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Effect of Bifidobacterium *longum* suspension and bacterial supernatant on serum lipid and kidney function in male albino Wistar rats.

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Abstract

We report effect of *bifidobacterium longum* suspension and supernatant on serum lipid profile and kidney function in male Wistar rats. Groups of 6 rats each were fed standard diet (controls) and atherogenic diet daily for 28 days. Other rats were fed atherogenic diet supplemented with gavage 1ml of 0.5 per 1×10^8 CFU/1ml *Bifidobacterium longum* suspension and supernatant daily for 14 (n=6) and 28 (n=6) days. compared with control lipid profile and kidney function was increased in at rogenic diet group whenever in the bacterial suspension for 14 days reduced ($P < 0.05$) serum triglyceride (TG) and very low density lipid (VLDL), and given for 28 days, reduced total cholesterol, TG, low density lipoprotein and VLDL but increased high density lipoprotein (HDL). In kidney functions creatinine was decreased with significant in14 and 28 days. In urea there was non-significant decreasing in period 14 days but there was significant decreasing in 28 days.

In supernatant that gave orally to the rats daily 14 (n=6) and (n=28), in period 14 days all the parameters (TC, TG, VLDL, LDL) was a decrease with no significant and HDL parameter increased compared with atherogenic diet group. In period 28 days there was decreasing with significant differences in parameters (TC, TG, LDL) and without significant differences in parameter VLDL compared with the atherogenic diet group

The histological investigation in the kidney of rats that given bacterial suspension and supernatant gave improvement in kidney function and less deposition in lipids compared with atherogenic diet group for 28 days

Keywords: *Bifidobacterium longum* (suspension and supernatant), lipid profile, kidney function.

1. Introduction

Lactic acid bacteria (LAB) are more spread microorganisms which can be found in any environment rich mainly in carbohydrates, such as plants, fermented foods and the mucosal Surfaces of humans, terrestrial marine animals. In the human and animal bodies, LAB are part of the normal microbiota or microflora, they are inhabited in the gastrointestinal and genitourinary tracts, which is comprised by a large number of different bacterial species with a diverse amount of strains (1) (2). Lactic acid bacteria are gram positive, non-spore, catalase-negative bacteria characterized as fastidious, acid-tolerant and fermentative microorganisms. (LAB) bacteria are associated with habitats rich in nutrients, such as food products and plant materials, lactic acid bacteria are inhabitant, human oral cavity, Vagina and gastrointestinal tract (3). Probiotic bacteria have a positive effect in lowering serum cholesterol levels through many mechanisms one of them was de-conjugation of bile salts (4).

Bifidobacteria are added to foods for probiotic purposes, providing protection against gastrointestinal infections and inflammatory bowel disease, antagonizing pathogens, and conferring antiallergenic properties on the immune system (5).

Probiotic bacteria as "live microorganisms which when administered in adequate amount confer a health benefit on the host that defined by Food and Agriculture Organization / World Health Organization (6) (7).

Probiotic of bacteria has a positive effect in lowering serum cholesterol levels through de-conjugation of bile salts and considered to support the host health (4)

. Also some strains of lactic acid bacteria reduce serum cholesterol levels, presumably by breaking down bile in the gut, thus inhibit their reabsorption (8).

Probiotics can prevent the colonization of pathogens by producing antimicrobial compounds, competing for the adhesion to the gut epithelial surface (9**).**

HFD affect the kidney by increasing serum urea and creatinine levels and decreased level of nitric oxide (NO), increased oxidative stress and renal alteration suggesting association between lipid peroxidation, obesity (10). The hyperlipidemia and associated with oxidative stress and nitric oxide inactivation by reactive oxygen species (ROS) and diminish NO bioavailability (7).

The renal functional and histopathological studies revealed that the oral administration of probiotic has provided appreciable Reno protection and possibly alleviated the symptoms of Chronic Kidney Disease (CKD) (11).

2. Materials and Method

Rats

Healthy adult male albino of Wistar (*Rattus norvegicus*) weighing 100 to 160g, aged 4-7 weeks were used. The rodents were acclimatized 7 days in a room at 20 ± 2°C with a 12/12h light/dark schedule fed a standard diet drink tap water *add libitum*, at The Animal House, College of Science, University of Wasit, Iraq. Standard control and atherogenic diets were prepared as described by *Saso et al*. [12] and delivered

through a stomach tube *Shan Ghai , China* at dose of 0.086 gm/kg B.W. with 1 ml.

