Oral administration of acidophilus milk supplemented with calcium pyruvate modulates biochemical parameters and weight gain in rats fed high fat diet

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Abstract: This study aims to evaluate the protective effect of acidophilus milk supplemented with calcium pyruvate on reduction of obesity in rat model. For that purpose, Albino rats were randomized into three groups; rats in the first group (I, control) were fed high fat diet while rats in experimental groups were fed the same diet and orally administrated with acidophilus milk or acidophilus milk supplemented with 3% of calcium pyruvate for rats in group III. After five weeks of intervention, levels of plasma triglycerides, cholesterol, glucose, liver markers, and IL-6 and body weight were significantly \( p < 0.05 \) increased in rats of group I compared to rats in both experimental groups. Also, levels of plasma IL-10 were significantly increased in the experimental groups compared to rats in group I. Results of this study indicated that supplementation of acidophilus milk with calcium pyruvate could decrease the weight gain and modulate plasma triglycerides, cholesterol, glucose, liver markers, IL-6 and IL-10 in rat model. Indeed, further research on the molecular mechanism of anti-obesity effect of calcium pyruvate is still needed.

Keywords: Acidophilus fermented milk, Functional ingredients, High fat diet, Lactobacillus acidophilus, Pro-inflammatory marker

Introduction

Obesity becomes one of the main public health concern in recent decades in different countries (WHO, 2014) because it is associated with different metabolic syndrome including non-alcoholic fatty liver, type 2 diabetes and cardiovascular disease (Pospisilik et al., 2007; Brown et al., 2015; Franks and McCarthy, 2016). Obesity is an abnormal excessive accumulation of fat that may impair health due to the imbalance between calories consumed and expend whereas obese patients have body mass index (BMI) greater or equal 30 (WHO, 2014). Increase high fat diet intake like fast foods is associated with increased capacity for energy harvest (Turnbaugh et al., 2006), increased bacterial translocation leading to an increase in circulating LPS resulting metabolic endotoxemia (Cani et al., 2008; Kim et al., 2012), increased intestinal permeability through reduction of tight junction protein (Shen et al., 2014) and enhanced lipogenesis (Huang et al., 2013).

Probiotics are live commensal microorganisms which, when consumed in adequate quantities, confer a health benefit to the host (FAO/WHO, 2001). Recently, Elshaghabee (2017) defined probiotics as live microbial strains with health impact on host when they consumed daily with enough amounts (not less than \( 10^6 \) to \( 10^8 \) CFU/g). Different probiotic strains (in free or microencapsulated form) have been studied extensively for their application as new therapy or as dietary intervention for reducing the risk of obesity and its related metabolic diseases (Tennyson and Friedman, 2008; Sanz et al., 2013; Bhathena et al., 2013; da Silva et al., 2013, Li et al., 2018). Acidophilus milk either sweet or fermented is produced using Lactobacillus (L.) acidophilus as main starter culture for milk (3.6% fat and 8.5% solid not fat) (Mital and Garg, 1992). L. acidophilus in tablet form or in acidophilus milk has many beneficial effects these are including anti-cancer activity especially in colon (Takano et al., 1985), inhibition of different pathogens (Gilliiland and Speck, 1977; Abd EL-Salam et al., 2004) and hypocholesterolemic effects (Nielsen et al., 1985; Ynnin and Welchen, 2000; El-Dieb et al., 2010).
Pyruvate is an intermediate substance of the glycolysis and it presents a key substrate for energy production by mitochondria (Axelsson, 2004; Kang et al., 2012). Supplementation of diet of Zucker rats with 9% of calcium pyruvate could reduce the weight gain of rats during 25 days of intervention (Ivy et al., 1994). Also, pyruvate salt, as a dietary electron acceptor, could modulate the metabolites profile of fructose fermentation by fecal slurries collected from obese subjects (Elshaghabee et al., 2016a). Recently, combination of rope skipping and calcium pyruvate (50mg/kg) could effectively enhance the fat metabolism and optimize body shape of sixty female college students (Gong and Jiang, 2018). The present study aims to investigate whether oral administration of acidophilus milk supplemented with calcium pyruvate mainly causes modulation of plasma metabolic profiles, reducing the pro-inflammatory cytokine, and weight gain resulting reduction the development of obesity using rat model.

**Materials and Methods**

**Propagation of cultures**

*L. acidophilus* NRRL -B-4495 was obtained from Northern Regional Research Laboratory (NRRL), Peoria, USA. It was sub-cultured in MRS broth medium (Merck, Darmstadt, Germany) and incubated aerobically at 37°C for 18 h.

**Preparation of acidophilus milk**

Acidophilus milk was manufactured from skim milk as described by Azlin et al. (1997). Calcium pyruvate (Shanghai Helios Technology Co., Ltd, Shanghai, China) was mixed with acidophilus milk using laboratory mixer (Davis Instrument Co., Illinois, USA) at final concentration of 3%. This concentration of calcium pyruvate was used after preliminary sensory evaluation test for the tested product. The viable counts (using MRS agar pH 5.8 at 37°C for 72 h) of *L. acidophilus* in acidophilus milk were 7.85±0.57 Log CFU/g.

