Cholesterol-Lowering Effects of Lactobacilli and Bifidobacteria Probiotics in vitro and in vivo

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Authors’ contributions

This work was carried out in collaboration between all authors. All authors designed the study and wrote the protocol. Author SME wrote the first draft of the manuscript and analysis a part of the in vitro experiment. Author AAS managed the biochemical parameters analysis and revised the written manuscript. Authors JBA managed the biological experiment and collected the literature searches. Author AFAS performed the strains preparation of the in vitro experiment of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRB/2018/v2i4540

Received 20th May 2018
Accepted 26th July 2018
Published 31st July 2018

ABSTRACT

Two lactobacilli strains; Lactobacillus acidophilus ATCC 20079 and Lactobacillus plantarum ATCC 20179 and two bifidobacteria strains; Bifidobacterium bifidum GSGG 5286 and Bifidobacterium longum ATCC 15707 were studied their abilities to reduce the cholesterol content in vitro. It was investigated that the in vivo cholesterol-lowering effect of L. plantarum ATCC 20179, B. bifidum GSGG 5286 and mixture of both probiotics (L. plantarum ATCC20179 and B. bifidum GSGG5286) on hyperlipidaemic rats for 8 weeks. All lactobacilli and bifidobacteria strains assimilate the cholesterol content in laboratory media. It was observed the highest assimilation of cholesterol was in L. plantarum ATCC 20179 and B. bifidum GSGG 5286 strains. In vivo, L. plantarum ATCC 20179 group was more effective in improving serum lipid profile levels [total cholesterol (TC), triglycerides (TG), low density lipoprotein – cholesterol (LDL-C), high density lipoprotein – cholesterol (HDL-C), very low density lipoprotein – cholesterol (VLDL-C) and Atherogenic Index (AI)], liver enzyme activities (ALT, AST and ALP), malonaldehyde (MDA), hydrogen peroxide (H₂O₂) and
total antioxidants capacity (TAC) levels than mixed-organisms and B. bifidum groups, respectively of hyperlipidaemic rats. It was concluded that L. plantarum ATCC 20179 showed more favourable results than B. bifidum GSGG 5286 in relation to cardiovascular risk factors in hyperlipidaemic rats.

Keywords: Lactobacilli; bifidobacteria; cholesterol assimilation; hyperlipidaemic rat.

1. INTRODUCTION

Hyperlipidemia is an abnormality of lipid metabolism, characterized by an elevation of TC, TG, and LDL-C, and/or a decreasing of HDL-C in circulating levels [1]. Hyperlipidaemia is a significant risk factor for developing cardiovascular diseases (CVDs) that is a leading cause of death in many countries [2]. The World Health Organization (WHO) has predicted that by 2030, approximately 23.3 million people will die from CVDs, and CVDs are projected to remain the single leading cause of death [3]. Dietary fat is one of the most important environmental factors associated with the incidence of CVDs; high cholesterol and saturated fat diets have been shown to promote atherosclerosis [4]. Cholesterol is essential for several physiological functions. It is necessary for the formation of certain hormones and vitamins, and it is an essential component of cell membranes and nerve cells. However, high levels of cholesterol or other lipids are considered risk factors for cardiovascular and other atherosclerotic diseases [5]. Currently available hypolipidemic drugs such as fibrates, statins and bile acid sequestrants have been associated with a number of side effects in patients [6]. Many adverse effects have been attributed to statins such as cognitive decline, hyperglycaemia, increased risk of cancer, cognitive loss, neuropathy, sexual dysfunction, pancreatic and hepatic dysfunction [7]. Fibrates and statins can increase the risk of skeletal muscle toxicity [8]. Bile acid sequestrants do not have major systemic side effects as they are not absorbed and remain in the intestinal tract to cause gastrointestinal side effects [9]. Thus, there is a considerable interest in in the development of lipid-lowering drugs from natural products in the recent years. The reduction of serum cholesterol levels could be effected by consumption of appropriate food containing low cholesterol, dietary fibre [10], soy protein [11], plant sterols [12] or Lactobacillus casei bacteria [13]. Probiotics have been defined as ‘live microorganisms’ which when administered in adequate amounts in the diet, deliver health benefits to the host [14]. The main probiotics are lactic acid bacteria, such as Lactobacillus, Bifidobacterium and Enterococcus, which have been suggested to lower cholesterol levels in vitro or in vivo by different mechanisms [15-17]. Supplementation of the diet with fermented dairy products or foods containing bifidobacteria and lactic acid bacteria have been reported to lower serum cholesterol levels [18,19].