Bifidobacterium longum

Lactic acid bacteria (LAB) were isolated from 6 fecal samples of 2-4 week old human infants born at The AL-Hussein Medical Center, Al-Kut, Wasit , Iraq. Infants were breast fed by mothers with no trace of antibiotic verified in sterile liquid peptone water, MRS-broth and culture on MRS agar [13]. LAB isolates were verified by catalase, carbohydrate fermentation test and API 20-A system (Biomerieux) for anaerobic bacteria and compared with Whitman, 2012 and Al-Saadi, 2016 [13] [14] LAB were characterized by growth in the bottom of the MRS- broth, growth at different temperatures, and resistance test of bacteria to bile salts and determination of the ability of *Bifidobacterium* to tolerate low pH [14]. Colonies of *Bifidobacterium longum* were collected by loop from the MRS-agar and suspended with saline compared with McFarland standard (0.5 per 1×10^8 CFU/1ml) [15].

Diets

The groups of 6 healthy male albino Wistar rats each were fed a standard control diet or a fatty atherogenic diet daily for 28 days. Other rats were fed the atherogenic diet but supplemented with 1 ml bacterial suspension of *Bifidobacterium longum* probiotic standardized to 0.5 per 1×10^8 CFU/1ml (MacFarland) daily for 14 (n=6) or 28 (n=6) days**.**

Blood lipids

Blood was drawn by heart puncture with a disposable syringe (5ml) left at room temperature for coagulation. Clotted blood was centrifuged for 10 minutes at 3000 RPM and serum stored at - 20 C for serum lipids. Serum TC, HDL and TG were determined by kits (BIOLABO, France) as described [16] [17]. Serum LDL) and VLDL were calculated from the Friedewald equation:

LDL -C = Total cholesterol (TC) – (HDL-C+ VLDL-C)
VLDL-C= Triglycerides / 5

Kidney histology

Twenty-four hrs. after the last injection, rats were sacrificed by subcutaneous injection of 0.1 ml Xylene and 0.5 ml Ketamine. The abdomen was exposed and livers excised and fixed in 10% formalin for histopathology [18]. Tissue was stained with hematoxylin and eosin for high power microscopy and photomicrography.

Statistical analyses of Mean ± SEM were done with SPSS ver. 20 *(Steel and Torrie, 1980)* [19]. Analysis was performed with a one–sample T- Test for body weight and one-way (ANOVA). Significant differences were determined by least significant differences (LSD) ($P \leq 0.05$).

3. Results and discussion

The statistical analysis showed that level of total cholesterol (TC) was reduced with a non-significant difference ($p \geq 0.05$) after treated period for 14 days compare with atherogenic diet; While, the long term-treatment for 28 days showed that mean level of (TC) in each treatment also non-significant . Also there was no significant reducing in TG in two period 14 and 28 days of bacterial suspension and bacterial supernatant treatment compared with atherogenic diet. HDL was elevated significantly after using bacterial suspension and supernatant in each periods compared with atherogenic diet group. (LDL) slight decreased without significant deference when compare with atherogenic diet group in period 14 days. In contrast, mean serum of (LDL) level after 28 days showed a marked decrease significantly compared with atherogenic group. In VLDL significant reducing in each period compared with atherogenic group. This suggested that *B.longum* suspension and *B.longum* supernatant may have affected total cholesterol biosynthesis which resulted to lowering in the level of total cholesterol in the blood. Also probiotic food containing Bifidobacteria results in the decreased total cholesterol and LDL-C and increased HDL-C [20]. The probiotic was lowering the blood cholesterol level in similar pattern as in agreement observation which reported by [21]. Same result was obtained by [22] who conclude, that probiotic strains have great potential as possible therapy for reducing cholesterol levels. In contrast, lactobacilli with BSH activity have the ability to survive and colonize the lower small intestine where the enterohepatic cycle takes place. BSH activity is considered an important colonization factor and an essential criterion for the selection of probiotic isolates with cholesterol-lowering properties [23]. Based on the ability of certain probiotic Lactobacilli and Bifidobacteria to deconjugated bile acids enzymatically, The BSH activity mechanism increases the rate of excretion. Such mechanism could be used in controlling serum cholesterol levels by colonic microbes. In addition mechanisms proposed for cholesterol removal including: cholesterol incorporation in the cell wall, adhesion to the cell wall and enzymatic reduction via cholesterol reductase [24] [25]

Table1. Serum lipids (mg/dl) in rats fed a standard control diet, an atherogenic diet, suspension and supernatant of bacteria for 14 days.

