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Histopathological Effects of *Bifidobacterium* spp. in Balbc Mice Infected with *Salmonella typhimurium*

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Abstract

The present study involved isolation and characterization of *Bifidobacterium* from stool of healthy persons and infants. A total of 66 isolates has been collected randomly from healthy persons and infants in Al-Najaf Al-Ashraf city during the period 1/2012-4/2012. Identification of isolates has been carried out depending on their characters on selective media, biochemical tests and polymerase chain reaction technique (PCR). Our results showed that all 66 isolates belong to *Bifidobacterium spp.*, while none of these isolate were belong to *B. infantis* according to the results of PCR.

Furthermore, to explain the role of *Bifidobacterium spp.* in decreasing infection by pathogenic bacteria, experimental study has been carried out. *Salmonella typhimurium* were used as pathogens that infect Balb/C mice which were used as a model for experimental study. The result revealed that mice which administered with *Bifidobacterium* and infected with *S. typhimurium* showed no important clinical signs of salmonellosis with no pathological of histopathological change when compared with mice that were infected with *S. typhimurium* (-ve control). Also, the result revealed that Gentamicin had little effect in the treatment of mice administered with *Bifidobacterium* and infected with *S. typhimurium*, when compared with mice infected with *S. typhimurium* only.

Keywords: ***Probiotics; S. typhimurium; PCR; Histopathology; Pathogenicity***

Introduction

Bifidobacterium spp. is one of the most probiotic that widely used. These micro-organisms are common members of the human gut microbiota and they predominate in breast-fed infants [1].

Bifidobacterium is a Gram positive bacterium, non-motile, non-spore forming, irregular rods and obligate anaerobic [2]. The typical habitate of some *Bifidobacterium* is human, honeybee intestinal tract and warm-blooded animals. *Bifidobacterium* among the common commensal intestinal microflora of human and animals comprising get to 3% of the total fecal microflora

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of adult and get to 91% of the total microflora in infant gut of breast fed babies while it forms up to 75% in formula fed infants [3,4].

Classification and identification of *Bifidobacterium* species by conventional methods that depended on carbohydrates fermentation pattern has been demonstrated to be strain–specific rather than species–specific [5] while molecular identification depending on 16SrDNA has been demonstrated to be a punchy and minutest method to determine phylogenetic relationships among *Bifidobacterium* which revealed more than 99% of sequence similarity within the group of *Bifidobacterium* [5-7]. Currently, *Bifidobacterium* comprises over 30 species with correctly published names, one of which also has subspecies [8-10].

Several studies referred to the role of *Bifidobacterium* species in maintaining general health because of beneficial effect of *Bifidobacterium* on human health such as (i) stimulation of immune response. (ii) reducing the growth of many potential pathogens. (iii) Inhibition of costiveness, diarrhea and other intestinal infection. (v) enhancement of lactose–tolerance and (iv) it could be used to recover the intestinal microflora after an antibiotic treatment [11,12].

The mechanism by which *Bifidobacterium* could exert their useful effect involved the interaction between *Bifidobacterium* and mucosa epithelial cells which play a significant role in barrier defense, discriminating between invasive micro organisme from one site and harmless microorganism and dietary antigen on the others and as cytokine producer [13]. Also, other studies demonstrated that *Bifidobacterium* and other lactic acid bacteria can enhance for example fucosylation of glycolipids in the cells of small intestine by modification of host environment [14,15]. This study aimed to explain the role of *Bifidobacterium* species in decreasing the effect of pathogens in animal lab.

Materials and Methods

Collection of specimens

Stool samples were collected from healthy infants and normal persons during the period from 2012/1-2012/4 in sterile scrow cop to avoid any possible contamination and mixed with normal saline to form a suspension, then 0.1 ml of suspended stool was cultured on LCL agar and MRS agar, incubated at 37°C for 24 hrs. under anaerobic condition.

Identification of *Bifidobacterium spp*.

- Conventional methods: A suspected colonies appears on LCL and MRS agar were further identified identified according to cultural and biochemical tests that describe previously [16].
- Molecular identification of *B. infantis*: Alkaline lysis method described by Sambrook and Russell [17] was carried out for extraction of DNA for identification of *Bifidobacterium spp*. by PCR technique. In order to amplified *16S rDNA* gene in genomic DNA a set of primers has been used (Pbi (F, 5´-CCGGAATAGCTCC-3´ and R, 5´-GACCATGCACCACCTGTGAA-3`) for *Bifidobacterium spp*. while Bil (F, 5´--AGTTGATCGCATGGTCTTCT-3´ and Inf R 5´- CCATCTCTGGGATC-3´ were used for identification of *B. infantis* [18]. PCR amplification solution consist of 5 µL of PCR master mix (Top DNA polymerase 1U, 250 µM of each dATp, dGTp, dCTp and dTTp, Tris-HCL (PH 9.0), 10 mM, KCL, 30 mM and 1.5 mM MgCL, stabilizing and tracking dye), 2.5 µL of each primer and 5 µL of extracted DNA. Complete the final volume of mixture to 20 µL by adding nuclease free water

and spin the mixture for 1 min. PCR was conducted with the following condition: 92°C for 5 min followed by 35 cycle of 92°C for 30 sec, 52°C for 30 sec, 72°C for 1 min with final extension at 72°C for 10 min.