The objective of this study was to select the best strains of probiotics lactobacilli and bifidobacteria with maximum cholesterol removal ability in vitro. The in vivo study was conducted to investigate the efficacy of the best twice probiotic strains and mixture culture of L. plantarum and B. bifidum in lowering lipid profile levels in albino rats fed on a high fat diet (HFD).

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

MRS broth was obtained from Oxoid Limited, Wade Road, Basingstoke, England. Ethanol, hexane, glacial acetic acid and sulphuric acid of HPLC grade were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Water-soluble cholesterol (polyoxyethanyl cholesterol sebacate), Cholesterol, bile salts, potassium hydroxide and o-phthalaldehyde, were purchased from Sigma–Aldrich (St. Louis, USA). Casein and cellulose were purchased from PanReac Loba Chemie Pvt Ltd (India). Sugar, corn oil, starch and sheep tail were purchased from the local market, Egypt.

2.2 Bacterial Strain

The bacterial strains used in this study e.g. Lactobacillus acidophilus ATCC 20079, Lactobacillus Plantarum ATCC 20179, Bifidobacterium bifidum GSGG 5286 and Bifidobacterium longum ATCC 15707 were obtained from Regional Center for Food and Feed, ARC, Giza, Egypt.

2.3 Animals

Thirty adult male Wistar albino rats weighing 130-135 g were obtained from the Central Animal
2.4 Methods

2.4.1 Standard inoculums

Standard inoculums were prepared by inoculation of the conical flask (100 ml in the volume containing 50 ml of MRS broth pH 6.2) for 24 h at 37°C with loop each of *L. acidophilus*, *L. plantarum*, *B. bifidum* and *B. longum*. Achieved viable cells counts were determined by serial dilution and subsequent enumeration on MRS agar.

2.4.2 Measurement of cholesterol assimilation

The cholesterol removal was performed using the procedures described by [20]. Thirty milligrams of water-soluble cholesterol (polyoxin thanyl cholesterol sebacate) (Sigma, St. Louis, MO, USA) were dissolved in 10 ml Milli-Q water and Filter-sterilized using 0.45 um Filter (Millipore, Corp, Bedford, MA, USA) to obtain a stock solution of cholesterol. The sterilized MRS broth (Oxoid) containing 0.3% bile salt oxgall (Sigma) and 100 µl/ml cholesterol stock solution was inoculated with 1% activated probiotic cultures (*L. acidophilus*, *L. plantarum*, *B. long* and *B. bifidum*) and incubated at 37°C for 24 h. After the incubation period, cells were centrifuged at 4000 × g for 20 min at 4°C. Briefly, 1 mL of the supernatant was mixed with 1 mL KOH (33%, w/v) and 2 mL 96% ethanol. The mixture was vortexed for 1 min followed by incubation at 37°C for 15 min and cooled to room temperature. Upon cooling, 2 mL of Milli-Q water and 3 mL of hexane were added to the mixture followed by vortexing for 1 min. The mixture was then allowed to settle until the separation of the two layers. The upper hexane layer was collected and evaporated under nitrogen gas. Two millilitres of o-phthalaldehyde reagent (50 mg OPA dissolved in 100 mL glacial acetic acid; Sigma) was added and vortexed for 1 min to dissolve the residues. To this, 0.5 mL of sulphuric acid (98%; Sigma) was added and vortexed for 1 min followed by resting for 10 min at room temperature before measuring the absorbance at 550 nm using UV-spectrophotometer (Pharmacia Novaspec II, Cambridge, England). The cholesterol concentration was read off a standard curve prepared using the cholesterol stock solution. All experiments were conducted in triplicate and assayed twice (n = 6). The ability of probiotics to assimilate cholesterol was expressed as the percentage of cholesterol removed as follows:

\[
\text{% of cholesterol removed} = \frac{100 - \text{residual cholesterol}}{100} \times 100\%
\]

