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Physiological Effects of a Combination of Cinnulin with Probiotics

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ABSTRACT

The search for an optimal combination of natural immunomodulators led us to study the biological effects of the combination of a cinnamon extract Cinnulin PF and probiotic LactoSpore. We found that this combination has strong synergetic effects on phagocytosis and on regulation of cholesterol and blood sugar levels. In addition, the Cinnulin/LastoSpore combination also reduced intestinal damage in mouse model of colitis.

Keywords: Glucan, Cinnamon, Colitis, Probiotics

1. INTRODUCTION

Probiotics are live microorganisms that, when delivered in sufficient amounts, confer health benefits on the host. Lactic acid bacteria and bifid bacteria are the most common types of microbes used as probiotics however certain bacilli may also be used. Probiotics are commonly consumed as part of fermented foods with specially added active live cultures, such as in yogurt and soy yogurt. Lately, their addition to dietary supplements has gained popularity.

For our experiments, we decided to use LactoSpore, with clinically demonstrated hypocholesterolemic and various gastrointestinal effects. LactoSpore is *Bacillus coagulans* (originally *Lactobacillus sporogenes*) in the form of spores that are able to withstand the acidic environment of the stomach and start to proliferate within the gastrointestinal tract.

With a recent wave of studies showing synergistic effects of carefully designed combinations of bioactive molecules (Vetvicka and Vetvickova, 2011; 2012; 2013), we combined the LactoSporeand a cinnamon extract Cinnulin PF, with the documented effects of fasting blood sugar and on regulation of glucose metabolism (Roussel *et al*., 2009). Cinnamon extracts were found to increase glucose uptake and glycogen synthesis and trigger the insulin cascade system (Jarvill-Taylor *et al*., 2001). This hypothesis was supported by additional findings showing that a dietary combination of pre- or probiotics with additional substances such as soy have synergetic effects (Larkin *et al*., 2009).

2. MATERIALS AND METHODS

2.1. Animals

Female, 8 week old BALB/c mice were purchased from the Jackson Laboratory (Bar Harbor, ME). All animal work was done according to the University of Louisville IACUC protocol. Animals were sacrificed by CO_2 asphyxiation.

2.2. Diet

All diets Laboratory Rodent Diet 5001 enhanced with glucan and/or cholesterol) were formulated and prepared by Purina (Richmond, IN). Diet ingredients for all groups were identical except for the proportion of samples and/or cholesterol.

2.3. Materials

Cholesterol, streptozocin, dextran sulfate sodium and cholesterol were obtained from Sigma Chemical Co. (St. Louis, MO). LactoSpore was purchased from Sabinsa Corp. (Piscataway, NJ), Cinnulin PF from Integrity Nutraceuticals (Spring Hill, TN, USA).

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2.4. Phagocytosis

The technique employing phagocytosis of synthetic polymeric microspheres was described earlier (Vetvicka *et al*., 1982; 1988). Briefly: peripheral blood cells were incubated with 0.05 mL of 2-hydroxyethyl methacrylate particles (HEMA; 5×10^8 mL^{-1}). The test tubes were incubated at 37°C for 60 min., with intermittent shaking. Smears were stained with Wright stain. The cells with three or more HEMA particles were considered positive. The same smears were also used for evaluation of cell types.

2.5. Glucose Evaluation

The mice were given drinking water freely and were not fed 24 h prior to measurement of blood glucose level. In some experiments, blood glucose was determined in hyperglycaemic mice which were pretreated with streptozotocin (250 mg kg^{-1} ip.) 12 days before the start of feeding with glucan (Hatanaka *et al*., 1992).

2.6. Biochemical Analysis

Mice were deprived of food for 24 hr and sacrificed. Serum was collected via the retro-orbital sinus and stored at -80°C for less ten a week. Biochemical analyses were performed by Antech Diagnostics (Indianapolis, IN).

2.7. Colitis

Experimental colitis was induced as described before (Cooper *et al*., 1993). Briefly, mice were getting 3.5% Dextran Sulfate Sodium (DSS) in drinking water for 20 days. To assess the extent of colitis, body weight, stool consistency and blood in the stool was monitored.

