Acid Bacteria isolation: A total of 15 fermented dairy samples were collected from different markets in Salah-Aldin governorate, 25g from each sample was dissolved in 225 ml of normal saline and makes optimal serial dilutions and from the last diluted was cultured 0.1ml on each plate contains MRS-CaCO₃ agar medium by spreading method and culturing anaerobically at 37 °C for 48 hrs on in anaerobic jar according to (Winn et al., 20016). Isolates on MRS-CaCO₃ agar were diagnosed according to the color, shape and diameters of colony, then sub-culturing for each isolate on the same medium. Isolates colony were preparing to diagnosis by makes the smear slides and staining used gram stain, then completed the microscopic, cultural and biochemical characterizes to identify the *Lactobacillus* species and selected the *Lactobacillus casei* (LC) according to that illustration in (Holt et al., 1994).

After diagnosis the *Lb. casei* it was tested the isolates for ability to growth in different pH levels from 2 to 8 (Chou and Weimer, 1999) and growth in different bile salts concentrations from 0.05 to 5.0 mg/ml according to (Lee et al., 2000), Moreover the ability of *Lb. casei* cells to adhesion on epithelial cells was tested according to (NAS-NRC, 2002).were done.

Laboratory Animals Initialization: Forty, 35(±3) day-old male growth (Albino-Sprague Dawley Rats), were individually weighed, wing banded, and housed in heated battery brooders under 12 hrs, fluorescent lighting daily with feed and water provided *ad libitum*. The rats were fed the basil diet formula that prepared according to (Seely and Van, 1981). The rats (except the control group) were orally dosage from the *S. typhimurium* bacteria at 1.5×10^8 cell/ ml, till the symptom of diarrhea infection were appeared on most of the rats. The diarrhea infection rats were separated to three groups, one of its was control positive which leaved without treatment, and the two other groups were treated with probiotics and the antibiotics.

The experimental were designed to the following treatment groups: T1) Control group: Rats orally dosage with one ml distilled water/animal/day; T2) Infection group: Rats orally dosage with *Salmonella typhimurium* and leaved till end the experimental T3) Probiotic treated group: Infection Rats that orally dosage with one ml from probiotics; T4) Antibiotic treated group: Infection Rats that orally dosage with one ml from 0.05 mg Ciprofloxacin/kg animal/day.

The probiotic's bacteria was *Lb. casei*, and the orally dosage consist 1.0 ml which divided at 0.5 ml each 12 hrs. The experimental design consisted of four dietary treatments, each group were contains two replicates of five rats per dietary treatment, and the rats were maintained to 10 days period after infection to became healthy. At the end of experiment 5ml of blood from eight rats (4 rats from each replicate) from each treatment, were collected by bled from eyes artery.

blood was taken in tubes that contain EDTA for hematological parameters determination. Like hemoglobin concentration, total erythrocyte counts and differential leucocytes were made according to the method of (Schottelius et al, 1988) and (Tietz , 2005). Other quantity was transfer to tubes as free from EDTA to assay the biochemical parameters as follow. After blood samples were centrifuged at 3000 cycle/mint. for 15 minutes and the supernatant liquid which represent blood plasma was collected using micropipette and transferred to Eppendorf tube, then kept in a freezer at -20 °C until assay the biochemical parameters that included glucose, cholesterol, urea, creatinine, total protein, albumin, globulin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were assay according to the methods in (Bezkorovainy , 2001). the blood plasma parameters were assayed according to the procedures that mention in the instructions of each kit set from (BIOLABO or BIOMERIEUX, France).

The small intestine was collected after the rats were slaughter. Then, determine the intestine microbial counts after preparing the optimal serious dilution, then was spreading on MacConkey agar and MRS agar media and incubation to appear the colony of microbial species, according to (Holt et al., 1994). Data were statical analyzed used ANOVA analysis, the general linear model was used for the Statically Analysis System (SAS, 2001) significant treatment differences were assay using Duncan's multiple-range test (Duncan, 1955). All significant statements are based on probability level at 0.05.