Parameter (gm/dl)	**Group**			
	Control	**Atherogenic diet**	**Bacterial suspension**	**Bacterial supernatant**
Total cholesterol	78.53 ±6.20 a	96.40 ±0.10 a	85.53± 0.03 a	88.65 ±8.73 a
Triglyceride	78.63 ± 5.30 ab	93.18 ± 9.62 a	80.14± 6.28 ab	82.99 ±5.29 ab
High Density lipoprotein	47.44 ± 4.70 ab	35.100 ±3.39 b	43.88± 2.74 ab	40.82 ±2.13 ab

Low density lipoprotein	19.83 ± 5.81 a	42.67 ±10.12 b	25.79± 8.22 ab	31.23 ±8.02 ab
Very low density lipoprotein	15.73 ± 1.06 ab	18.63 ±1.92 a	16.03±1.26 ab	16.59 ±1.06 ab

Table2. Serum lipids (mg/dl) in rats fed a standard control diet, an atherogenic diet, suspension and supernatant of bacteria for 28 day

Parameter (gm/dl)	**Group**			
	Control	**Atherogenic diet**	**Bacterial suspension**	**Bacterial supernatant**
Total cholesterol	78.53± 6.20 ab	96.40±10.10 a	77.47±5.71 ab	84.23±6.42 ab
Triglyceride	78.63± 5.31 ab	93.18±9.62 a	75.85±5.05 ab	79.71±6.04 ab
High Density lipoprotein	47.44±4.69 a	35.10±3.39 b	49.69±3.45 a	44.87±2.63 ab
Low density lipoprotein	19.83±5.81 a	42.67±10.12 b	15.06±4.93 a	24.72±6.78 ab
Very low density lipoprotein	15.73±1.06 ab	18.63±1.92 a	15.17±1.01 ab	15.94±1.21 ab

3.1 Effects of bacterial suspension and supernatant on kidney function

Creatinine was decreased in each groups for two period compared with atherogenic diet group probiotic microorganisms can utilize urea, uric acid and creatinine and other toxins as nutrients for growth. Overloaded and impaired kidneys lead to buildup of these poisonous wastes in the bloodstream. Probiotic microorganisms multiply and metabolize larger quantities of uremic toxins, facilitating the increased diffusion of these toxins from the circulating blood into the bowel across the lining of the intestinal walls. However, probiotics have potential dietary supplement uses (to help maintain healthy organ functions), and drug uses (to prevent or treat disease) [26].

Table3. Kidney function (mmol/L) in rats fed a standard control diet, an atherogenic diet, suspension and supernatant of bacteria for 14 days.

Parameter (mmol/L)	Group			
	Control	Atherogenic diet	Bacterial suspension	Bacterial supernatant
Creatinine	0.40 ± 0.04 a	0.68 ± 0.06 b	0.48 ± 0.03 a	0.43 ± 0.05 a
Urea	39.00 ± 2.52 a	47.83 ± 3.67 b	41.33 ± 2.67 ab	44.50 ±1.77 ab

Table4. Kidney function (mmol/L) in rats fed a standard control diet, an atherogenic diet, suspension and supernatant of bacteria for 28 days.

Parameter (mmol/L)	Group			
	Control	Atherogenic diet	Bacterial suspension	Bacterial supernatant
Creatinine	0.40 ± 0.04 a	0.68 ± 0.06 b	0.38 ± 0.03 a	0.43 ± 0.04 a
Urea	39.00 ±2.52 a	47.84 ± 3.67 b	36.17 ± 2.82 a	40.00 ± 2.23 ab

3.3 Histology of kidney

The glomerular hyper filtration and structural changes in the kidney may be the precursors of more severe glomerular injury associated with prolonged obesity [27]. As in figure (1).

The effects of probiotics on renal function improved kidney function [28], there was less deposition of fat in kidney tissues. As in figure (2).

