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RESEARCH ARTICLE

In Vivo Assessment the Anti-obesity Potentials of Enteric *Lactobacillus spp*

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Abstract

Consumption of probiotics impacted gut microbiota and have potential effects on chronic diseases, such as obesity. This study investigated the anti-obesity potentials of two putative probiotics, *Lactobacillus fermentum* L1 (Ll) and *Lactobacillus paracasei* L2 (L2), individually and in combination (L1+L2) against diet-induced obese rats model. The *Lactobacillus* suspensions were orally administered, to experimental rats, at a daily level, with two consecutives of approximately 1×10^8 CFU/rat for up to ten weeks. Feeding rats with a combination of L1+L2 was more effective in improvement some of obesity related biomarkers. Rats supplementation with a combination of L1+L2 attenuated the body weight gain in rats that shifted Lee index towered normal value (0.294±0.05).The L1+L2 combinations exhibited hypoglycemic activity, represented by amelioration of glycemic parameters significantly ($P<0.05$), where, they reduced the levels of; fasting blood glucose (80.43±2.46mg/dl), fasting serum insulin (0.29±0.03ng/dl), and improved insulin resistant disorder (1.05±0.04) comparison with positive control group rats. Concurrently, lactobacilli combinations (L1+L2) pronounced anti- dyslipidemia and improved serum lipid profile of experimental rats, hat the level of blood lipids were significantly ($p<0.05$) dropped; total cholesterol (TC) to 80.0±2.56 mg/dl, triglycerides (TG) to 79.2±2.07 mg/dl, and low-density lipoproteins cholesterol (LDL-C) to 52.71±2.56 mg/dl. The satiety-related hormones (leptin and adiponectin) levels in rat sera also were altered by supplementation of rats with L1+L2, leptin level was reduced to 1.12±0.05 ng/ml, while adiponectin level was rose to 2.35±0.04 ng/ml.

Keywords: *Probiotics, Lactobacillus fermentum, Lactobacillus paracasei, Anti-obesity potentials, Dyslipidemias, Glycemic parameters, Leptin and adiponectin.*

Introduction

Obesity is medical condition in which excess body fat has accumulated to an extent that it may have a negative effect on health [1].People are generally considered obese when their body mass index (BMI) is over $30kg/m^2$ [2].Obesity now considered a disease, and it's the most serious public health problems of the 21st century [3].

The key to weight management of obesity is the lifestyle changes, including increased physical activities and dietary modification. Success rates of long-term weight loss maintenance with lifestyle changes are low, ranging from 2-20% [4].Obese patients often resort to available medical treatments. The intensive method such as weight loss medications and bariatric surgery are the only methods currently available [5].

Most of approved medications for obesity have been removed from the market after a while of launch, due to various adverse effects and the failure of obese individuals to maintain long-term weight loss.

While surgical interventions can be effective in reducing excess weight for some individuals, the procedure is highly invasive, may be unsuccessful due to a narrow focus on body weight, and requires a massive effort in adopting a new lifestyle [6]. These realities implore the clinical community to develop novel approaches to handle this ever-growing problem, among the potential solutions, using of probiotics therapy, generally they considered safe for human health, have shown some promise.

Probiotics have been extensively studies and widely thought to be the intervention of choice in manipulating gut microbiota composition [7]. Human gut harbors 10^{13}-10^{14} microorganisms, which are collectively termed the gut micro biota [8], the majority of these are bacteria, most of which are friendly [9]. Friendly bacteria have complicated linked to physiological processes that affect many organ systems including cardiovascular, neural, immune, and metabolic [10].

Two bacterial phyla are dominant in the gut, Bacteroidetes and Firmicutes. Studies in experimental animals and in human elucidated that the body weight seems be related to the balance of these two phyla of bacteria [11].Several experiments have confirmed an increase in the ratio of Firmicutes to Bacteroidetes, also known as the F/B ratio, in obese individuals [12]. Lean individuals have a higher proportion of bacteria from the Bacteroidetes phylum, while obese individuals have more from the Firmicutes phylum [13].Studies suggests that manipulation of gut microbiota through dietary or other means may confer beneficial effects by restoring gut functional integrity and reverse dysbiosis that is characteristic of obesity [14].

Probiotics have been shown to influence the composition of the gut microbiota, improve gut integrity and restore the microbial shift characteristics of obesity [15].Actually, they making person harvest fewer calories from diet foods. However, it is important to keep in mind that these mechanisms aren't understood very well, more research required, further its strain-dependent feature [16].

Among the well-studied probiotics are lactic acid bacterial (LAB) strains belonging to *Bifidobacteria, Lactobacilli*, which have an established safety record and have been given GRAS (generally recognized as safe) status by the United States Food and Drug Administration [17]. Over the past several years, promising preclinical studies investigating the effects of probiotic supplementation on the development of obesity have emerged.