Results and Discussion

Isolation and diagnosis of *Salmonella spp* from Diarrhea Infections: In the present research, 100 clinical samples were collected from diarrhea infections samples. According to the results, the *Salmonella spp* isolates from the infection were appeared at 15 isolates from total samples. The negative samples for bacterial growth may be for other pathogenic species or it's taken from patients that having doses of antibiotics before taking the samples (Eykan , 2001). All collected samples from specimens were firstly isolated into suitable media as XLD agar and S.S. agar, after incubated at 37 °C for 24 hours. The biochemical diagnostic tests were conducted with (Koneman et al, 2006; Willey et al., 2008 and Prescott, 2010) aimed to isolating and identification of *Salmonella spp*. The Purification was conducted using SS agar as selective media.

All isolates were demonstrated by their ability to grow on XLD and SS agar media, these were conducted as selective and differential medium because it cans to metabolize thiosulfate compound which found in XLD media to produce hydrogen sulfide, to formation of black centers colonies, and it appeared as colorless with black center color on SS agar which differentiated from *Shigella* colonies. A single colony was chosen for plating on the blood agar media, which conducted as enrichment media and used to determine of bacterial ability for lysis of blood. The results appeared, as the bacterial isolates were capable to lysis the blood completely, noted this case from the pale zone in agar around the colony.

The bacterial isolates were executing the gram stain for colony isolates, they appear as negative response for stain, single or in short strep bacilli. The

biochemical testing investigated as positive oxidase, positive catalase, while the IMViC test appeared as (-, +, -, -) respectively. Also, the isolates performed as positive motility, and the incubation on Triple sugar iron media appeared as negative results for fermented of each glucose, sucrose and lactose, and the urease production was changeable in results. The results were explained that these isolates as *Salmonella typhimurium* which isolated at 15 isolates.

Sensitivity of Bacterial isolates to antibiotics: All bacterial Isolates were screened for their resistance against some commonly antibiotics Table 1. The results were showed that all bacterial isolates were appeared as completely resistant to ampicillin, colistin and tetracycline antibiotics. Also, with trimethoprim antibiotics were resistant from isolates. The bacterial isolates were appeared as completely sensitivity to the Amikacin (AK), Gentamicin (CN), and Ciprofloxacin (CIP) antibiotics, these findings are close to the result reported by (Pattanayak , et al, 2013).

Table 1. Ability of bacterial isolates on resistance against commonly antibiotics

Isolates Species	AK	CN	CIP	AM	CT	TMP	TE
<i>Salmonella typhimurium</i>	S	S	S	R	R	R	R
AK: Amikacin; CN: Gentamicin; CIP: Ciprofloxacin; AM: Ampicillin; CT: Colistin; TMP: Trimethoprim; TE: Tetracycline.							

Isolation and identification of lactic acid bacteria

In this study, the lactic acid bacteria (LAB) have been isolated by culturing on MRS media from dairy products (Yogurt) that provided from local markets. The diagnosis process was performed depending on morphological of colonies and biochemical parameters after obtains a subculture of pure colonies from each isolate on MRS media (De Man et al., 1960). *Lb. casei* was selected from other isolated Lactic Acid Bacteria which obtained successfully on MRS media because it was contains all nutrients needed to grow well. The identification of *Lb.casei*, was completed by anaerobic cultivations and ability to utilize the CaCO₃ when cultivated on MRS- CaCO₃ and the colony was appeared as restricted and creamy color in central of pellucid zone for the *Lb. casei* compare with other species of lactobacillus , which cultivated aerobically and not able to utilize the CaCO₃ (Gilliland , 1990 ; and Cogan , 1999) Also was differed from *Lb. casei* in capable to grow in concentrate 6.5% NaCl in MRS medium, and acts as capable to ferment of inositol, mannose and not fermented sorbitol, while the *Lb. casei* had a reverse action. These morphological and biochemical characterizations were in agreement with found in Berge's Manual guide at (Holt et al., 2005).

