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Coencapsulation of Synbiotics for the evaluation of *in vivo* antidiabetic activity

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ABSTRACT

Glucose is the main source of energy for the body cells and different organisms possess different levels of glucose levels in their body. Change in levels of blood glucose leads to either hyperglycemia or hypoglycemia. When the body fails to regulate blood glucose levels correctly and the levels go beyond the normal range, one becomes diabetic. Diabetes is a chronic disease and can even lead to death. Synbiotics; a combination of probiotics and prebiotics show beneficial health effects to the host and the effects vary with strain of probiotic and type of prebiotic used. The current study was performed to determine the hypoglycemic potential of the coencapsulated prebiotic (lactulose) and probiotic (Lactobacillus casei subsp. casei 17@10^9 cells/ml) in vivo. Encapsulation was done using Sodium Alginate (3.5%) & Calcium Chloride (75mM), resulting in the formation of calcium alginate beads of synbiotics. Glibenclamide was taken as positive control. Alloxan (150 mg kg^{-1} b.wt. i.e. 50 mg kg^{-1} b.wt.) was used to induce diabetes in animals. Acute and subacute studies were conducted on alloxan induced diabetic mice. Results showed that encapsulated form of synbiotics were more efficient in lowering down the blood glucose levels as compared to unencapsulated form.

Keywords: Synbiotics, Hyperglycemia, *Lactobacillus casei subsp. casei 17*, Lactulose

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INTRODUCTION

Glucose is the primary source of energy for the body's cells. Normally in mammals, the body maintains the blood glucose level at a reference range between about 80-120 mg/dl. Diabetes goes from 7th leading cause of death to 5th leading cause of death. It is evident from the figures that in 2011, diabetes accounted for about 4.6 million deaths worldwide.

Synbiotics refer to nutritional supplements combining probiotics and prebiotics in a form of synergism [1]. Prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health [2]. Lactulose is a disaccharide comprising of fructose and galactose and is used in treatment of chronic constipation. Lactulose is also a food ingredient, better known as galactofructose, with sweet taste and offering beneficial health benefits on digestive health. It is the energy source for beneficial saccharolytical bacteria like *Bifidobacteria* and *Lactobacilli* which can metabolise it to produce short-chain fatty acids. Galactofructose acts as a prebiotic within the colonic microflora, increasing numbers of bifidobacteria and improving transit time in healthy volunteers; it is the bifidogenic effect [3]. Lactic acid bacteria (LAB) and bifidobacteria are the most common types of microbes used as probiotics and are commonly consumed as a part of fermented foods with specially added active live cultures [4,5].

The currently available oral antihyperglycemic agents for clinical use have characteristic profile of side effects. Management of diabetes with agents devoid of any side effects is still a challenge to the medical system. This has led to an increase in the demand for products with antihyperglycemic activity having fewer side effects.

Synbiotics are a major field of attention now days as help confer protection against various diseases without any potent side effect. Functional foods are commonly used to modulate the composition of the gut microbiota or stimulate the growth and activity of bacteria in the digestive system contributing to the maintenance of the host health or prevention of disease.

In experimentations, synbiotics are either given in encapsulated or unencapsulated form [6]. Hence the main objective of the study was to determine which of the two forms i.e. encapsulated or unencapsulated; would be more potent of decreasing the blood glucose levels in alloxan induced diabetic mice.

MATERIALS AND METHODS

Strains of microorganisms
Strains of *Lactobacillus casei subsp. casei 17* was procured from National Dairy Research Institute (NDRI), Karnal, Haryana. The culture so obtained was revived in the de Man–Rogosa–Sharpe broth (MRS broth) at 37 °C. The bacterial culture was grown and maintained for further use.

Animals
Swiss albino male mice (18-22 g) which were maintained on a standard laboratory diet (Kisan Feeds Ltd., Mumbai, India) and water *ad libitum* were employed in the study. The animals were divided into respective groups each of a maximum of six animals, were housed individually in the departmental animal house and were exposed to 12 hr cycles of light and darkness.

Experiment: Induction of diabetes
Alloxan (1,3-Diazinane-2,4,5,6-tetrone), a glucose analogue was injected intra-peritonially at the interval of 24 hours, for 3 subsequent days for the induction of diabetes at the rate of 50 mg/kg b.wt. per dose, i.e. the total alloxan introduced was 150 mg/kg b.wt [7,8,9].

Glucose level was checked with the help of Glucometer (Ascentia Entrust) on the 4^{th} day, by taking blood from the tail vein. Animals having blood glucose level more than 120 mg/dl were selected for the study.

The Experimental Animal Design:
The animals were grouped according to the following scheme:

Group I: (Untreated control) Mice were fed the basal feed only.
Group II: (HG + drug) Hyperglycemic mice which were treated with Glibenclamide (10 mg/kg b.wt.) with normal diet.
Group III: (HG + CES) Hyperglycemic mice which were dosed with co-encapsulated synbiotics (2:2 ratio of prebiotic and probiotic at the rate of 10^9 cells/ day/ mouse)
Group IV: (HG + UES) Hyperglycemic mice which were dosed with unencapsulated synbiotics (2:2 ratio of prebiotic and probiotic at the rate of 10^9 cells/ day/ mouse)

Follow up of the experiment:
The animals received an oral dose of 150 µL of either constituents {LB 17 (10^9 cells/ml) and Lactulose} for 30 days.

Acute study: The blood glucose level was checked on 1^{st} day of dosing for acute study at 0^{th} hr, 2^{nd} hr, 4^{th} hr, 6^{th} hr, and 24^{th} hr for the acute study to observe effects of doses on the levels of blood glucose.