The resulting of PCR products were electrophoresed in 1% agarose gel to yield a first amplicon of 914 bp for *Bifidobacterium spp* and 837 bp for *B. infantis* [18].

Experimental Procedure

Animals

A total of mature Balb/c mice (male and female) were obtained from college of veterinary /Kufa University and housed in clean plastic cages at room temperature in animal house at the college of Education for girl's/Kufa University for adaptation and reproduction. Care was taken to avoid any unnecessary stress.

All animals were feed and watered freely in the cages with tap water. After 4 months of reproduction, the final number of animals which adapted for experimental study was 50 males, their age ranged between 8-16 week and their weight ranged between 23-33 g.

Estimation of infectious dose (ID)

Preparation of*salmonella typhimurium* suspension: Colony forming units per ml (CFU/ml) of *S. typhimurium* obtained from college of Medicine /Kufa University have been calculated according to the method of Miles et al. [19] by inoculation BH Iagar with 5 colonies of *S. typhimurium,* incubated at 37°C for 24 hrs. Centrifugation was carried out at 8000 rpm/10 min and washed the pellet with PBS for three times and resuspended the pellet with 1 ml of PBS. A ten-fold dilution was done and calculates the CFU/ml for each diluents and select the diluents which had the following concentrations for drenching the mice: (9×10^6 CFU/ml), (7×10^7 CFU/ml), (8×10^8 CFU/ml), (5×10^9 CFU/ml), (4×10^{10} CFU/ml) and (7×10^{11} CFU/ml).

Inoculation of mice: Thirty-five healthy male mice were selected for estimation the infectious dose and divided into seven groups each group contained five mice. Each one of six group of the mice were drenching orally with 1 ml of each calculated (CFU/ml) diluents by using syringe while the control group drenched with phosphate buffer saline (PH=7.2).

To estimate the infectious dose, all groups were noticed for 30 days to calculate the live and dead mice which were determined by selecting a group of mice that's howed clinical signs are similar to those found in Salmonella with no mortality [20]. After the appearance of clinical signs, two mice were sacrificed for necropsy for isolation of *S. typhimurium* from internal organs.

The effect of *Bifidobacterium spp*. in mice

Inoculation of mice with *Bifidobacterium* spp.: Two groups of healthy male mice which contained 5 mice in each group were drenching with 1 ml of 8×10^8 CFU/ml of *Bifidobacterium* spp orally by using syringe [19]. All mice were undergone daily observation and samples of mice stool were collected daily for isolation of *Bifidobacterium* spp.

Experimentally induction of Salmonellosis

Three groups of healthy male mice contained 5 mice in each group were used in this experiment in addition to two groups which prepared as a *Bifidobacterium* spp. treated. All mice in each group were administered orally with 1 ml of infectious dose of *S. typhimurium* and observed daily pre-and post-infection, this observation included the important clinical examination such as temperatures, appetite, presence of diarrhea, dehydration, and body weight for clinical signs.

After appearance of clinical signs which referred to infected of mice with salmonellosis, two groups of mice, one treated previously with *Bifidobacterium* spp. were injected with Gentamicin twice daily (each 12 hrs).

Blood samples were collected via cardiac puncture technique after necropsy and put it in a tube containing EDTA, mixed gently and it was used for hematological test. Multiple sections of liver, intestine and stomach were prepared and stained with hematoxyline stain.

Statistical analysis

Stagraphic plus 5.1 software was used for statistical analyses (Manugistics, Rockville, MD, USA). ANOVA was created to evaluate significant differences between means with post hoc Fisher's least significant differences (LSD) test at $P<0.05$. Data are expressed as mean and standard deviation (SD) of duplicate measures determined in four independent experiments.

Results

Isolation and identification of *Bifidobacterium*

Initial identification of *Bifidobacterium* spp. depended on colonies morphology, microscopic examination of cell and biochemical tests. Microscopic examination of *Bifidobacterium* spp. showed that it was a gram positive branched or pleomorphic rods appeared as Y and V shape. The colonies appeared small in size, white to cream in color and non- motile on MRS agar. All isolates showed negative haemolysis when growing on blood agar base. The optimum growth temperature of *Bifidobacterium* spp. isolates were 37°C and it could grow well at 40°C under anaerobic condition.