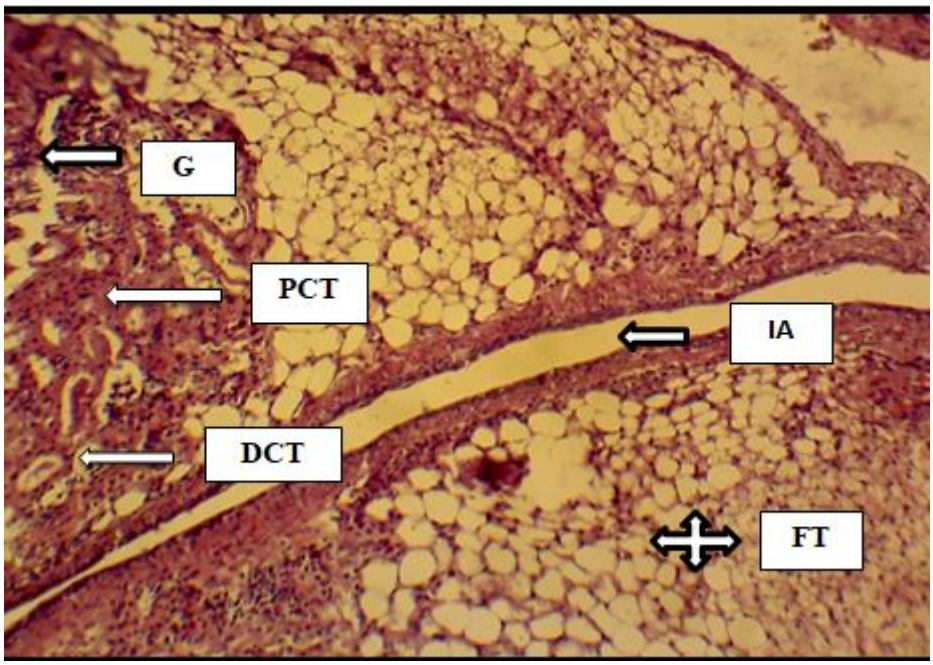

Figure (1): Light photomicroscopic picture (cross-section) of the kidney tissue in atherogenic group (A) showing, interlobular artery (IA); adipose tissue (AT); glomerulus (G); proximal connective tissue (PCT) and distal convoluted tubule (DCT)(H and E 40x).

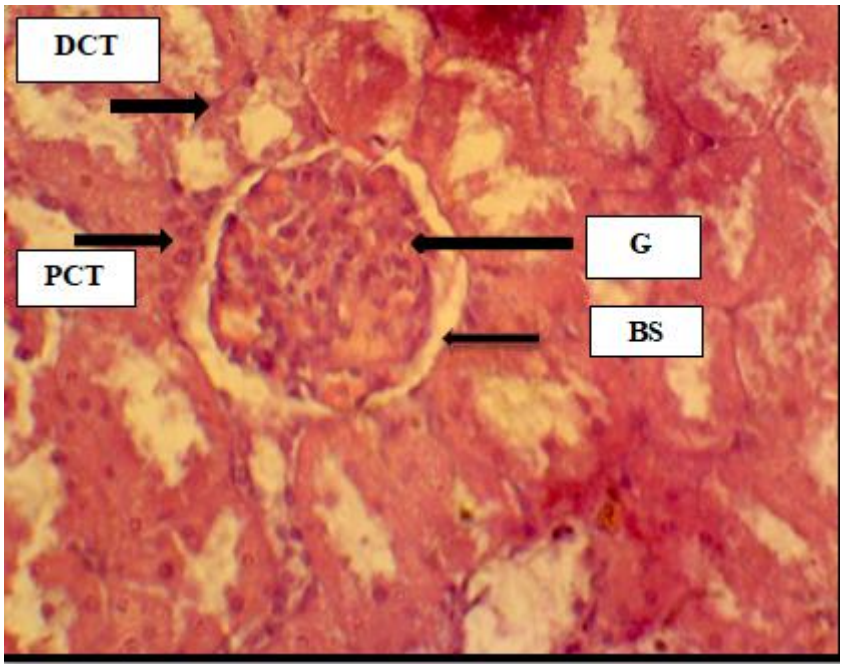

Figure (2): Light photomicroscopic picture (cross-section) of the kidney tissue after treatment by *B.longum* group (C2) for 28 days showing, distal convoluted tubule; proximal convoluted tubule (PCT); glomerulus (G) and bowman's space (BS)(H and E 400x).

4. **Conclusion**:

The administration of bacterial suspension and bacterial supernatant (as probiotic) have an effective role on reducing the serum lipid profile (hypolipidemic action) and improving the kidney function also , improving in histological characters of kidney r that associated with long-term treatment (28 days) .

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