2.8. Statistics

Student's t-test was used to statistically analyze the data.

3. RESULTS

Phagocytosis is one of the most important immunological reactions traditionally connected with effects of natural immune modulators. Therefore, we started our experiments by comparing the effects of oral administration on stimulation of phagocytic activity of peripheral blood neutrophils. For these studies, we used a model of synthetic microspheres based on 2-hydroxyethyl methacrylate, which are routinely used for their low negative charge resulting in minimal false positivity. Our data showed that only the combination of Cinnulin with probiotics resulted in significant stimulation of phagocytic activity. The same pattern was found during all the experiments (**Fig. 1**).

For cholesterol and blood sugar experiments we used the Laboratory Rodent Diet 50001 consisting of 23.9% protein, 4.6% fat, 5.5% fiber, with 75.5% total digestible nutrients. The diet was supplemented with either individual samples or cholesterol corresponding to the final daily doses of 100 µg Cinnulin, 10^7 LactoSpore (probiotics) or 16 µg of cholesterol, resp. First, we studied effects of long-term feeding on cholesterol levels, but no significant differences were found (data not shown). Therefore, we used a model of a diet enhanced with cholesterol (Vetvicka and Vetvickova, 2007). Levels obtained after 14 day cholesterol feeding were used as positive control. The effects were followed for 40 days, individual groups were sacrificed in 10-day intervals and cholesterol levels were measured. Results shown in **Fig. 2** showed that, whereas Cinnulin alone was effective only after 40 days, probiotics were already active after 10 days. The Cinnulin-probiotics combination significantly lowered cholesterol levels in hypercholesterolemic animals through all tested intervals.

The next part of our project was devoted to the effects of our samples on blood sugar levels. Feeding with Cinnulin did lower the level of glucose, but the changes were not significant. However, when combined with LactoSpore, the reduction was significant after both 7 and 14 days (**Fig. 3**). Next **Fig. 4** summarized our finding in fasting blood glucose. Both samples significantly reduced the glucose levels, with Cinnulin alone having the same effects as the Cinnulin-LactoSpore combination. Our study was further supplemented by measuring the effects of diet supplementation on levels of cholesterol, triglycerides, LDL and HDL in serum of animals with STZ-induced diabetes. Application of STZ significantly increased total cholesterol, triglycerides and LDL levels and lowered levels of HDL. Addition of probiotics slightly affected tested substances, but the results were of little consequence. Cinnulin effects were much stronger and in all substances significant. Simultaneous addition of Cinnulin and LactoSporenoticeably lowered levels of total cholesterol and LDL (**Table 1**).

In the finalpart of our study, we focused on inflammatory bowel disease, which was repeatedly shown to be influenced by substances with immunomodulatory activities. First, we measured the colon length in mice with experimentally-induced colitis.