All isolates were catalase negative and oxidase negative. The ability of isolates to grow on simmon citrate was variable and some isolates had the ability to ferment glucose without gas production as shown in KIA test which revealed an acid /acid. All isolates showed negative results for indole, methyl red and Vogues Proskauer test.

Molecular identification of *Bifidobacterium* spp.

Molecular method by using PCR technique for amplification specific gene (16SrDNA gene) was used for specific identification of *Bifidobacterium spp*. The results of amplification by PCR for 16 SrDNA were shown in FIG. 1. *Bifidobacterium spp*. was identified by PCR when amplicon was described with molecular weight (914) bp. All isolate showed +ve result for DNA amplification. On the other hand, identification of *Bifidobacterium* spp. to species level was carried out by amplification of 16SrDNA a specific gene for identification of *B. infantis* which identified when one amplicon with molecular weight (837) bp was amplified.

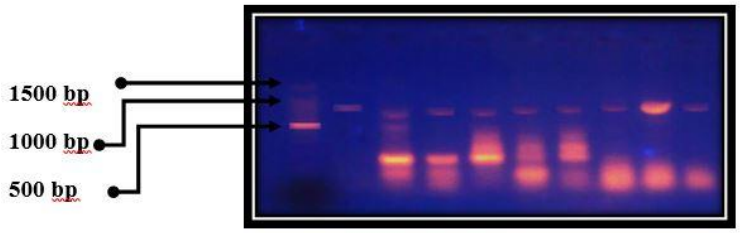

FIG. 1. **Agarose gel electrophoresis of 16srDNA gene (914 bp) foridentification of *Bifidobacterium spp*. Line 1: Ladder DNA (100bp). Line 2 - 11*: Bifidobacterium spp*. isolates.**

To identify *Bifidobacterium* spp. isolated from human feces, the species-specific PCR technique was used. The results showed that none of these isolates were belong to *B. infantis.*

Experimental study of the results

The result revealed that the infectious dose of *S. typhimurium* in mice drenching with bacteria was (8×10^8 CFU/ml) by calculating dead and live in each group during (30) days as shown in TABLE 1.

As we showed in this dose the mice were alive with clinical signs of salmonellosis such as dullness, diarrhea, weakness, fever, loss of appetite, decreased of body weight. On the other hand, administration of 1 ml of (8×10^8 CFU/ml) of *Bifidobacterium* spp. showed the isolation of *Bifidobacterium* spp. in mice stool without any change with their appearance which referred to the adaptation of bacteria and their ability to colonize the intestine normally.

TABLE 1. **Estimating of infectious dose (ID) of *S. typhimurium*in mice.**

Groups	Dose*	Alive	Dead	Percent mortality
1	7×10^{11}	1	4	80%
2	4×10^{10}	2	3	60%
3	5×10^9	4	1	20%
4	8×10^8	5	0	0%
5	7×10^7	5	0	0%
6	9×10^6	5	0	0%
7	PBS	5	0	0%
No. of rabbits in each group = 5. Total no. of rabbits = 35. *The dose calculated as **(**CFU/ml).				

Experimental induction of Salmonellosis

After estimating of infectious dose, five groups of healthy male mice (5 mice in each group) were conducted to clinical observation and other examination as follows:

Daily observation of clinical signs: The +ve control group of mice didn't show any important clinical signs during the experimental period which continued for (30) days in a comparison to the clinical signs that appeared on mice in -ve control group which include; dullness, diarrhea, weakness, fever, loss of appetite, decreased of body weight. On the other hand, group which treated previously with *Bifidobacterium* spp. showed less clinical signs which include: increase of body weight, abdominal enlargement.

Hematological test: The result of hematological analyzer which compared with control +ve and control -ve are shown in TABLES 2 and 3. Our data referred to a significant increase in WBC, RBC, HCT, MCV and a significant decrease in HGB, MCH, MCHC, PLT, LYM%, LYM in manipulated group in comparison with +ve control, while there was a significant increasing in WBC, RBC, HGB, HCT, MCV, PLT, LYM% and a significant decrease in MCH, MCHC, LYM in treated group compared with -ve control.