2.4.3 Preparation of concentrated inoculums

The strains of *L. plantarum* ATCC 20179 and *B. bifidum* GSGG 5286 were concentrated to $10^{10}$ CFU/ml and $10^9$ CFU/ml, respectively in a sterile saline solution (0.9%).

2.4.4 Animal diet

Normal diet, vitamin and mineral mixtures were prepared according to [21]. The high fat diet included 20% fat sheep tail, 1% cholesterol and 0.5% bile salt then completed to 100% with corn starch.

2.4.5 Experimental design

Rats were individually housed in metal cages and kept under normal laboratory conditions (temperature 25°C ± 2°C, relative humidity 55± 5% and 12/12 h light/dark cycle) of animal house in the Regional Center for Food and Feed, Agriculture Research Center, Giza, Egypt and were fed on normal diet and offered water *ad libitum* for one week. After this adaptation period, rats were randomly assigned into five groups; Group I was fed on a normal diet (ND group, n = 6), while the other experimental groups were fed on a high fat diet (HFD group, n = 24) for further subsequently three weeks. After that, all animals fasted for 12 h. Blood was collected, and the serum was obtained to determine the serum levels of TC, TG, HDL-C, LDL-C and VLDL-C to indicate the success of hyperlipidemia induction. Finally, all rats were fed for additional more four weeks and administered by probiotic bacteria as follows: Rats of Group I and Group II were orally administered of sterile saline solution (10 mL/kg b.w); Group III was orally administered of $10^9$ CFU/ml *L. plantarum* ATCC 20179 (10 mL/kg b.w.); Group IV was orally administered of $10^9$ CFU/ml *B. bifidum* GSGG 5286 (10 mL/kg b.w.) and Group V was oral administered with mixture culture $[10^9$ CFU/ml *L. plantarum* ATCC 20179 and $10^5$ CFU/ml *B. bifidum* GSGG 5286 (10 mL/kg b.w.)].

At the end of the last fourth week, blood samples were collected from the retro-orbital vein using a glass capillary tube after the animals were fasted for 12 h and then centrifuged the whole blood at
speed of 4000 rpm/min in 4°C for 10 min to obtain serum samples. Plasma samples were obtained by collecting blood in heparinized tubes and then centrifuged for the speed of 4000 rpm/min at 4°C for 10 min. Serum and plasma samples were stored at -80°C before biochemical analysis. The liver and heart were removed, rinsed with a sterile physiological saline solution, blotted dry with sterile filter paper, and weighed quickly. Relative organ weight was calculated as the ratio of the absolute organ weight to body weight.

### 2.4.6 Analyses of biochemical parameters

Serum TC, TG, HDL-C, LDL-C, AST, ALT and ALP were determined according to [22,23] using kits obtained from Biosystems S.A., Barcelona, Spain. Serum MDA, plasma H₂O₂ and TAC were analyzed using kits obtained from Bio-diagnostic, Giza, Egypt. Determinations were carried out according to manufacturer’s instructions. Serum VLDL was estimated by calculation based on Friedwald et al. [24] formula. Atherogenic Index was calculated by using the formula of Schulpis et al. [25].

### 2.4.7 Histopathological studies

Liver and heart from each rat of all groups were fixed in 10% buffered formalin and embedded in paraffin wax. Microtome thickens sections of 3-4 µm were prepared according to the standard procedure and stained with haematoxylin and eosin. Sections were then examined for pathological findings of such as centrilobular necrosis, fatty and lymphocytes infiltration by the light microscope of Leica DM3000 Microsystosms, CMS GmbH, Wetzlar, Germany [26].