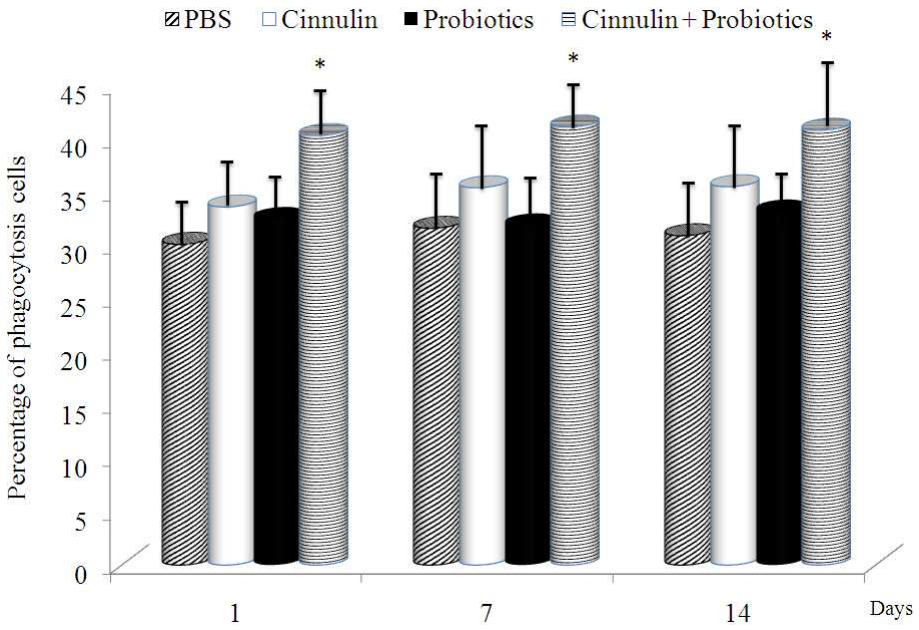

Fig. 1. Effect of long-term feeding with Cinnulin, LactoSpore (probiotics), or Cinnulin/LactoSpore combination (Cinnulin + Probiotics) on phagocytosis of HEMA particles by peripheral blood neutrophils. Each value represents the mean of the three independent experiments +/- SD. *Represents statistical difference from control (PBS).

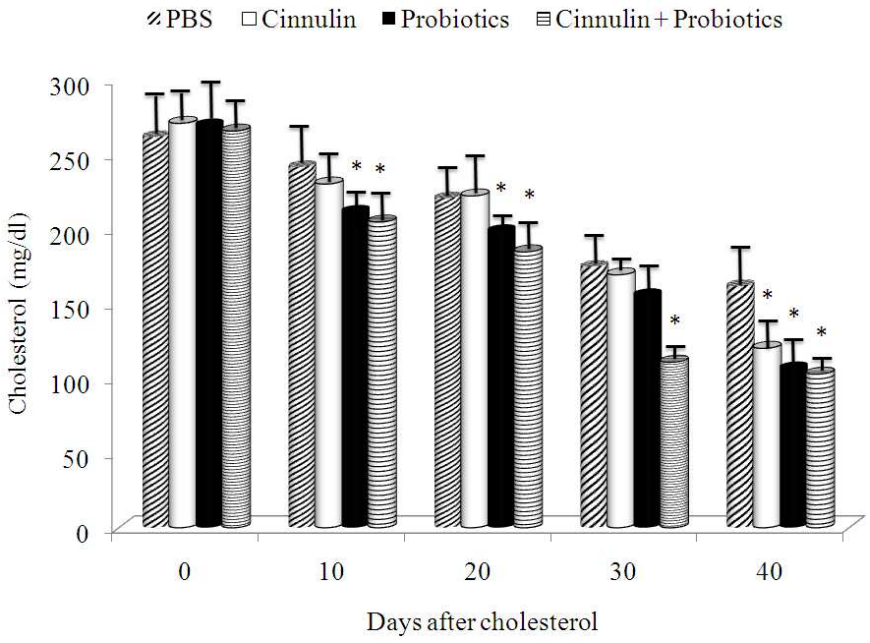

Fig. 2. Effect of long-term feeding with Cinnulin, LactoSpore (Probiotics), or Cinnulin/LactoSpore combination (Cinnulin + Probiotics) on blood cholesterol levels in experimentally-induced hypercholesterolemia. The feeding with tested material started after two weeks of cholesterol-high diet. Each value represents the mean of the three independent experiments +/- SD. * Represents statistical difference from control (PBS)