Effects of orally dosage of *Lb. casei* on some parameters of salmonellosis infection rats

Blood Parameters: The table 2. Was illustrated the effect of orally dosage of *Lb. casei* on WBC, Granulocytes percentage, Monocytes percentage, Lymphocytes, Hgb, RBC count in blood of rat's infection with salmonellosis and treatment for 10 days. The count of WBCs (T3), in infection rats with salmonellosis that orally

dosage from *Lb. casei* were appear as, not differ significantly ($p < 0.05$) with control group. While the rat's infection with salmonellosis when treated with ciprofloxacin (T4) were show as decreased the WBCs count to become $5.16 \times 10^3/\text{mm}^3$. The differential of WBC counts was also show in the granulocyte's cells percentage were decreased while, with the monocytes, lymphocytes cells were increased significantly when compared with its percentage in control group (T1). Also, the orally dosage from the ciprofloxacin was caused to decreased significantly the Hb and RBCs counts to $10.21 \times \text{g/dl}$ and $4.93 \times 10^6/\text{mm}^3$ respectively compared with control group (T1).

Table 2. Effect of orally dosage from *Lb. casei* on some blood parameters in salmonellosis infection rats

Treatments types (1 ml of Bacterial suspensions/Animal/Day)	Parameters tested					
	WBCs ($\times 10^3/\text{mm}^3$)	Granulocyte %	Monocyte %	Lymphocyte %	Hgb (g/dl)	RBCs ($\times 10^6/\text{mm}^3$)
T1	5.86 b ± 1.42	62.50 b ± 1.74	3.00 c ± 0.18	34.50 g ± 1.06	12.72 b ± 1.63	5.35 b ± 1.42
T2 (Infection group)	8.94 a ± 0.88	50.60 c ± 1.97	3.10 c ± 0.99	46.30 f ± 1.32	11.43 a ± 1.34	4.27 a ± 1.05
Treatment groups						
T3	6.27 b ± 1.25	55.43 f ± 1.08	4.70 b ± 1.11	39.87 b ± 0.89	11.81 b ± 2.11	5.01 b ± 0.13
T4	6.16 b ± 0.62	65.27 a ± 3.41	2.78 c ± 0.07	29.34 h ± 1.53	10.21 c ± 0.89	4.93 b ± 0.93

- Similar letters on the means of each column indicate significant differences at probability 0.05.
- Means values were taken from eight replicates. \pm standard error.

T1: control group; T2: Salmonellosis Infection group: orally dosage (1.5×10^8) cells/ml, from *S. typhimurium*; T3: Probiotic treatment of salmonellosis rats' group: orally dosage (1.5×10^8) cells/ml, from *Lb. casei* T4: Antibiotic treatment of salmonellosis rats' group: orally dosage from Ciprofloxacin at 0.05 mg/kg body weight.

Biochemical parameters of serum blood: The effect of orally dosage of *Lb. casei* as probiotics on some biochemical parameters of rat's serum infected with salmonellosis for ten days periods was shown in (Table 3). The orally dosage from *S. typhimurium* (T2) resulted in significantly ($p < 0.05$) decreasing the glucose, cholesterol and creatinine significantly to became 86.2(mg/dl), 132.1(mg/dl) and

0.92(m/dl) respectively, and the other parameters were not significantly differed when compared with same parameters in serum of control rats' group (T1). The orally dosage from *Lb. casei* (T3) had effects in modulate all the negative effects that's caused by the *S. typhimurium* bacteria in biochemical tests and became significantly not differ or nearly with the same parameters at control serum of rats group. While the use of ciprofloxacin as antibiotics (T4) were caused a negative effect especially in significantly increased the urea, creatinine and decreasing the globulin when compared with same parameters in the serum of rats in control group. The results of this study clearly indicated that Cholesterol, Glucose were significantly improved as a result of orally dosage with probiotics, concerning the cholesterols it's decrease when dietary probiotics, our previous study was in agreement with (Capcarova and Kolesarova, 2012; Capcarova et al, 2011 b), whom indicated that supplementation of probiotics were caused in decreased the glucose and cholesterol content in hens (Capcarova and Kolesarova, 2012). The other results of our study as glucose, was increased with orally dosage with probiotics and the influence as transport properties of small intestine epithelium and increase absorption of glucose could be interpreted as a positive effect for the rats (Lodemann et al., 2006).