TABLE 2. **The effect of *Bifidobacterium* spp. and drug in physiological parameter in mice infected with *Salmonella typhimurium* (compared with positive control).**

Parameters	**Control (+)**	***Salmonella*+ drug**	***Salmonella*+ *Bifidobacterium***	***Bifidobacterium*+ Drug + *Salmonella***	**LSD**
WBC	2.5×10^3/ µL	1×10^3/ µL	4.8×10^3/ µL	1.7×10^3/ µL	1.2×10^3
RBC	6.6×10^6/ µL	5.69×10^6/ µL	8.21×10^6/ µL	4.6×10^6/ µL	0.8×10^6
HGB	12.8 g/dL	9.1 g/dL	12.7 g/dL	6.9 g/dL	2.4
HCT	33.20%	31.10%	45.60%	23.10%	6.8
MCV	49.8 fl	54.7 fl	55.5 fl	50.2 fl	2.2
MCH	19.2 Pg	16.0 Pg	15.5 Pg	15.0 Pg	1.7
MCHC	38.6 g/dL	29.3 g/dL	27.9 g/dL	29.9 g/dL	4.2
PLT	1805×10^3 µL	110×10^3 µL	806×10^3 µL	95×10^3 µL	211.6×10^3
LYM%	92.40%	0%	72.7%	0%	20.8
LYM	2.3×10^3 µL	0×10^3 µL	3.5×10^3 µL	0×10^3 µL	0.7×10^3

Pathological changes

As it is shown in FIG. 2A, 2B, and 2C there are no significant pathological changes in +ve control group and group administered with *Bifidobacterium* spp. in comparison with -ve control which show a significant pathological change. The animals were examined after 3 and 5 days post infection. Two mice were sacrifice necropsy in each time.

Three-day post-infection: After 3 days of inoculation the mice were sacrificed and showed the following significant change as follows:

- **Group -ve control:** Enlargement was observed in the size of liver, spleen, mesenteric lymph nodes and heart with decrease in the size of stomach. Large intestine showed sever congestion and filled with feces. As it is shown in FIG. 2B.
- **Group treated with *Bifidobacterium spp*.:** Enlargement in the size of liver, spleen, mesenteric lymph nodes and heart and decrease in the size of stomach. Large intestine showed sever congestion and filled with little amount of feces (FIG. 2D).
- **Group treated with *Bifidobacterium* and Gentamicin:** As it is shown in FIG. 2E there is enlargement in the size of liver, mesenteric lymph nodes and heart and increase in the size of stomach. Large intestine showed sever congestion and filled with feces.
- **Group treated with Gentamicin only:** Enlargement in the size of liver (little), mesenteric lymph nodes and heart with normal size of stomach. Large intestine showed sever congestion and filled with little amount of feces (FIG. 2F).

TABLE 3. **The effect of *Bifidobacterium* spp. and drug in physiological parameter in mice infected with *Salmonella typhimurium* (compared with negative control).**

parameter	**Control (-)**	***Salmonella*+ drug**	***Salmonella*+ *Bifidobacterium***	***Bifidobacterium*+ Drug+*Salmonella***	**LSD**
WBC	0.6×10^3/ µL	1×10^3/ µL	4.8×10^3/ µL	1.7×10^3/ µL	1.3×10^3
RBC	1.2×10^6/ µL	5.69×10^6/ µL	8.21×10^6/ µL	4.6×10^6/ µL	3.2×10^6
HGB	4.0 g/dL	9.1 g/dL	12.7 g/dL	6.9 g/dL	3.6
HCT	6.00%	31.10%	45.60%	23.10%	8.7
MCV	50 fl	54.7 fl	55.5 fl	50.2 fl	2.5
MCH	33.3 Pg	16.0 Pg	15.5 Pg	15.0 Pg	4.4
MCHC	66.7g/dL	29.3g/dL	27.9g/dL	29.9 g/dL	10.8
PLT	68×10^3 µL	110×10^3 µL	806×10^3 µL	95×10^3 µL	277.3×10^3
LYM%	0%	0%	72.7%	0%	60.8
LYM	0×10^3 µL	0×10^3 µL	3.5×10^3 µL	0×10^3 µL	1.6×10^3

- **Histopathology:** Multiple sections were prepared from liver and intestine of mice from each group. The results of histopathology were shown in FIG. 3 and 4.
- **Group treated with PBS:** The results showed no changes in the morphology of mucosa and epithelium (FIG. 2A and 3A).

- **Group infected with s*almonella***: Amucosal autolysis and some back-ground changes were generally noted when a small proportion of the intestinal tissues were examined, including lymphoid hyper plesia and sub mucosal oedema. Periportal inflammation was unexamined accurately and low number of littersneutrophils and lymphocytes were considered a background level observation, also it had pyogranulonas in their liver compared with that adminstered previously with *Bifidobacterium* which showed no pyogranulonas in their liver (FIG. 2B and 2C & FIG. 3B and 3C).