### 2.4.8 Statistical analysis

All data were recorded as mean ± SE. Statistical analysis utilized the Statistical Analysis System software package. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan’s multiple range tests at a level of P<0.05.

### 3. RESULTS AND DISCUSSION

#### 3.1 Probiotics Cholesterol Assimilation in vitro

The removal percentage of cholesterol by four bacterial probiotic strains grown in MRS supplemented with 0.3% bile salt oxgall at 37°C of cholesterol by four probiotic bacteria strains grown in MRS supplemented with 0.3% bile salt oxgall at 37°C for 24 h is shown in Fig. 1. All probiotic bacteria strains had the cholesterol removal ability at 24 h of incubation. In particular, probiotic strains of *L. plantarum* ATCC 20179 and *B. bifidum* GSGG 5286 as showed significantly (p < 0.05) higher percentage of cholesterol removal ability (71.67 and 64.17%, respectively) after 24 h of incubation in comparison with the other two strains. Therefore strains *L. plantarum* ATCC 20179 and *B. bifidum* GSGG 5286 were used for in vivo study. These results were agreement with those observations that *L. plantarum* L26 more active capability to remove cholesterol from the culture medium compared to *P. plantarum* L14/1, *P. acidilactici* L25, *L. pentosus* and *Enterococcus faecium* N 15 strains [27]. Cholesterol was partially removed from the medium after culturing of *L. acidophilus* RP32 in the presence of bile salts may due to the disruption of the cholesterol micelles caused by bile salt deconjugation and precipitation of cholesterol with the free bile salts as the pH of the media dropped from acid production during growth [28]. Another study showed that *L. plantarum* PH04 strain was able to produce bile salt hydrolase enzyme in vitro [29]. Increased bile- salt hydrolase of *L. plantarum* PH04 strain may explain the increased ability of this strain to remove cholesterol. Also, *L. plantarum* EM showed the highest cholesterol removal than *L. sakei* DC1 or *L. acidophilus* ATCC 43121. Thus, strong cholesterol attachment to *L. plantarum* EM regardless of its cell state might be due to the unique in chemical and structural properties of the *L. plantarum* EM cell wall that promote binding to cholesterol compared to those of other LAB cell types [30].

#### 3.2 Body Weight and Relative Percentage of Liver and Heart to Body Weight

Data in Table 1 shows body weight and relative percentage of liver and heart to body weight in rats fed on a high fat diet. The initial body weights of rats were recorded at the start of the study. At the last of the fourth week, it was observed that the final body weights of all four groups were significantly increased especially group II compared with group I. The results are in concordance with previous studies [31-33], which demonstrated that body weight increased significantly in rats fed on HFD compared with rats fed on a normal diet. Therefore, the groups of rats fed on the high fat...
diet plus the low and high oral administration dose of L. plantarum NCU116 demonstrated a significant decrease in the body weights at the end experiment (P < 0.05) [34]. The relative percentage of liver and heart to body weight were significantly increased in group II compared to group I, which were significantly decreased (P < 0.05) by administration two probiotic strains or a mixture. The increasing in the relative percentage of the liver to body weight in cholesterol-fed rats might be due to excessive accumulation of lipids in these tissues. In addition, there were nonsignificant changes in the relative percentage of liver and heart to body weight in group III compared to group I. Recent researches demonstrated that some strains of lactobacilli and bifidobacteria have anti-obesity effect and reduced liver weight than the HFD group [35]. Mice fed on the HFD and supplementation with L. acidophilus or B. longum BORI significantly decreased liver weights [36]. Also, rats were fed high-fat diet supplemented with probiotic L. plantarum LS/07 was significant lower ratio liver/body weight compared to HFD group [37].