A majority of the reviewed studies focused on intervention with *Lactobacillus* spp. and have demonstrated considerable anti-obesity effects and the potential for probiotic based therapies in the treatment and prevention of obesity [18]. One of the early studies conducted over an eight-week period showed that *Lactobacillus rhamnosus*PL60 reduced body weight without reducing energy intake, and significantly reduced white adipose tissue. Signals of apoptosis and uncoupling protein-2 (UCP-2) mRNA levels were increased in adipose tissue, while fatty acid synthase and serum leptin were simultaneously reduced; the authors attributed these effects to the production of t10, c12-conjugated linoleic acid by the probiotic [19].

In animal studies the *Lactobacillus* association to obesity biomarkers was also proved. In murine model, mice orally given *Lactobacillus rhamnosus* GG for 13 weeks, tracking results indicated weight loss, and improve of insulin sensitivity, concurrently, GG increased the expression of fatty acid oxidative genes in the liver while it decreased gluconeogenic genes [20].

Other study revealed that milk fermented by *Lactobacillus gasseri* SBT2055 can be effective in regulating the gene expression of leptin (satiety hormone) in rats and reduces the adipocytes size [21]. Multiple strains of probiotics are assumed to be more effective than that of individual strains, although *in vivo* evidence is lacking to determine whether combining individual strains is more effective in improving the host health [22].

For example, a study assessed the effects of combination of two lactobacilli, *L. rhamnosus* LGG and *L. sakei* NR28 for 3 weeks feeding in mice. The combined lactobacilli produced a significant reduction in epididymal fat mass, as well as obesity-related biomarkers such as acetyl-CoA carboxylase, fatty acid synthase, stearoyl-CoA desaturase-1 in the liver of HFD mice [23].

The goal of this study was the assessment the anti-obesity potentials of two locally isolated probiotic *Lactobacillus* strains, individually and combination in diet-induced obese rodent model, by investigating the impact of probiotics ingestion on some of obesity parameters, such as, glycemic response, serum lipid profile, and satiety hormones.

Materials and Methods

Microorganism and Growth Conditions

Lactobacillus fermentum L1 (Ll) and

Lactobacillus paracasei L2 (L2) were used in the study, both were previously isolated from human infants' feces, and suggested to be promising probiotic strains with regards bactericidal and hypolipidemic potentials [24].They were refreshed from 30% glycerol cultures stocks at -80°C on de-Man, Rogosa and Sharpe (MRS) agar medium (Himedia, India), supplemented with erythromycin 5µg/ml, cultures were incubated at 37°C under anaerobic conditions (anaerobic jar supplied with Gas Pak, BioMerieux, France) for 48h. Several subculture steps were carried out on MRS agar to obtain pure cultures of isolates. The identity of both L1 and L2 was confirmed by DNA sequencing of the 16S rRNA. GenBank BLAST analysis confirmed the identification of both strains.

Animals

Six week- old male Albino rats with an average weight 111.48±0.02 g was used in the study, they were obtained from National Control Center for Drugs and Researches (NCCDR) / Iraq. Rats were kept in plastic cages (two animals/cage), and maintained on a 12:12 light: dark cycle at controlled temperature 25 ± 2°C, during the entire duration of experimentation (10 weeks), and the rats were given free access to food and water.

Experimental Design

The rats kept on normal diet for the first week, after acclimation week the

experimental rats (n=50) were assigned randomly to five different groups (A, B, C, D, E) ten rats per group. The group A animals were left on normal rodent chow diet, (negative control group). The group B, C, D, and E, animals were received high fat and carbohydrates (HFC) diet, normal rodent chow diet was enriched with additional 10% cholesterol and 10% sucrose (BioMerieux, France). The group B (positive control group) maintained on HFC nutrition throughout experiment.

Stable colonization of group C rats gastrointestinal tract (GIT) were achieved by daily two consecutives orally administration with 100 µl (10^8 CFU/ ml, 0.5 McFarland's standard) of L1 suspension for ten weeks. Same protocol was followed for colonization of group D rats CIT with L2 suspension. Group E rats were daily received a combination of L1+L2 in dose 10^8CFU/ml for each strain. Groups C, D, and E were treatment groups.

The experimental design was approved by the Ethics Committee at Baghdad University/ College of science for the care and use of laboratory animals. The rats weight, illness symptoms, and survival rate (number of alive/total number of mice) was recorded weekly throughout the study. Weight gain (obesity index) was analyzed in accordance to Lee index [25], it was calculated as follow:

$$\frac{\text{Cube root of body weight (g)}}{\text{Naso-anal length (Cm)}}$$

Animals representing values > 0.300 were classified as obese, and that representing values ≤ 0.300 considered as normal.

Gastrointestinal Tract Colonization

Rats gut furnishing by ingested *Lactobacillus* strains was monitored by viable cell count (CFU) in freshly collected feces samples at time intervals of 2, 4, 6, 8, 10 /weeks of experiment. Briefly; fecal samples were collected in sterile Eppendorf tubes, weighted, homogenized, and 10-fold serial dilution was prepared, 100 µl aliquot was inoculated on MRS agar plates, and viability was expressed as ($\log_{10}$ CFU / gm feces).