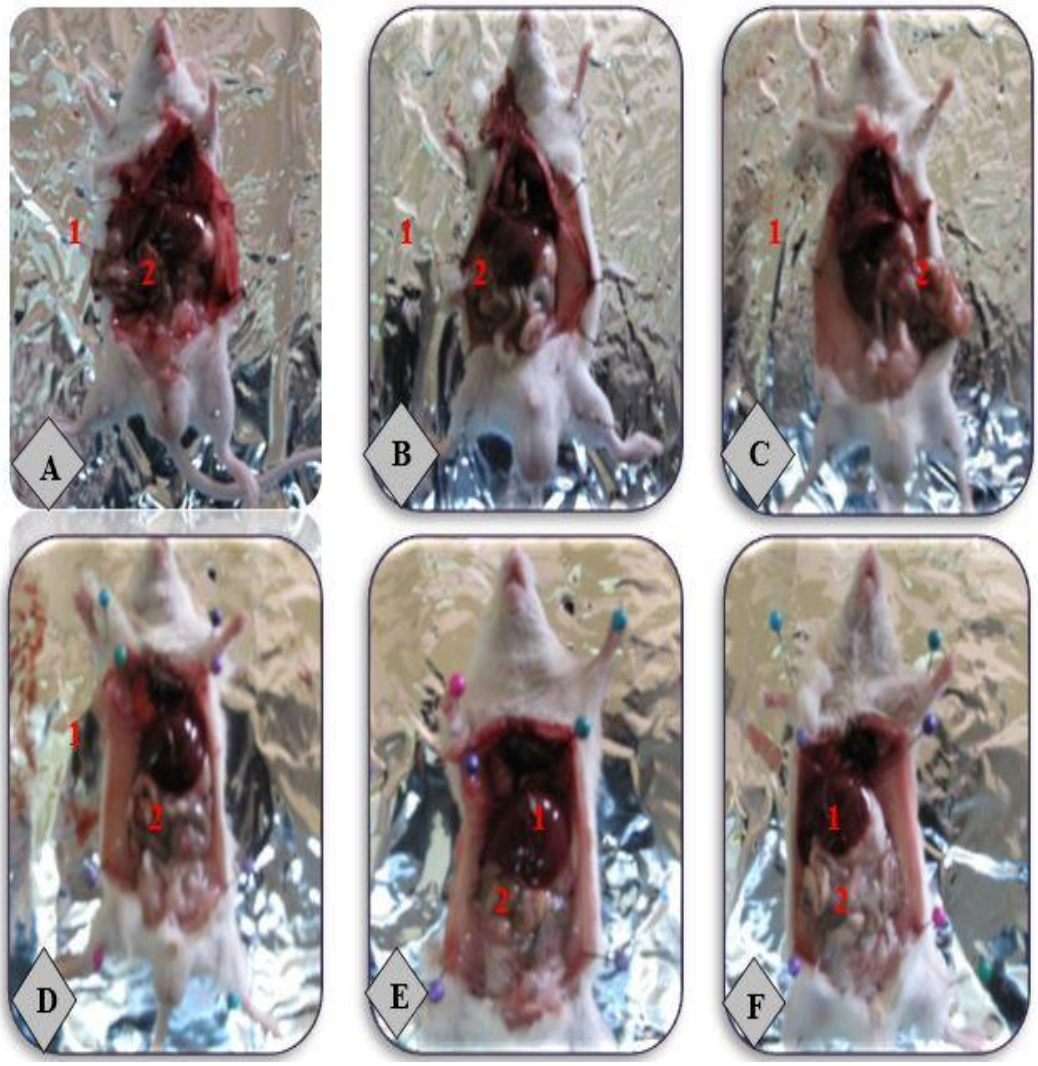

FIG. 2. **Pathological feature of BALB/C mice: A- administered with PBS (+ve control) B: infected with *Salmonella typhimurium* (-ve control) C- administered with *Bifidobacterium spp.* D- administered with *Bifidobacterium spp*. and infected with *Salmonella typhimurium*. E-administered with *Bifidobacterium spp.*, infected with *Salmonella typhimurium* and treated with Gentamicin. F- Infected with *Salmonella typhimurium* and treated with Gentamicin.1- Liver, 2- intestine.**