The Moral elevation of the serum protein is due to the role of folic acid in stimulating protein synthesis increase in general (Protein synthesis) through impact positive in the liver and stimulate its action. Our study was in agreement with (Scott, 2002) who indicated, that the composition of the blood protein is important in increasing the natural growth of the body and the major part of these increases in plasma protein level and the albumin.

Table 3. Effect of orally dosage from *Lb. casei* on serum blood biochemical tests in salmonellosis infection rats

Treatments types (1 ml of Bacterial suspensions/Animal/Day)	Serum Parameters tested						
	Glucose (mg/dl)	Cholesterol (mg/dl)	Urea (mg/dl)	Creatinine (m/dl)	Total protein (g/l)	Albumin (g/l)	Globulin (g/l)
T1	93.8 b ±3.05	146.3 a ±6.34	39.3 3 c ±1.76	1.22 a ±0.05	8.80 a ±0.6 9	3.84 a ±0.05	4.96 a ±0.09
T2(Infection group)	86.2 d ±4.17	132.1 d ±5.67	38.8 9 c ±2.13	0.92 b ±0.02	9.13 a ±0.8 1	3.57 a ±0.04	5.56 a ±0.07
Treatment groups							
T3	94.7 a ±2.81	142.1 c ±6.11	41.3 3 b ±1.27	1.31 a ±0.01	8.50 a ±0.0 5	3.23 a ±0.03	5.27 a ±0.07
T4	91.3 c ±4.19	143.5 c ±7.06	46.1 2 a ±0.83	1.41 a ±0.06	7.50 b ±0.5	3.90 a ±0.07	3.60c ±0.15

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- Similar letters on the means of each column indicate significant differences at probability 0.05.
- Means values were taken from eight replicates. ± standard error.

T1: control group; T2: Salmonellosis Infection group: orally dosage (1.5×10^8) cells/ml, from *S. typhimurium*; T3: Probiotic treatment of salmonellosis rats' group: orally dosage (1.5×10^8) cells/ml, from *Lb. casei* T4: Antibiotic treatment of salmonellosis rats' group: orally dosage from Ciprofloxacin at 0.05 mg/kg body weight.

Serum blood enzymes activity: The blood enzymes activity as Alanine amino transferase (ALT), Aspartate amino transferase, (AST) and Alkaline phosphatase (ALP) of salmonellosis infection rats after ten days orally treated with *Lb. casei* of rats were illustrated in (Table 4). The results were indicated that the salmonellosis infection rats group (T2) and the rat's group that orally dosage of probiotics (T3) was significantly ($p < 0.05$) increased of ALP activity while the AST and ALT enzymes activity were not different significantly when compared with the same enzymes in serum of rats in control group (T1). The intakes of ciprofloxacin antibiotics from rats (T4) were caused to increase significantly the three enzymes activity when compared with the control group. These results of AST, ALT and ALP activities of the serum may be due to the liver and the kidney functions and rats' health. These results were in agreement with (Kamgar et al., 2013). The amelioration effects of orally treated for rats with probiotics may be refers to the inhibits the accumulation of lipids and preventing cells toxicity by lipids (Lipotoxicity) in liver and kidney of treated rats (Oliveira et al, 2012). Also. (Zhu et al., 2011) noted that the probiotics from LAB capable to inhibits formation of the compounds which effecting on increasing the oxidation process such as (*t*-BHP) tert-butyl hydro peroxide and causing in destroying the liver cells.