Identification of feces recovered *Lactobacillus* strains were confirmed by API 50 CHL (BioMerieux, France). All the counting was done triplicates.

Preparation of Organ Samples

At the end of the experimental period, following 12 h of fasting, the rats were anesthetized with ethyl ether inhalation. Blood was collected using a cardiac puncture; blood samples left at room temperature for 1 h, sera collected by centrifugation (3000g /15min at 4°C), the carefully obtained clear

sera were kept at -20 °C until use. The liver, spleen, kidney, testis was removed and weighted to measure weight changes.

Measurement the Obesity Biomarkers

Glycemic Response

Fasting Blood glucose (FBG) was detected by Trinder glucose oxidase method [26]. Fasting serum insulin (FSI) was determined using rat insulin ELISA Kit (Mercodia, Sweden), homeostasis model assessment (HOMA) index was used to calculated approximate insulin resistance (IR) from the formula:

(FBG ×FSI /22.5)

Sample representing value > 1.4 was considered as IR [27]

Lipid Profile Assessment

Serum lipid profile in respect of serum total cholesterol (TC), serum total triglyceride (TG), high density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels were enzymatically determined with corresponding to commercial rat sandwich ELISA kit (LSBio, USA).

Satiety-related Hormones

The leptin and adiponectin levels were measured in collected sera samples, using rat leptin ELISA Kit (CUSABIO, USA) and rat adiponectin ELISA Kit (CUSABIO, USA), respectively.

Statistical Analysis

One-way analysis of variance (ANOVA) was used for data analysis. All results are expressed as mean ±SD. $P<0.05$ were considered statistically significant.

Results

Animals weight and obesity index; rats weight were monitored throughout the study (10 weeks) as an index of general well-being, and animals weight gain was measured using Lee index, results are represented in Table.1. Rats in group B showed significant body weight gain vs. animals in group A ($P<0.05$), consequently Lee index of group was shifted towered obese (0.340±0.06). Non-significant body weight gain was recorded in the tree treatment groups. Otherwise, the mixed *Lactobacillus* (L1+L2) feeding for rats (group E) was induced improvement in obesity index (0.294±0.05), comparable to rats of B group.

The survival rate (number of alive/total number of rats), no more than one mortality has been recorded within experimental group. No illness symptoms were observed except of inactivity of few animals within different groups, and this may be attributed to the body weight gain and locking of animals in the limited space of cages. GIT colonization; rats in the groups C and D were individually received a daily two consecutive dosage of 10^8 viable cells of L1 and L2 for ten weeks, following the same feeding protocol group E rats were received a combination of L1+L2.

The colonization of rats GIT was tracked by isolation of viable lactobacilli from feces samples intervals. The analysis of obtained data showed that the followed protocol was sufficient to establish sufficient furnishing of rats GIT. The both adopted *Lactobacillius* were properly achieved GIT colonization and maintained high population levels, and was no significant difference ($P>0.05$) in the number of viable cells among the experimental groups throughout the feces sampling period.

L1 and L2 were re-isolated from the feces during the administration period at average levels 3.31±0.12 – 5.78±0.14 $\log_{10}$ CFU/g feces and 4.05±0.19–6.45±0.22 $\log_{10}$ CFU/g feces, respectively, and the average level of lactobacilli shedding to the feces sample in group E rats was, 4.75±0.15 – 5.97±0.21 $\log_{10}$ CFU/ g feces (Fig.1).

Table.1: Rats body weight change and obesity index

Groups	Initial wt.(g)	Final wt.(g)	Lee index
A	110.3±0.04	326.0±13.52	0.267±0.04b
B	110.9 ± 5.48	481.21±16.73	0.342±0.06a
C	112.48±6.33	373.1±12.94	0.306±0.06a
D	111.6±4.29	361.21±12.67	0.320±0.07a
E	112.3±0.04	352.70±9.83	0.294±0.05ab

Data are expressed as mean ± SD. n= 9-10 animals. ($p<0.05$)