**Animals and feeding protocol**

Male Albino rats, 10 weeks old (ca. 220 g), were housed in plastic cages individually and maintained on basal diet and water *ad libitum* for 2 weeks, until they reached 350 - 380 g. Thereafter, rats were randomly allocated to three groups. During five weeks of intervention, rats in the control group (n = 8) were accustomed to high fat diet (HF) (Tanida et al., 2008). While rats in the experimental groups (n = 10) were accustomed to HF diet and orally administrated (Hsieh et al., 2013) with acidophilus milk or acidophilus milk mixed with 3% calcium pyruvate. The composition of basal and high fat diet was as previously described by Kawasaki et al. (2009). Animal care and experimentation performed in this study were conducted according to the guide for care and use of laboratory animals, approved by the local experimental ethics committee of Research Institute of Ophthalmology, Giza, Egypt.

**Blood collection and determination of biochemical parameters in plasma samples**

At the end of the experiment, rats were anaesthetized and blood samples (one mL) were collected as previously described by Elshaghabee (2016b). Spectrophotometric kits of plasma alanine transaminase (ALT), aspartate aminotransferase (AST), triglycerides, cholesterol and glucose were purchased from Biological Co. (Giza, Egypt). Plasma Interleukin (IL)-6 (Northemann et al., 1989) and IL-10 (Said et al., 2010) were determined using commercial ELISA kits (BIOZOL GmbH, Eching, Germany).

**Statistical analysis**

Data were expressed as mean ± standard error (SE). One – way ANOVA and Duncan test were used to assess the significance of differences between groups with *P* < 0.05 being considered significant.

**Results and Discussion**

Levels of triglycerides (295.50±19.85 mg/mL), cholesterol (125.82±9.51 mg/mL), ALT (40.52±3.18 U/L), AST (69.82±5.95 U/L), glucose (245.81±15.53 mg/mL), IL-6 (10.56±2.45 pg/mL) and weight gain (550.00±30.01 g) of rats were significantly (*P* < 0.05) increased in rats fed high fat diet (control group) at the end of intervention period as shown in Figures. (1, 2, 3, 4 and 5) respectively. These results are in the same line of results obtained by Kawasaki et al. (2009) who reported that feeding rats with high fat diet resulted in increased levels of plasma triglycerides, cholesterol and ALT and induced obesity and liver steatosis after five weeks of intervention. Levels of triglycerides, cholesterol, ALT, AST, glucose, IL-6 and weight were lower by 38.9, 22.0, 29.5, 35.0, 24.5, 22.0 and 18.18% respectively for rats in Group (II) compared to control group. However, it was 46.3, 33.3, 51.50, 47.4, 33.3, 37.8 and 29.1 % respectively for rats in Group (III).

Several research groups reported that supplementation with different probiotic strains e.g. *L. gasseri* (Hamad et al., 2008), *L. paracasei* CNCM I-4270, *L. rhamnosus* I-3690 and *B. animalis* subsp. *lactis* I-2494 (Wang et al., 2015), *L. acidophilus* La-5, *L. casei* DN001 and *Bifidobacterium* Bb-12 (Zarrati et al., 2013) could decrease the development of obesity through reduction levels of plasma triglycerides, cholesterol, ALT, AST and glucose either in animal models or human. In contrast, Dahi (traditional Indian fermented milk) containing *L. acidophilus* NCDC 13 had no significant changes on body fat composition, liver and muscle adiposity (Arora et al., 2012). Levels of plasma IL-10 were significantly increased with 45.1 % and 53.2 % in rats fed high fat...
diet mixed with acidophilus milk (Group II) and when rats fed the same diet mixed with acidophilus milk containing 3% calcium pyruvate (Group III) compared to control group. This result supports results obtained by Poutahidis et al. (2013) and
Fig. 4 Mean values of concentration of interleukin (IL)-6 and IL-10 in plasma of rats

Fig. 5 Mean values of weight (g) of rats

HF: Rats fed high fat diet, HF+A: Rats fed high fat diet mixed with acidophilus milk, HF+AC: Rats fed high fat diet mixed with acidophilus milk and calcium pyruvate. a, b and c: Different superscript letters represent the significant differences between each parameter ($P < 0.05$), i.e. same letters mean no significant difference between each parameter.
Elshaghabee (2016b) who reported that eating yoghurt containing *L. reuteri* and Karish cheese containing ABT probiotic culture could decrease levels of IL-6 and enhance secretion of IL-10.

Calcium pyruvate is fermentable salt by different gut microbiota (Elshaghabee et al., 2016a) and it could reduce weight gain and enhance fat metabolism (Gong and Jiang, 2018). Results in Figure (1) showed a significant reduction in levels of plasma triglyceride and cholesterol in rats fed acidophilus milk supplemented with calcium pyruvate which are in agreement with results obtained by Ivy et al (1994).

**Conclusions**

Development of fermented dairy foods by supplementation with functional ingredients may be one of the useful dietary intervention for reduction the risk of obesity. In this study, supplementation of acidophilus milk with calcium pyruvate ameliorates the obesity in rat model and this effect may attribute to block the inflammation process through reduction levels of IL-6 and increase levels of IL-10. Furthermore, it could enhance different plasma biochemical markers. Finally, this study provides evidences supplementation of acidophilus milk with calcium pyruvate may be a promising therapeutic agent in prevention of obesity.

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**References**


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