3.3 Levels of Serum Lipids Profile

The differences between experimental group levels are shown in Table 2. The levels of TC, TG, LDL-C and VLDL-C as well as and AI were significantly elevated and the level of HDL-C was reduced in the HFD-fed group. At the end of last fourth week, all three probiotic groups showed

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>134.50±1.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>133.83±1.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>133.33±1.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>133.67±1.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>134.00±1.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>213.00±1.34&lt;sup&gt;e&lt;/sup&gt;</td>
<td>274.67±1.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>221.67±0.67&lt;sup&gt;d&lt;/sup&gt;</td>
<td>242.50±1.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>230.83±0.87&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Relative percentage of liver to body weight (%)</td>
<td>3.46±0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.20±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.51±0.08&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.10±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.80±0.04&lt;sup&gt;c&lt;/sup&gt;</td>
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</tbody>
</table>
| Relative percentage of heart to body weight (%) | 0.36±0.02<sup>c</sup> | 0.43±0.02<sup>a</sup> | 0.37±0.02<sup>c</sup> | 0.41±0.04<sup>b</sup> | 0.39±0.01<sup>b</sub>

Results are expressed as the means ± SE (n = 6). Different superscripts in a given row denote significant difference, p < 0.05.
lower TC, TG, LDL-C and VLDL-C levels as well as AI compared to group II. While HDL-C level was significantly increased in all probiotic groups compared with group II. Moreover, the oral administration of *L. plantarum* ATCC 20179 strain exhibited the highest decrease of serum TC, TG and LDL-C, TG and VLDL-C levels, followed by *B. bifidum* GSGG 5286 strain and a mixture, respectively. Based on our results, different strains lead to change responses of serum lipid parameters in groups. Our recent findings are consistent with these results that the *L. plantarum* LS/07 (LPH) group had more decreased TC and LDL-C levels such as *L. plantarum* Biocenol LP96 (LPP) group compared with the HFD group. It was illustrated that a high fat diet changed the intestinal microflora composition; in particular, the number of *Lactobacillus* spp. was reduced [38]. Also, results of our study showed that HDL-C level in the III and V groups were significantly higher than those recorded in the IV group. Moreover, the III and V groups were more efficient for decreasing the AI level when compared with the IV group. The HDL is considered to have anti-atherogenic properties since there is a negative correlation between HDL and risk of cardiovascular disease. It is referred to as the ‘good’ cholesterol because HDL is involved in the transport of cholesterol from peripheral tissues to the liver and thereby reducing the amount stored in the tissue and the possibility of developing atherosclerotic plaques [39]. The possible mechanisms involved in the hypocholesterolemic effect include an assimilation of cholesterol by growing cells, the binding of cholesterol to the microbial cellular surface, the deconjugation of bile by bile salt hydrolase (reduces cholesterol reabsorption, increases cholesterol excretion of deconjugated bile salts, and increases cholesterol uptake by low-density lipoprotein receptor pathway in the liver as a compensatory response), inhibition of hepatic cholesterol synthesis and/or redistribution of cholesterol from plasma to the liver through the action of short-chain fatty acids, the end products of carbohydrate fermentation in the gut [40].

### 3.4 Serum Hepatic Enzymes

Both ALT and AST activities are commonly measured clinically as a part of a diagnostic evaluation of hepatocellular injury in order to assess biochemical indicators of hepatic dysfunction [41]. In the present results, the activities of three hepatic marker enzymes i.e., ALT, AST and ALP in the HFD group were significantly higher than those in the control group (Table 3). These enzymes activities were significantly (p<0.05) lower in III, IV and V groups than those in the HFD group. In the previous study, the activities of ALT and AST in the HFD-fed group were significantly higher than the control group. In contrast, hypercholesterolemic rats treated with probiotics mixture containing two lactobacilli (*L. reuteri* and *L. plantarum*) and three bifidobacteria (*B. longum*, *B. lactis*, and *B. breve*) strains had significantly attenuated hepatic activities of ALT and AST [42]. Another study was also observed that *L. acidophilus* and *B. bifidum* BGN4 supplemented groups were significantly inhibited serum activities of aspartate transaminase and alanine transaminase compared to the HFD group [36]. The lowest activities of ALT, AST and ALP enzymes were noticed at group III compared with IV and V groups. Moreover, oral administration *L. plantarum* ATCC 20179 strain normalized the AST enzyme activity.