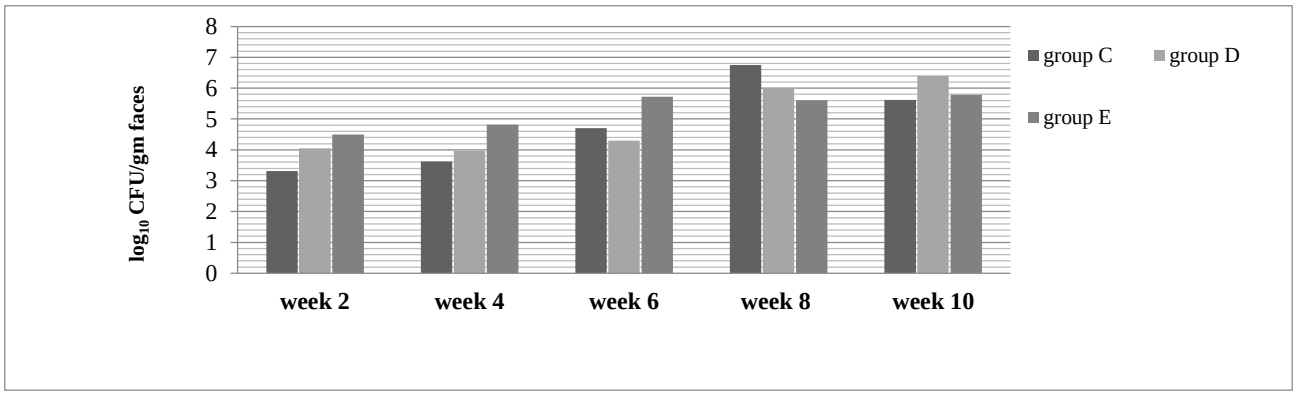

Figure. 1: Viability of Lactobacillus (L. fermentum, L. paracasei, and L. fermentum + L. paracasei) in faces samples of rats, in three evaluated groups C, D, and E. Data are presented as mean ±SD, (n=9-10), (p<0.05)

Organs weight changes; the HFC diet led to a significant increase in weights of liver, spleen, and kidney in group B animals vs. group A, except the testis, relatively maintained its normal weight in all experimental groups (Table.2). Feeding of rates individual probiotic (group C and group D) reduced mentioned organ weight; however, the reduction rates did not reach statistical significance. Conversely, group E animals, that supplemented with mixed lactobacilli suspensions showed a significantly lower kidney weight (group E: 1.92± 0.04g vs. group B: 2.40±0.2g).

Table 2: Effect of *Lactobacillus* ingestion on vital organs weights of rats in the end of experiment (10weeks)

Organ	*Group A*	*Group B*	*Group C*	*Group D*	*Group E*
Liver	8.12 ±	10.95±1.415	8.97±0.99	9.05±0.04	8.65±0.2
spleen	0.79±0.02	0.98±0.095	0.92±0.03	0.97 ± 0.025	0.83±0.07
Kidney	2.0±0.04	2.4±0.2	1.88±0.26	2.0±0.06	1.92±0.04
Testis	3.1±0.05	3.2±0.05	3.05±0.075	3.17±0.06	3.0±0.085

Data are expressed as mean± SD, (n=9-10), (p<0.05)

Glycemic Response

The HFC diet for 10 weeks produced hyperglycemia in the group B animals compared to the group A, that a significant increase in the level of FBG (group B:93.10±5.06mg/dl vs. group A:84.0±3.51mg/dl), was recorded. The FBG levels in treatment groups (C, D, and E) also were elevated, with non-significant difference among groups (Fig.2). Nevertheless, the mixed probiotics interventions offered significant (p<0.05) hypoglycemic effects, that the BFG levels was remarkably reduced in group E (80.43±2.46mg/dl) comparable with group B animals. The FSI level was affected by probiotic Ingestion (Fig.3), a significant increase was recognized in group B animals (0.59±0.04ng/dl)) compared to group A (0.28±0.01ng/dl) and group E (0.29±0.03ng/dl), respectively. Consequently, the group B animals represented IR disorder (2.34±0.09) according to HOMA-IR index, while introducing of *Lactobacillus* combination (L1+L2) to the group E rats, significantly recovered glucose insensitivity, as the IR dropped to 1.05±0.04 (Fig.4).

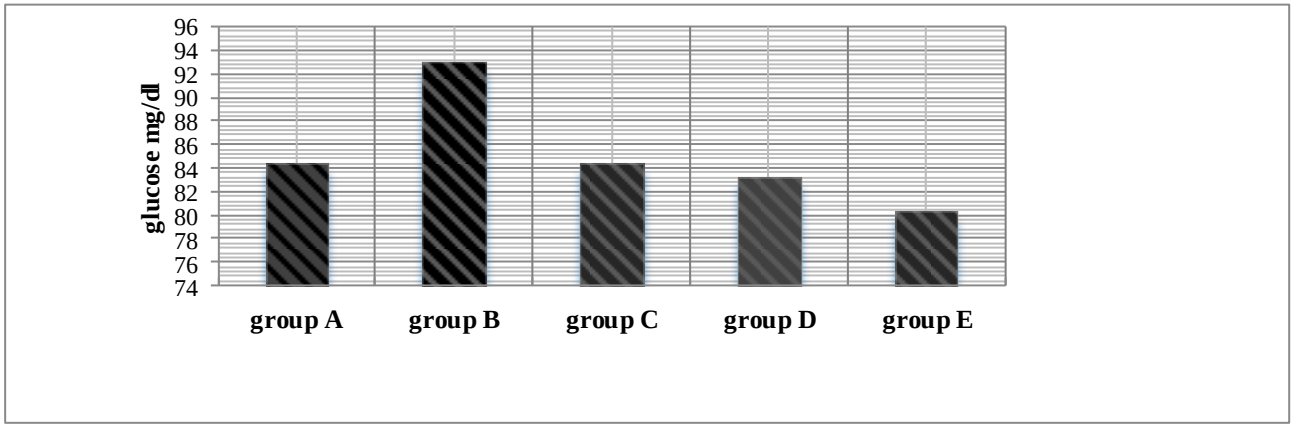

Figure.2: Fasting blood glucose (FBG) levels (mg/dl), in experimental groups (A, B, C, D, E). Data are presented as mean ±SD (n=9-10), (p<0.05)