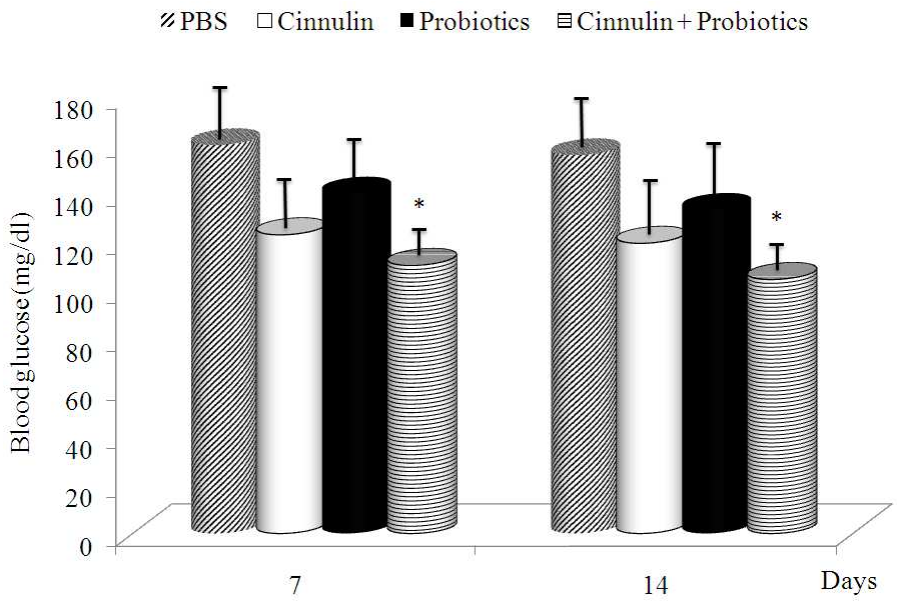

Fig. 3. Effect of long-term feeding with Cinnulin, LactoSpore (Probiotics), or Cinnulin/LactoSpore combination (Cinnulin + Probiotics) on blood glucose levels in mice with experimentally-induced hyperglucaemie. Each value represents the mean of the three independent experiments +/- SD. * Represents statistical difference from control (PBS)

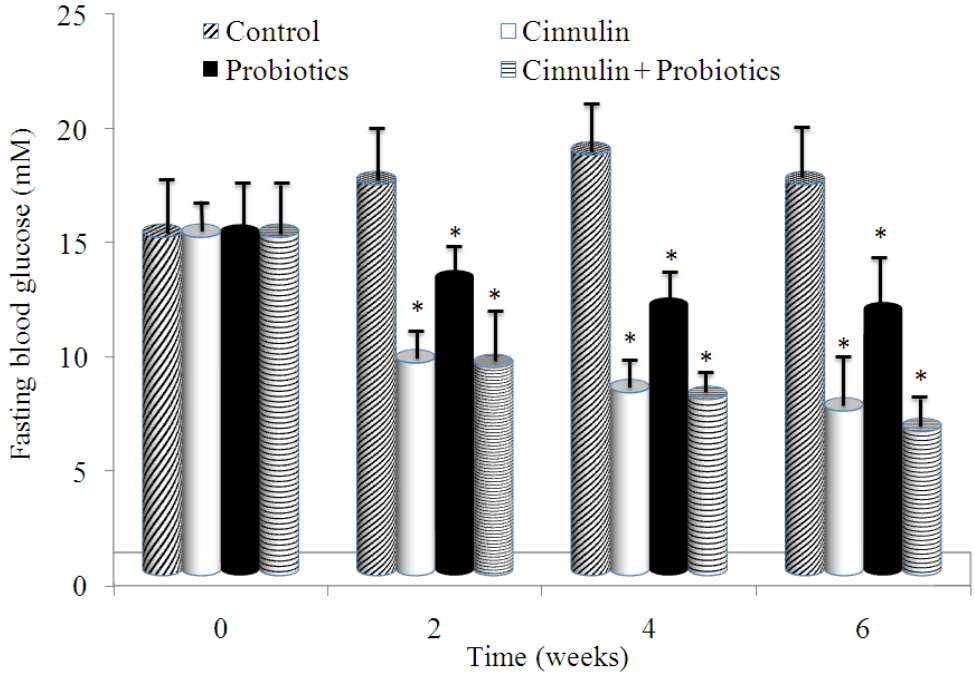

Fig. 4. Effect of long-term feeding with Cinnulin, LactoSpore (Probiotics), or Cinnulin/LactoSpore combination (Cinnulin + Probiotics) on fasting glucose levels in mice. Each value represents the mean of the three independent experiments +/- SD. * Represents statistical difference from control (PBS)

Table 1. Effects on serum lipid proteins in STZ-induced diabetic mice

Group	Cholesterol	Triglycerides	LDL	HDL
Control	144.1± 4.4	96.4± 4.2	105.4± 2.8	44.8± 2.1
STZ	281.5± 3.7	222.5± 6.6	209.9± 3.8	25.3± 1.8
Probiotics	272.7± 4.4	211.3± 3.8	206.7± 4.2	29.1± 2.9
Cinnulin	205.2± 3.5*	119.9± 4.6*	155.9± 3.8*	39.8± 4.2*
Cinnulin + Probiotics	149.4± 8,8*	112.4± 6.7*	132.9± 4.2*	33.8± 4.3*

(mg/dl) Results represent a mean of three experiments +/- SD. * Represents statistical difference from STZ-induced diabetic mice.