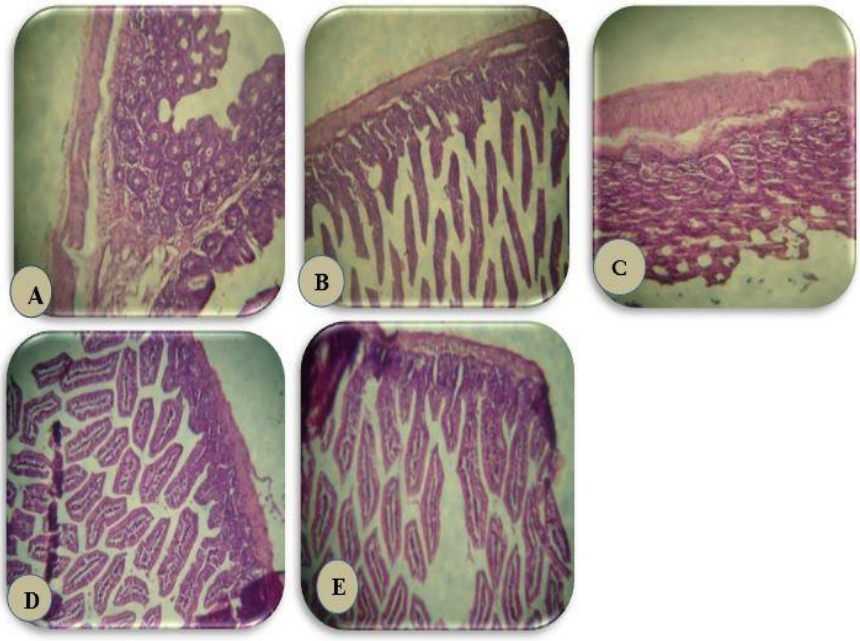

FIG. 3. **Histopathological finding: photomicrographs of H&E stained paraffin sections through intestine of Balb/C mice: (A) +vecontrol ;(B) mice infected with *Salmonella typhimurium*; (C) mice administered with *Bifidobacterium* spp. and infected with *S. typhimurium*; (D) mice administered with *Bifidobacterium* spp., infected with *S. typhimurium* and treated with Gentamicin (E) mice infected with *S. typhimurium* and treated with Gentamicin. (100X).**

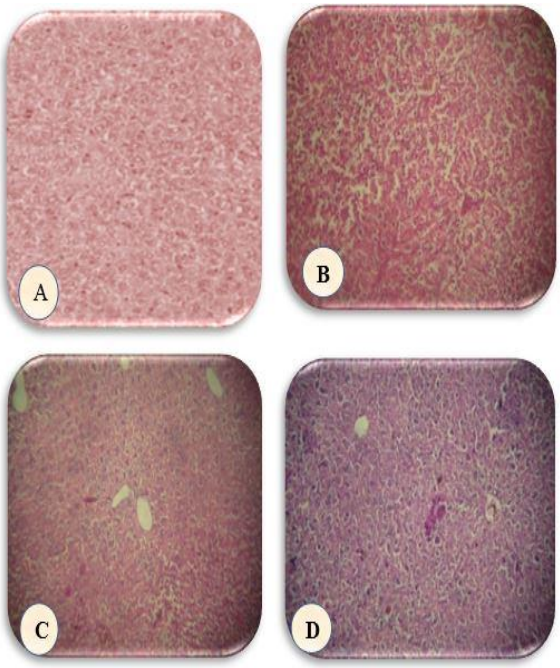

FIG. 4. **Histopathological finding: Photomicrographs of H&E stained paraffin sections through liver of Balb/C mice: (A) +ve control (B) mice infected with *Salmonella typhimurium* (C) mice administered with *Bifidobacterium* spp. and infected with *S. typhimurium* (D) mice administered with *Bifidobacterium* spp. infected with *S. typhimurium* and treated with Gentamicin (100X).**

- Inoculation of *S. typhimurium* in intestine resulted in proliferated (multifocal) vascular degeneration and decomposition of the intestinal necrosis in the tip of the villi associated with bacterial adhesion and invasion, erosion and ulceration of the epithelial layer and necrosis of the underlying lamina propia. No polymorphonuclear leukocyte infiltration orinflammatory changes bordering these areas were observed.

Group treated with *Salmonella* and *Bifidobacterium*: When mice were inoculated previously with *Bifidobacterium spp.* and infected with *S. typhimurium* (FIG. 2C and FIG. 3C) the results showed no morphological changes in mucosa and epithelium.

Discussion

Identification of *Bifidobacterium* spp.

Bifidobacterium are natural microorganism of the intestinal tract of newborn,nourished with breast milk. It is able to survive or multiply at a relatively high temperature (46°C).

The biochemical tests used for identification of *Bifidobacterium spp*. to species level were variable. All isolates showed negative results for MR and Gelatinase. This finding suggests that some isolates still produce acid as a result of glucose fermentation but they have a lower concentration of hydrogen ions because of returning toward neutrality which caused by further degradation of the organic acid to carbonates, and onto carbon dioxide and possibly formation of ammonium compounds from the protein in the medium, whereas other positive isolates had the ability to overcome the buffering capacity of the system by producing and maintaining stable acid end product from glucose fermentation [16].

A variable result was also shown with the simmon citrate test which was due to ability of some isolates to utilize citrate as a sole source of carbon depending on citritase or citrate dismutase, while other isolates can metabolize citrate rapidly via Tricarboxylic Acid cycle in presence of fermentation or lactic acid production.