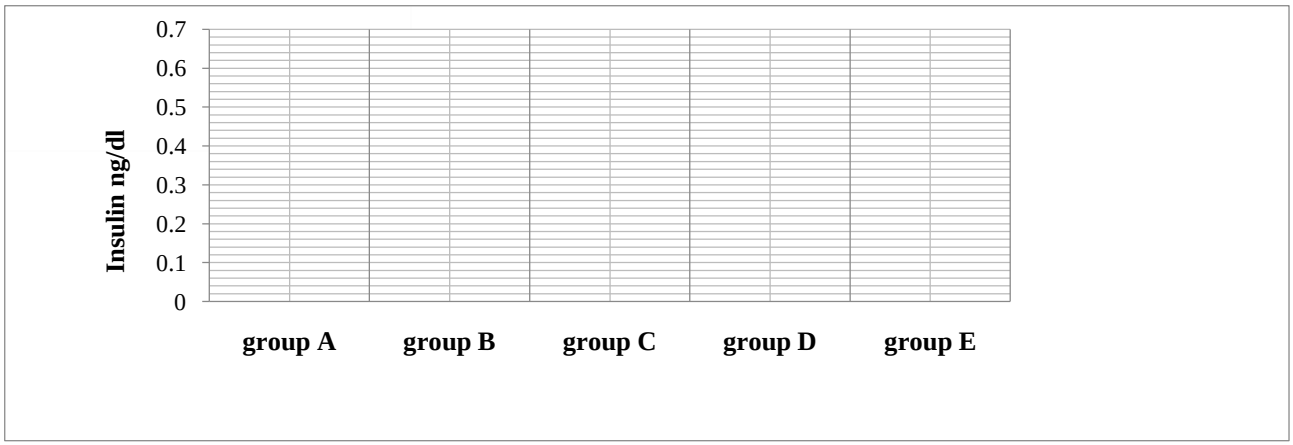

Figure. 3: Fasting serum insulin (FSI) level (ng/dl), in experimental groups (A, B, C, D, E).Data are presented as mean ±SD ((n=9-10), (p<0.05)

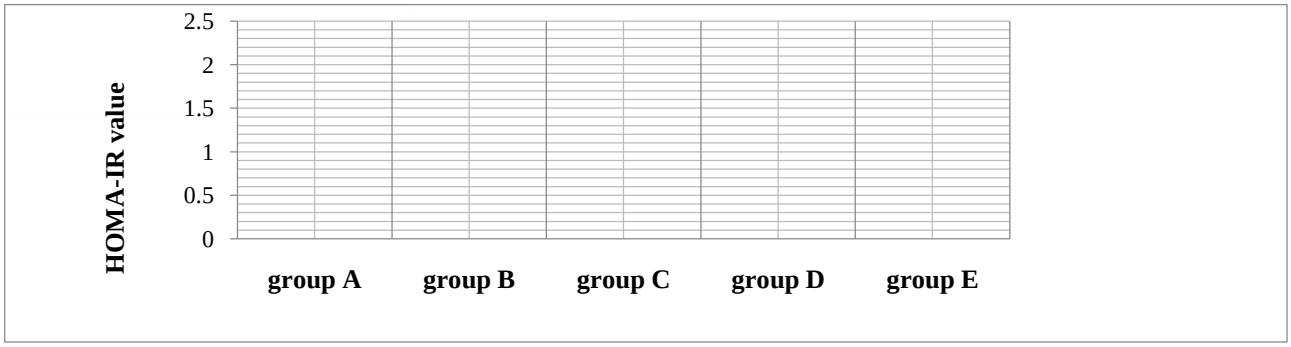

Figure.4: Insulin resistance represented (IR) as HOMA-IR index in experimental groups. Data are presented as mean ±SD ((n=9-10), (p<0.05)

Serum lipid profile; Rats supplemented with HFC diet for 10 weeks showed dyslipidemia. The group B animals dyslipidemia biomarkers (TG, TC, and LDL-C) were rose significantly ($p<0.05$) compared with the control group A (Fig.5), while HDL-C level was non-significantly changed. Supplementation of rats with *Lactobacillus* suspensions, individually and in combination significantly ($p<0.05$) improved lipid profile in treatment groups (C, D, and E) compared to group B, in that, there was obvious increase in levels of: TC (80.0±2.56mg/dl), TG (79.2±2.07mg/dl), and LDL-C (52.71±2.56 mg/dl), in group E animals.