### 3.5 Serum MDA, Plasma H$_2$O$_2$ and TAC Levels

As a biomarker of lipid peroxidation, serum malondialdehyde (MDA) level is considered as an expression of the oxidative stress caused by cholesterol [43]. The concentrations of serum MDA in the hyperlipidemic groups were higher compared to the lower lipid groups, indicating

### Table 2. Effect of *L. plantarum* ATCC 20179, *B. bifidum* GSGG 5286 and a mixture on serum lipids profile levels (mg/dL) of rats fed HFD

<table>
<thead>
<tr>
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<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>125.6±0.95$^a$</td>
<td>248.50±1.18$^{b}$</td>
<td>145.67±0.95$^{a}$</td>
<td>158.83±1.25$^{a}$</td>
<td>152.83±0.70$^{a}$</td>
</tr>
<tr>
<td>TG</td>
<td>90.33±0.88$^{a}$</td>
<td>232.50±0.76$^{a}$</td>
<td>103.17±0.79$^{a}$</td>
<td>123.67±0.71$^{a}$</td>
<td>113.50±0.76$^{a}$</td>
</tr>
<tr>
<td>HDL-C</td>
<td>63.50±0.99$^{a}$</td>
<td>30.83±0.95$^{d}$</td>
<td>46.57±0.11$^{b}$</td>
<td>51.00±1.06$^{b}$</td>
<td>79.13±0.90$^{c}$</td>
</tr>
<tr>
<td>LDL-C</td>
<td>44.10±0.36$^{a}$</td>
<td>171.17±1.56$^{a}$</td>
<td>71.53±1.38$^{d}$</td>
<td>87.93±2.20$^{c}$</td>
<td>79.13±0.90$^{c}$</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>18.07±0.18$^{a}$</td>
<td>46.50±0.15$^{a}$</td>
<td>20.63±0.16$^{a}$</td>
<td>24.73±0.14$^{a}$</td>
<td>22.70±0.15$^{a}$</td>
</tr>
<tr>
<td>AI</td>
<td>0.98±0.02$^{a}$</td>
<td>7.10±0.26$^{d}$</td>
<td>1.73±0.05$^{d}$</td>
<td>2.45±0.10$^{b}$</td>
<td>2.00±0.06$^{b}$</td>
</tr>
</tbody>
</table>

Results are expressed as the means ± SE (n = 6). Different superscripts in a given row denote significant difference, p < 0.05.
increasing oxidative stress with progressive hyperlipidemia. These increased levels could be attributed to increased ROS production and/or deficiency of antioxidant defense system [44]. The hypercholesterolemia increases oxidative stress by increasing lipid peroxidation and decreasing antioxidant enzyme [45]. The results of present study in Table 4 showed a significant increase in the MDA and \( \text{H}_2\text{O}_2 \) levels as well as a significant decrease in the activity of TAC in HFD-fed group as compared to the control group. Similar to our findings, Lipid peroxide (TBARS) level was significantly (\( p < 0.01 \)) increased and the activities of antioxidant enzymes were reduced significantly in HFD-fed rats as compared to the normal healthy control rats [46]. Oral administration of two probiotic strains (\( \text{L. plantarum} \) ATCC 20179 and \( \text{B. bifidum} \) GSGG 5286) and a mixture groups caused reduction in the levels of MDA and \( \text{H}_2\text{O}_2 \) whereas improved the level of TAC in comparison to group II. On the other hand, oral administration \( \text{L. plantarum} \) ATCC20179 strain normalized the MDA and \( \text{H}_2\text{O}_2 \) levels. In another previous study, the plasma MDA decreased significantly in the \( \text{bifidobacteria} \) treatment groups as compared to the control group due to the reduction of plasma cholesterol level [47]. In addition, a fermented dairy drink containing \( \text{L. casei} \) 114001 has been shown to reduce MDA content in the blood plasma of male Wistar rats [48]. On the other hand, rats fed high-fat diets supplemented with \( \text{L. actobacillus} \) plantarum P-8 elevated antioxidant ability [49].