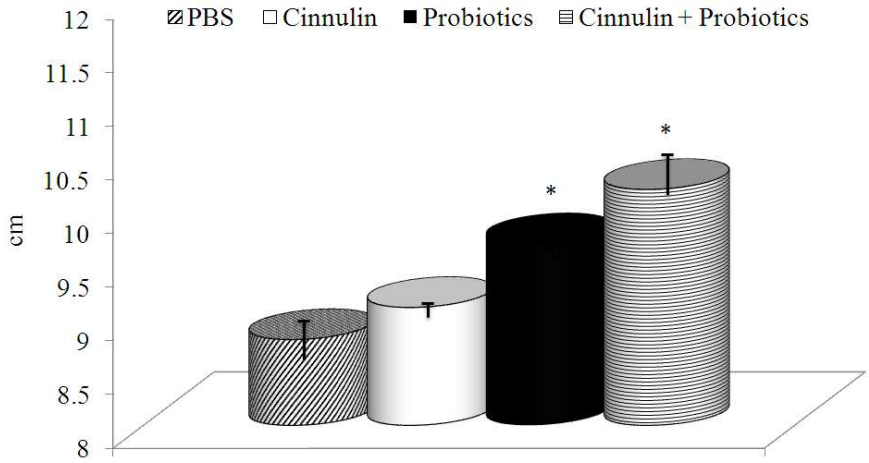

Fig. 5. Effect of long-term feeding with Cinnulin, LactoSpore (Probiotics), or Cinnulin/LactoSpore combination (Cinnulin + Probiotics) on colon length in mice with experimentally-induced colitis. Each value represents the mean of the three independent experiments +/- SD. * Represents statistical difference from control (PBS)

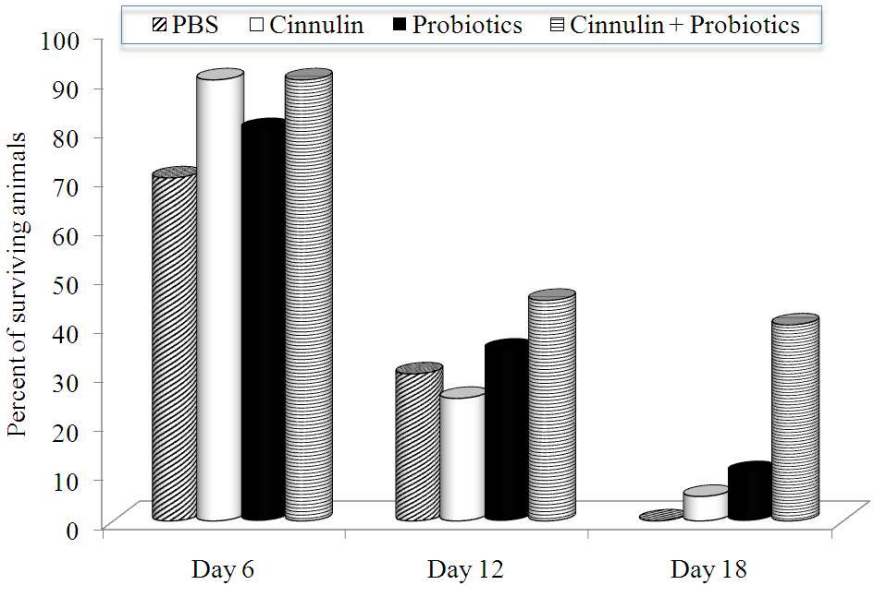

Fig. 6. Effect of long-term feeding with Cinnulin, LactoSpore (Probiotics), or Cinnulin/LactoSpore combination (Cinnulin + Probiotics) on survival rate in mice with experimentally-induced colitis.Results from 9 animals/group are given as percentage of surviving animals

We found that probiotics and the Cinnulin-LastoSpore combo significantly reduced the DSS-induced shortening of the gut length (**Fig. 5**). The combo's values were virtually identical with control mice (10.8 cm). Next, we measured the survival after longer exposure to the lethal dose of DSS and found that only the Cinnulin-LastoSpore combination significantly increased the survival of mice (**Fig. 6**).