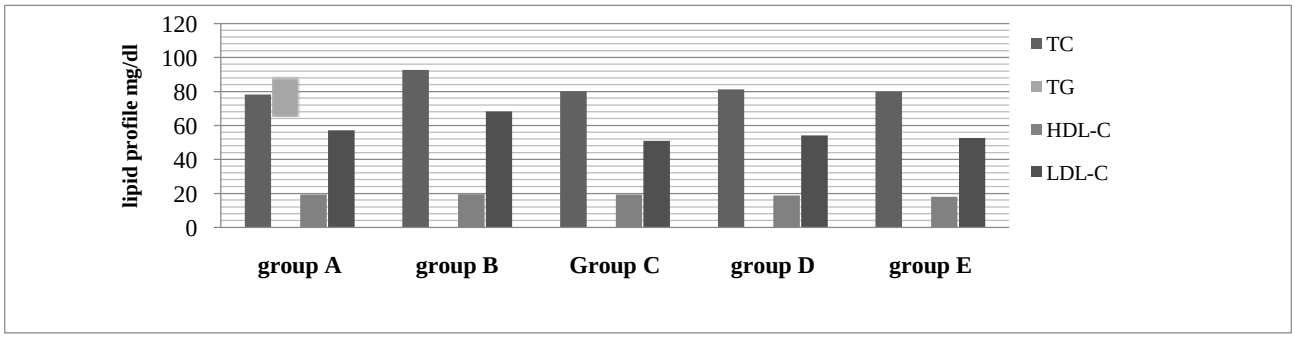

Figure.5: Serum lipid profile [total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL-C), and low-density lipoprotein (LDL-C)] levels in rat's sera in the end of experiment. Data are presented as mean ±SD ((n=9-10), (p<0.05)

Obesity biomarkers; the levels of satiety-related hormones homeostasis was altered Group B animals showed a significant ($P<0.05$) higher level of serum leptin (hyperleptinemia) compared with group a, rats that stayed on normal diet (group B: 2.96±1.13 ng/ml vs. group A: 0.88±0.06 ng/ml). A combination of Lactobacilli administration recovered this change, group E, rats displayed significant hypoleptinemia (1.12±0.05 ng/ml) when compared to the group B only (Fig.6). In the other hand, HFC diet induced hypoadiponectinemia in group B rats (1.98±0.06 ng/ml), administering the

lactobacilli also appeared to regulate the adiponectin imbalances (Fig.7), as the level of serum adiponectin was rose in group C and D rats (group C:2.03±0.11ng/ml and group D: 2.0±0.09 ng/ml), the group E rats that was received L1+L2 appeared significantly recovered the hypoadiponectinemia ($p<0.05$) disorder (2.35±0.04 ng/ml).

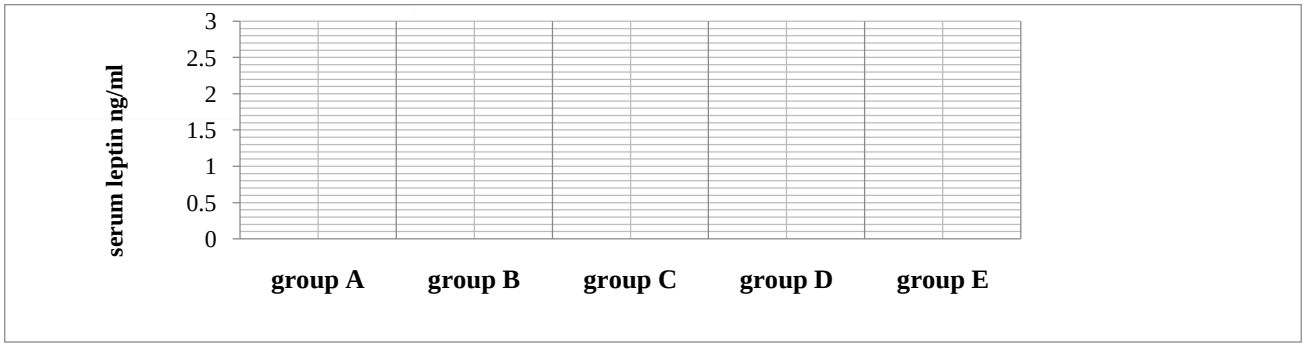

Figure.6: Serum leptin level in experimental groups. Data are presented as mean ±SD ((n=9-10), ($p<0.05$)