3.6 Histological examinations

3.6.1 Liver

Fig. 2 illustrates the effects of \( \text{L. plantarum} \) ATCC 20179, \( \text{B. bifidum} \) GSGG 5286 and a mixture on histopathology of liver tissues. Histological examination of rat liver fed normal diet from group I showed normal histological structure of hepatic lobule (Fig. 2a). In contrary, rat liver fed HFD from group II showed steatosis of hepatocytes (Fig. 2b) associated with mononuclear inflammatory cells infiltration and fibroplasia (Fig. 2c). Previous studies observed no cellular degeneration and necrosis in the liver of ND group rats, while there were marked fatty deposition and foamy degeneration of hepatocytes in the HFD group [41, 50]. However, liver of rats from group III showed no histopathological changes (Fig. 2d). It was found that more severe injuries in liver tissues of rats fed high-cholesterol diet compared to the liver tissues of rats fed \( \text{L. plantarum} \) NS5 and NS12 strains that partially ameliorated these injuries [51]. Liver of rats from group IV showed small vacuoles in the cytoplasm of some hepatocytes as well as sinusoidal leukocytosis (Fig. 2e) and Kupffer cells activation (Fig. 2f). Meanwhile, liver of rats from group V revealed Kupffer cells activation (Fig. 2g).

3.6.2 Heart

Fig. 3 illustrates the effects of \( \text{L. plantarum} \) ATCC 20179, \( \text{B. bifidum} \) GSGG 5286 and a mixture on histopathology of heart tissues.
Histological examination of rat heart fed normal diet from the group I showed the normal histological structure of myocardial cell (Fig 3a). Similar results showed that normal architecture with the regular morphology of myocardial cell of heart tissue in normal healthy control rats [45]. In contrary, rat heart fed HFD from group II showed focal necrosis of myocardial cells associated with inflammatory cells infiltration (Fig. 3b) and vacuolation of tunica media of myocardial blood vessel (Fig. 3c). However, the heart of rats from group III showed no histopathological changes (Fig. 3d). Some sections of heart tissue from group IV showed no histopathological changes (Fig 3e) whereas few sections from this group revealed focal necrosis of myocardial cell associated with inflammatory cells infiltration (Fig. 3f). Meanwhile, the heart of rats from group V revealed no histopathological changes (Fig. 3g) except small focal necrosis of myocardial cells associated with inflammatory cells infiltration in few examined sections (Fig. 3h).

Fig. 2. Histopathological changes in livers of treated and untreated rats. a: normal diet (ND group), b-c: high fat diet (HFD group), d HFD+ oral administration of 10^{10} CFU/ml L. plantarum ATCC20179, e-f: HFD+ oral administration of 10^8 CFU/ml B. bifidum GSGG5286 and g: HFD+ oral administration of mixture-organism (10^{10} CFU/ml L. plantarum ATCC20179 and 10^9 CFU/ml B. bifidum GSGG5286)
4. CONCLUSIONS

According to the results, in vitro study, it was observed that all lactobacilli and bifidobacteria strains were able to partially assimilate the cholesterol content in broth media. It was shown that *L. plantarum* ATCC20179 strain was the highest cholesterol removal compared to other studied strains. In vivo study, the oral administration for rats by $10^{10}$ CFU/ml *L. plantarum* ATCC20179, 10 mL/kg b.w. was showed the highest improving serum biochemical indicators for lipids profile, liver enzyme activities, MDA and $H_2O_2$ content as well as increasing HDL-C and TAC levels.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee”.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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