4. DISCUSSION

Cinnamon extracts were found to have significant biological effects. One of them, Cinnulin PF, represents the water soluble fraction containing relatively high levels of the double-linked procyanidin type A polymers of flavonoids (Rafehi *et al*., 2012). In adipocytes, it can act as a mimetic for insulin with almost identical activity (Jarvill-Taylor *et al*., 2001). In addition, these extracts were found to cause a major reduction in risk factors associated with diabetes and cardiovascular diseases (Roussel *et al*., 2009). Similarly, addition of probiotics into the food resulted in reduction of cholesterol and blood sugar levels in people with diabetes (Moroti *et al*., 2012) and reduced total or LDL cholesterol in hypercholesterolaemic patients (Larkin *et al*., 2009). Both our studies and studies by others using several bioactive substances showed that, in many cases, a well prepared combination of individual, highly active biomodulating molecules offered even better results (Vetvicka and Vetvickova, 2013; Ditteova *et al*., 2003; Vetvicka *et al*., 2010). These findings led us to study the activities of a Cinnulin/LactoSpore combination.

First, we measured the phagocytic activity using peripheral blood leukocytes and synthetic microspheres as a model. Our results showed that only a combination of Cinnulin with probiotics resulted in stimulation of this activity. These experiments represented the first evaluation of cinnamon extract on cellular immunity, as the only defense-related effects of Cinnulin were found in increase of TNF-α-induced production of intestinal lipoprotein (Qin *et al*., 2009).

Next, we focused on effects on regulation of cholesterol. Whereas Cinnulin alone was only marginally effective, the Cinnulin-probiotics combination showed strong synergistic effects both in normal and hypercholesterolemic animals. Since these effects of various probiotics are known (Kumar *et al*., 2012), we did not confirm the previous studies suggesting strong effects of Cinnulin.

The effects Cinnulin on blood sugar regulations have been more thoroughly studied. In human keratinocytes, Cinnulin caused changes in gene expression (Rafehi *et al*., 2012). Similarly, in adipose tissue of fructose-fed animals, cinnamon extract regulated plasma levels of adipose-derived factors and changed expression of multiple genes connected with metabolism of carbohydrates (Qin *et al*., 2010). In addition, Cinnulin has strong antioxidant effect in obese people with impaired fasting glucose (Roussel *et al.* 2009). Our findings are generally in agreement with previous studies but, again, in numerous instances, the synergistic effects of the Cinnulin-LactoSpore combination offered stronger effects.

Some of the Cinnulin effects were manifested on intestinal cells. Similarly, the growing body of experimental and clinical evidence supports the notion that the intestinal microbiota strongly influences function of intestinal tissue. Probiotics were repeatedly found to offer palative and/or protective effects in inflammatory bowel diseases including colitis (Greef *et al*., 2013; Viladomiu *et al*., 2013; Sung and Park, 2013). This led us to study the possible effects of the Cinnulin/LactoSpore combination on experimentally-induced colitis in mice. Our results showed that this combination is therapeutically effective in the DSS model of colitis. Even when the data suggested that the probiotic part of the combination is the responsible entity, there is a clear synergistic effect. We hypothesize that the effect of this combination might be extended via changes in the production of cytokines such as IL-4, IL-17 and IL-23 (Kim *et al*., 2012; Cox *et al*., 2012).

5. CONCLUSION

Taken together, the Cinnulin/LactoSporecombination represents a novel, fully natural supplement designed to improve physiological functions such as cholesterol and blood sugar level and improve the conditions of the gastrointestinal tract by treating various gastrointestinal disorders including colitis. As both components were preclinically and clinically tested, no health problems can be anticipated.

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