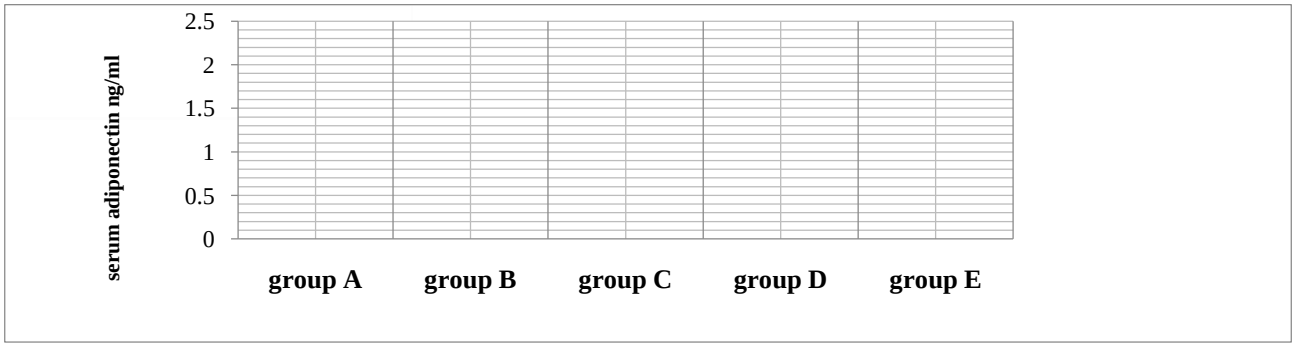

Figure.7: Serum adiponectin level in experimental groups. Data are presented as mean ±SD (n=9-10), ($p<0.05$)

Discussion

Obesity is becoming a problem of public health, due to its increased prevalence and the consequent repercussion of its comorbidities on the health of the population. Consumption of probiotics impacted gut microbiota and have potential effects on chronic diseases, such as obesity. In this study we investigated anti-obesity potentials of two putative probiotics, *Lactobacillus fermentum* L1 and *Lactobacillus paracasei* L2 (L2), individually and in combination of both against diet-induced obese rats model. The great similarity and homology between the genomes of rodents and humans make these animal models a major tool to study conditions affecting humans [28]. Feeding HFC diet daily to experimental rats resulted obesity in rats, lactobacilli oral ingestion protected rats against adiposity, represented by reduction of less index values.

It has been suggested *Lactobacillus* positively modulate gut microbiota, and consequently may help to reduce the risk of overweight or obese [29]. In murine model previously documented, that feeding *Lactobacillus* fermented yogurt inhibited the mice's fat accumulation and body weight gain, authors noted that both lactic acid bacteria and the resident microbiome affect the host immunity, which in turn affects obesity [30], The *Lactobacillus* combination (L1+L2) ingestion beneficially attenuated weight gain in group E rats, and shifted Lee index towered normality. Multiple strains of probiotics are proved to be more effective than that of individual strains [21].

Combination of *L. rhamnosus* LGG and *L. sakei* NR28 have demonstrated considerable anti-obesity effects and the potential for probiotic based therapies in the treatment and prevention of obesity [22]. The orally administrated lactobacilli achieved proper adhesions and colonization in rats gut represented by increased *Lactobacillus* shedding to the feces of rats (Fig.1).

Adequate attachment and adhesion of probiotic cells to the intestinal mucosa of the host considered an essential step in the successful interactions between probiotics and the host [31].Adhesion profiles varied widely among members of *Lactobacillus.* May be related to the diversity of cell surface architecture and the bacteria's ability to express certain surface components or secret specific compounds in response to the host

environment, consequently favored good furnishing of host gut, even temporarily [32].*Lactobacillus* display surface adhesins that facilitate attachment to the mucous layer in the host gut, the adhesins members generally grouped into; sortase-dependent [LPXTG] anchor protein motif, S- layer proteins, and fibronectin binding proteins [33].

These proteins have an array of functional implications, including facilitating colonization through the degradation of extracellular matrix of cells or by enabling close contact with the enterocytes surface [34]. In addition, lactobacilli bind to the enterocytes via electrostatic interaction, and steric forces [35].Dyslipidemia is another hallmark in the pathogenesis of obesity, it characterized by hypertriglyceridemia, (high serum TG level) and low levels of HDL-C [36]. Chronic dyslipidemia has been characterized as a major risk factor for cardiovascular risk, including atherosclerosis [37].

In recent years, attention has been given to the hypolipidemic potentials of probiotics to reduce lipids and cholesterol levels [38]. In this context, when rats were acclimated on high fat diet for 10 weeks and in concomitant, animals daily received lactobacilli supplementations, the treatment was significantly enhanced dyslipidemia in group B animals, while improvement of lipid disorders have been detected in *Lactobacillus* ingested groups, as the treatment group showed clear decrease in TC, LDL-C, and TG. Similarly, in study was involved *Lactobacillus plantarum* Q180, the obtained results demonstrated hypolipidemic effects of the used strain, represented by reduced body weight gain, and concurrently decreased triglycerides and LDL-C in mice acclimated on high fat diet [39].

Other *Lactobacillus* strain *Lactobacillus plantarum* DK211 (kimchi-derived) supplanted with a high protein whey beverage to rats-induced obesity for 4-weeks, the mixture prevented body weight gain, and improved serum lipid profile of treated rats [40].There are several proposed mechanisms of *lactobacillus* hypolipidemic actions. One mechanism, bile salt deconjugation activity of most lactobacilli that are catalyzed by bile salt hydrolase (BSH) activity [41].In addition, *Lactobacillus* has been reported to assimilate cholesterol [42], thereby lowering luminal cholesterol levels available for absorption. Moreover, *Lactobacillus* can produce ferulic acid (FA) [43], which can inhibit hepatic HMG-CoA reductase and promote the excretion of acidic sterol [44]. Hyperglycemic statues are causally related to a greater risk of several chronic disorders, including, diabetes, and obesity [45].