Table 4. Effect of orally dosage from *Lb. casei* on serum enzyme activity in salmonellosis infection rats

Treatments types (1 ml of Bacterial suspensions/Animal/Day)	Enzymes Activity		
	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
T1	41.28b ±1.25	16.22b ±2.32d	18.61c ±1.94e
T2(Infection group)	43.05b ±1.46	16.57b ±1.25	24.44b ±2.13
Treatment groups			
T3	42.21 b ±1.75	17.49 b ±2.18	24.13 b ±1.47
T4	48.62a ±2.94	24.25a ±1.87	28.68a ±2.16

- Similar letters on the means of each column indicate significant differences at probability 0.05.
- Means values were taken from eight replicates. ± standard error.

T1: control group; T2: Salmonellosis Infection group: orally dosage (1.5×10^8) cells/ml, from *S. typhimurium*; T3: Probiotic treatment of salmonellosis rats' group: orally dosage (1.5×10^8) cells/ml, from *Lb. casei* T4: Antibiotic treatment of

salmonellosis rats' group: orally dosage from Ciprofloxacin at 0.05 mg/kg body weight.

Intestinal Microbial balance: The infection of laboratory rats with salmonellosis by orally dosage of *S. typhimurium* bacteria (T2) were significantly increased ($p < 0.05$) the total counts of coliform species 105 (log 10 cfu /g of intestine) and decreased the counts of LAB in intestine tract to 74 (log 10 cfu /g of intestine), compared with the its counts that appeared at 90 and 84 (log 10 cfu /g of intestine) respectively in control group T1 (Table 5).

Table 5. Effect of orally dosage from *Lb. casei* on microbial intestine balance in salmonellosis infection rats

Treatment types (1 ml of Bacterial suspensions/Animal/Day)	Bacterial counts (log 10 cfu /g of intestine)	
	Coliform	LAB
T1	90 b \pm 5.4	84 b \pm 2.8
T2(Infection group)	105 a \pm 2.1	74 c \pm 4.1
Treatment groups		
T3	62 c \pm 3.5	95 a \pm 3.7
T4	51 d \pm 1.04	63 d \pm 1.48

- Similar letters on the means of each column indicate significant differences at probability 0.05.
- Means values were taken from eight replicates. \pm standard error.

T1: control group; T2: Salmonellosis Infection group: orally dosage (1.5×10^8) cells/ml, from *S. typhimurium*; T3: Probiotic treatment of salmonellosis rats' group: orally dosage (1.5×10^8) cells/ml, from *Lb. casei* T4: Antibiotic treatment of salmonellosis rats' group: orally dosage from Ciprofloxacin at 0.05 mg/kg body weight.

The orally dosage with *Lb. casei* (T3) was caused a positive balance of rat's gut microbiota through significantly increased the *Lb. casei* to 95 (log 10 cfu /g of intestine) against the coliform bacterial count at 62 (log 10 cfu /g of intestine). In addition, the orally dosage from ciprofloxacin was caused to decreased significantly the counts both of coliform and LAB and became at 51 and 63 (log 10 cfu /g of intestine) compared with its intestine bacterial counts in the rats of control group (Table 5). The probiotics used in this study consist of *Lb. casei* and its metabolites which may be caused significantly increased the LAB species of gut microflora and it play an important role in maintaining a positive balanced ecosystem. This bacterial species was usually capable to produce antimicrobial substances as bacteriocins (Ting et al., 2009), lactic and acetic acid which caused to decreased the rats gut acidity then became an optimal for pathogenic bacterial growth (Willis et al., 2007), also, were acts as competition for nutrients and adhesion sites with pathogenic bacteria (Walter et al., 2008), and it has important roles in increased of enzyme activity, antibody levels and macrophage activity (Fuller, 1989). The above mention actions were caused in prevention the enteric diseases which its important concern to the health (Higgins et al, 2007).

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