Our results was in agreement with other studies that found wide distribution of *Bifidobacterium spp* in stool of infants and person [2,21-23]. Also, our results were in agreement with Zinedine and Faid [24] who found only 3 strains of *B. infantis* from 26 isolates belonged to *Bifidobacterium*.

16SrDNA gene-targeted species-specific was used as accurate method for characterization of *Bifidobacterium* that inhabitate the human intestinal tract. A species-specific PCR technique performed with extracted DNA was also used to investigate the distribution of *Bifidobacterium* in the intestinal microflora of human adults and infants.

The results which shown in FIG. 1 were in agreement with Denis and Stephane [18] who revealed specific amplification of *Bifidobacterium* DNA which was achieved with primer Pbi F1 combined with primer Pbi R2 for all of the strains tested [25,26] and found that the 16SrRNA gene was considered to be globally present in bacteria and showed a high degree of sequence conservation. The highly conserved portions of 16S rDNA gene are perfectto designing primer that will amplify small subunits of rDNA gene [27]. The phylogenetic analysis of the genus *Bifidobacterium* was depending on the sequence homology analysis of this gene. Several studies confirmed using 16S rDNA gene for identification of *Bifidobacterium* such

as Baffoni et al. [28] who fostering hsp60 as the global primer in a simple PCR procedures while [29] compared the sequence of the genome of *B. animalis* subsp. *Lactis* ATCC 27673 with other strains using molecular tools for probiotic activity.

Experimental study

Salmonella organisms are responsible for the most common and important zoonotic disease [30]. Salmonellosis has remained one of the three most common meat associated disease in human [31]. Animal products are readily contaminated with microorganism and support their growth if not conveniently handled, processed, and preserved [32,33].

Probiotic has been involved in purveying the host with promoted resistance to zoonotic pathogens which cause gastroenteritis [34]. Administration of probiotics capable of colonizing the intestine is expected to induce long-term beneficial effects on intestinal health. *Bifidobacterium* is part of the normal intestinal flora of humans, the composition of which can be affected by such factors as diet and age. Several studies found that *Bifidobacterium* significantly reduced the colonization of *S. typhimurium* in Balb/c mice model, a model that has been extensively characterized enteropathogens [35,36] and the use of *S. typhimurium* in the model is considered as an effective prosthesis model of *S. typhi* infection in humans [36].

As mentioned, our results revealed that the infectious dose of *S. typhimurium is* 8×10^8CFU/ml was approximate to that noticed by Maxwell [37] which showed that the infectious dose ranged between 10^5-10^{10} CFU/ml. Moreover, the results were in agreement with other studies such as Al-Naqeeb [38] who found that the ID of *S. hadar* in mice was 1.7×10^7 CFU/ml and Al-Mansory [39] who recorded that the ID of *Salmonellaenteritidis* in rabbit was (2×10^8 CFU/ml).

In infection, *Salmonella* colonize the gastrointestinal tract of the mouse and then penetrates the ilealPeyers patches for induction a systemic infection [40]. The formation of biofilms in the reactor, which is known to increasing sterilizing resistance, play an important role in persistence of *Salmonella* [41]. As shown in our experimental study, the clinical signs caused by *S. typhimurium* in groups that not treated previously with *Bifidobacterium* were similar to those reported by Al-Naqeeb [38] who found the important clinical signs in mice such as fever, loss of appetite, thirst, diarrhea and past in consistency, decreased activity and these sign continued for 72 hrs.

Daily observation was in agreement with other studies associated with other type of *salmonella* species which caused the same clinical signs such as Al-Mansory [39] and Harab [42] which recorded the same clinical signs on rabbit infected with *Salmonella enteritidis* and *S. hadar* respectively. The appearance of clinical signs revealed that the pathogenic *salmonella* invaded the mucosa of small and large intestine and produced toxin such as endotoxin, cytotoxin, enterotoxin, exotoxin and stimulated the release of pro inflammatory cytokines and induced acute inflammatory reactions. The bacteria can disseminate from intestine to other organs and cause systemic infection [43]. The elevation of temperature is due to endogenous pyrogen in the cytokine IL-1 produced by macrophages in response to endotoxin. These endogenous pyrogens circulate the blood stream and act on stimulating the thermoregulatory center in the hypothalamus to cause fever [44,45].

Bifidobacterium is a specific catchier as potential probiotics for human beings as they constitute one of the prevalent populations of the normal colonic microbiota and are very well acclimatized to this ecosystem. Human *Bifidobacterium* strains had antimicrobial activity against *S. typhimurium* and showed that two among them expressed antagonistic

activity *in vivo*. As we mentioned in group treated previously with *Bifidobacterium* and infected with *S. typhimurium*, our results were in agreement with other studies that found invasion of *S. typhimurium* was suppressed in presence of *Bifidobacterium* and the addition of prebiotic to the diet may have an anti-adhesive effect in the host [36,34,40]. Breast feeding has been shown to decrease an infant's risk for developing neonatal necrotizing enterocolitis [46,47] and can limit the severity of gastroenteritis caused by rotavirus [46] compared to formula feeding, thought to be due in part to the predominance of *Bifidobacterium,* and the suppression of coliform and other potentially pathogenic bacteria in the intestine of breast fed babies [48-50].