The results indicated high impact of using mixed *Lactobacillus* (L1+L2) on glycemic response; they pronounced more reduction in FBG, FSI and improved IR disorder. This finding is in line with the previous studies, suggested a combination of probiotic species are more effective than single species products [46].

The detected hypoglycemic activity was also related to hypotriglyceridemic activity of both adopted *lactobacillus* strains, due to IR condition is also reflection of hypertriglyceridemia [47], and in consistency with previous studies reported the probiotic supplementation in rats improved glucose and lipid metabolism, suppressed glucose intolerance and delayed the onset of hyperglycemia, hyperinsulinemia, dyslipidemia, and oxidative stress [48].

The anti-hyperglycemic effects of probiotics are strain-dependent as well as type of animal models, not all studies reported beneficial effects of probiotics in this context [49], and hypoglycemic activity seems to be restricted to duration of probiotics supplementation, anti-obesity studies was claimed that the improvements in FBG and HOMA-IR were restricted to long-term trials > 8 weeks, while better insulin reduction was obtained in trials > 8 weeks compared to trials ≤ 8 weeks [50]. Possible mechanisms by which probiotic intake improve glycemic status and IR is unclear.

It may be related to decreased oxidative stress, the probiotic yogurt improves antioxidant status in type 2 diabetic patients [51]. Specific strains of lactic acid bacteria have antioxidant properties [52].Animal studies have also provided insights. Obese mice fed *Lactobacillus casei* strain Shirota had better insulin resistance through decreasing plasma levels of lipopolysaccharide-binding protein, a marker of endotoxemia, rather than reducing abdominal fat [53].

It was demonstrated that in obesity-induced rodent study, rats daily supplementation with probiotic Bifidobacterium *longum* led to reduced intestinal inflammatory activity index, which may also be the underlying mechanism by which probiotics affect glucose and lipid metabolism [54].

Probiotics are effective in improving the IR by reducing the concentration of endotoxin, increasing fecal PH, and reducing the production and absorption of intestinal toxins. Thus, modulating gut microbiota can be effective in improving glycemic status through the use of probiotics [55]. Other proposal mechanisms by which probiotic intake might improve IR include the adjustment of the energy metabolism, by increased the production of Glucagon-like peptide (GLP), GLP-1 and GLP-2 (appetite-reducing hormones).

GLP-1, moderates pancreatic and plasma insulin secretion, beta cell mass, and its function, while GLP-2 enhances insulin sensitivity in the liver, fat, and muscle [56]. Adipocytokines, such as leptin and adiponectin, are known to act as a regulator of energy homeostasis [57]. Leptin functions as part of a feedback mechanism that suppresses appetite, through its receptor at the hypothalamus, leptin level in serum is positively associated with increases in the body weight and adipocyte size [58].

This correlation was observed in this study, as the serum leptin levels were 2.4 times lower in the mixed *Lactobacillus* fed rats (group E) compared to positive control rats group B (2.69±0.13ng/ml). According to studies in rodents and man the expression and release of leptin depend on adipocyte size, therefore, Plasma levels of leptin are generally increased in obese individuals [59]. Leptin exerts anorectic effects through its hypothalamic receptor [58]. However, several evidences indicate that leptin's actions are not the result of its anorectic effects alone. For instance, leptin may have a central role in preventing the accumulation of hepatic TAG through the regulation of fat synthesis and its distribution and by modulating hepatic ß-oxidation [60].

Adiponectin has an important role in carbohydrate and lipid metabolism, also controls food intake and energy expenditure. Generally, serum adiponectin level is negatively associated with adipose tissue weight, and decreases in obese individuals [57].Adiponectin that is specifically produced by adipose tissue and regulates insulin sensitivity and tissue inflammation. Weight reduction reportedly leads to a significant increase in the adiponectin level [61]. High level of adiponectin increases insulin sensitivity, while a low adiponectin level contributes to IR in obesity and type 2 diabetes mellitus [62].

In current study rats were supplemented with *Lactobacillus* for ten weeks significantly improved adiponectin secretion, and this was associated with reduction of FPG, and enhancement of insulin sensitivity, which is consistent with previous studies, that demonstrated the efficacy of probiotic supplementation in adiponectin secretion or expression [63], and several studies showed that certain probiotic supplementation may improve adiponectin secretion or expression [64, 65].

Conclusion

- The enteric Lactobacillus, *L. fermentum* L1 and *L.s paracasei* L2 have anti-obesity potentials with multiple modes of actions in rodent model.
- A combination of both lactobacilli is more effective in amelioration some of obesity-biomarkers.
- Supplementing with enteric lactobacilli may be an alternative strategy for combating obesity and related disorders, or use as adjuvant to increase the effectiveness of available therapies.

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