Marteau et al. [51] reported that Probiotics, such as lactic acid bacteria and *Bifidobacterium*, had been availed in the treatment of allotted intestinal microbiota and diarrheal diseases. The inhibition of *S. typhimurium* by *Bifidobacterium* could also due to their ability to decrease the pH of large intestine by producing lactic acid and acetic acid there by restricting the growth of many potential pathogens and putrefactive bacteria [52]. Also, *Bifidobacterium* may inhibit the adhesion of pathogenic bacteria to the epithelial cell a first step of intestinal infection [53,54]. The ability of *Bifidobacterium* to increase the intestinal barrier against pathogens firstly by strengthening the physical resistance of the epithelial layer and secondly by modulating the immune system towards a pre-activated steady state [55,56].

One possible mechanism that could construe our results is the potential ability of *Bifidobacterium* to bind endotoxin. Actually, several strains of probiotic bacteria have already been shown to bind certain mycotoxins on their cell surface [57,58]. The less appearance of clinical signs in the group treated by *Bifidobacterium* have been shown to improve cellular immune responses, including phagocytosis, lymphocyte proliferation and cytokine production [59] as well as humoral immune responses Li et al. [60], Silva et al. [61] and also Shu et al. [62] suggested that this improvement seemed to associate with an increased resistance to intestinal infection with *Salmonella typhimurium* [34,63,64]. Suggested that the prebiotic might be binding specific receptors on the host epithelial cell surface, thus prevent the adherence and subsequent invasion of *S. typhimurium* [65] noted that certain commensalism produced molecules that could affect the host immune system and protected against intestinal inflammation. Other studies showed that probiotic microorganisms such as *Bifidobacterium* and *Lactobacillus* can exert their immune-modulatory effect through interaction with Toll-like receptor 2 (TLR2), which characterize components of cell wall such as peptidoglycan, lipoteichoic acid, and lipoprotein [66-68]. A study carried out by Chen-Yu [69] demonstrated that some *Bifidobacterium* strains protect the epithelial tight junctions (TJ) barrier against TNF-α –induced injury and promote the restoration of TNF-α-induced loss of epithelial barrier integrity which attributed to increased production of acetate and formate. Miyauchi et al. and Sultana et al. [70,71] reported that cell wall components derived from some *Bifidobacterium* and *Enterococcus* strains are also enhance epithelial TJ barrier markedly through a TLR2-mediated mechanism.

The results of haematological analyzer of mice infected with *Salmonella* were in agreement with the results of other studies such as Santos et al. [72] who found an increase in RBC, Hb and PCV in claves were experimentally infected with *Salmonella typhimurium*, Gupta et al. and Raghad [73,74] on the guinea pigs experimentally infected with *S. dublin* and Sharma et al. [75] on goat experimental infected with *Salmonella typhimurium*; Harab [42] and Raghad [74] on rabbit infected with *S. hadar*. The result of pathological finding of *S. typhimurium* in mice were in agreement with the result of Harab [42] who found the same result in rabbit infected with *S. hadar*.

The result of histopathology of the effect of a *Bifidobacterium* on mice infected with salmonellosis was in agreement with [40] who found the same effect of prebiotic Bimuno produced by *Bifidobacterium bifidum.* Several studies found that *Bifidobacterium* reduced the invading of *S. typhimurium* in the early phases of infection, therefore perform to retaining of *S. typhimurium* in the lower gastrointestinal tract or it significantly decline the colonization of *S. typhimurium* in all organs [40,65,76,77].

On the other hand the result of treatment was in agreement with Asahara [78] who found that treatment of conventional mice with certain *Bifidobacterium* (*B. breve* and *B. pseudocatenulatum*, 10^8 CFU per mouse) together with prebiotics barred the antibiotic-induced obstruction of colonizing resistance to labial infection with *S. typhimurium* and that the metabolic activity required to production low intestinal pH and organic acids was important in this anti-infectious property.

Conclusion

Wide distribution *Bifidobacterium spp*. in stool of healthy infants and person. Molecular technique depending on amplification of 16SrDNA represented a specific method for identification of *Bifidobacterium* in comparison with biochemical test. *Bifidobacterium spp*. had the effective role in decreasing the salmonellosis in mice infected with *S. typhimurium* in comparison with Gentamicin.

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