Subacute study: Subacute study was carried out for 20 days checking blood glucose levels on 6^{th}, 9^{th}, 13^{th}, 16^{th} and 20^{th} day to check the significant decrease in blood glucose levels among all the .

Analysis of Blood Glucose:
For acute and subacute studies, the blood sample was taken on the above said days from tail vein[10]. Glucose levels were checked using Glucometer from Entrust Ascentia.

Statistical Analysis: Results were expressed as Mean ± Standard Deviation (SD).

RESULTS

ACUTE STUDY: Acute studies showed a maximum decrease in blood glucose levels after 6 hours which is the peak of effect, but after 24 hours, the levels started increasing again. This is due to the fact that the effect of single

dose remains for initial 6 hours but the effect is decreased after 24 hrs as the mice were devoid of any treatment dose. The blood glucose level was reduced by 37% by encapsulated synbiotics as compared to 32.3% by unencapsulated synbiotics and 32% by glibenclamide [Table 1(a) and Figure 1(a)].

SUBACUTE STUDY: Subacute studies showed that the blood glucose levels of animals kept on decreasing and became stable till the 20th day. This continuous decrease in blood glucose level is due to subsequent doses given at the intervals of 24 hrs. This leads to more sustained results. Encapsulated synbiotics lead to a decrease in blood glucose level by 54% whereas unencapsulated synbiotics lead to a decrease by 51% and 46% by glibenclamide [Table 1(b) and Figure 1(b)].

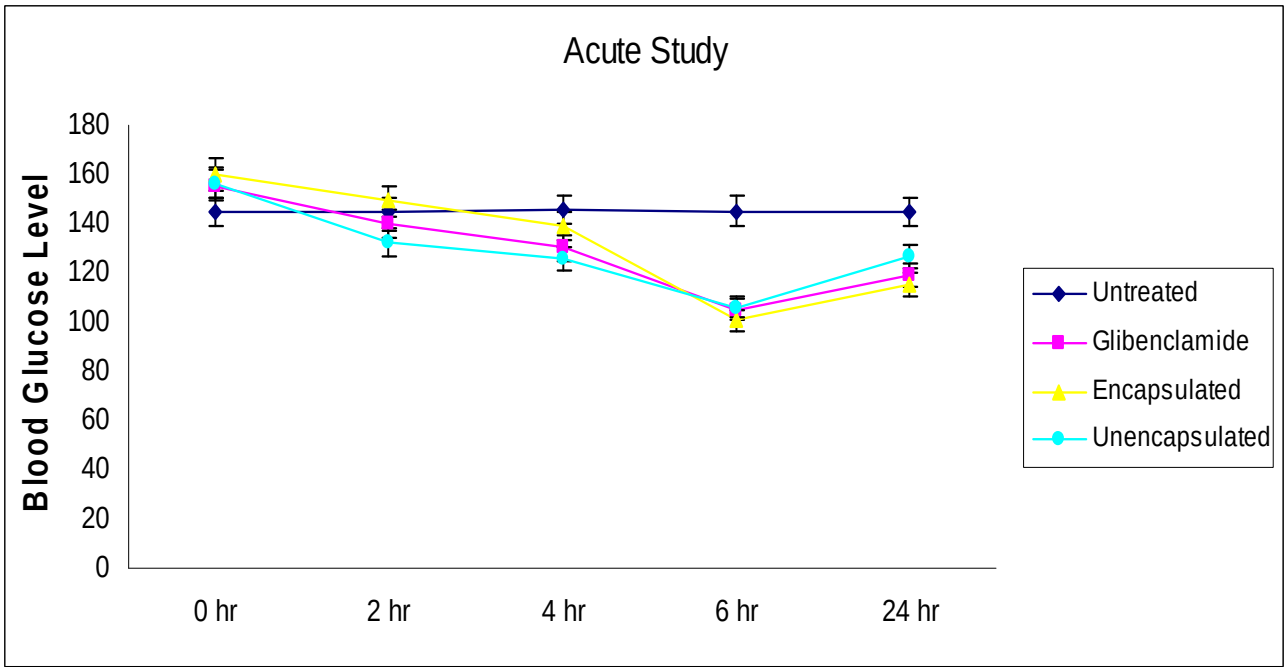
Table 1(a): Acute Study

ACUTE STUDY	0 hr	2 hr	4 hr	6 hr	24 hr
Untreated	145±5.54	144.3±6.37	146±6.62	145.2±5.81	144.8±6.25
Glibenclamide	155.3±5.03	140±3.37	130.1±3.08	105±3.43	119.1±4.95
Encapsulated	160±7.03	149.2±5.76	139±4.54	100.5±3.76	115.1±4.79
Unencapsulated	156.6±3.14	132±2.65	125.5±2.94	106.1±1.47	126.6±4.03

Table 1(b): Subacute Study

SUBACUTE STUDY	BEFORE	6th day	9th day	13th day	16th day	20th day
Untreated	92.8±6.32	158±6.25	148.2±6.87	150.6±5.92	149.9±5.75	153.1±6.52
Glibenclamide	102.3±2.45	160.6±3.43	121.1±2.09	118.8±1.94	94.6±2.36	85.3±2.60
Encapsulated	110±3.42	170.3±2.42	121.3±4.50	108.5±3.38	89.6±3.50	78±3.26
Unencapsulated	99.1±3.34	168±1.75	124.8±1.89	115.3±3.72	101±4.24	84.3±3.86

Figure 1(a): Acute Study



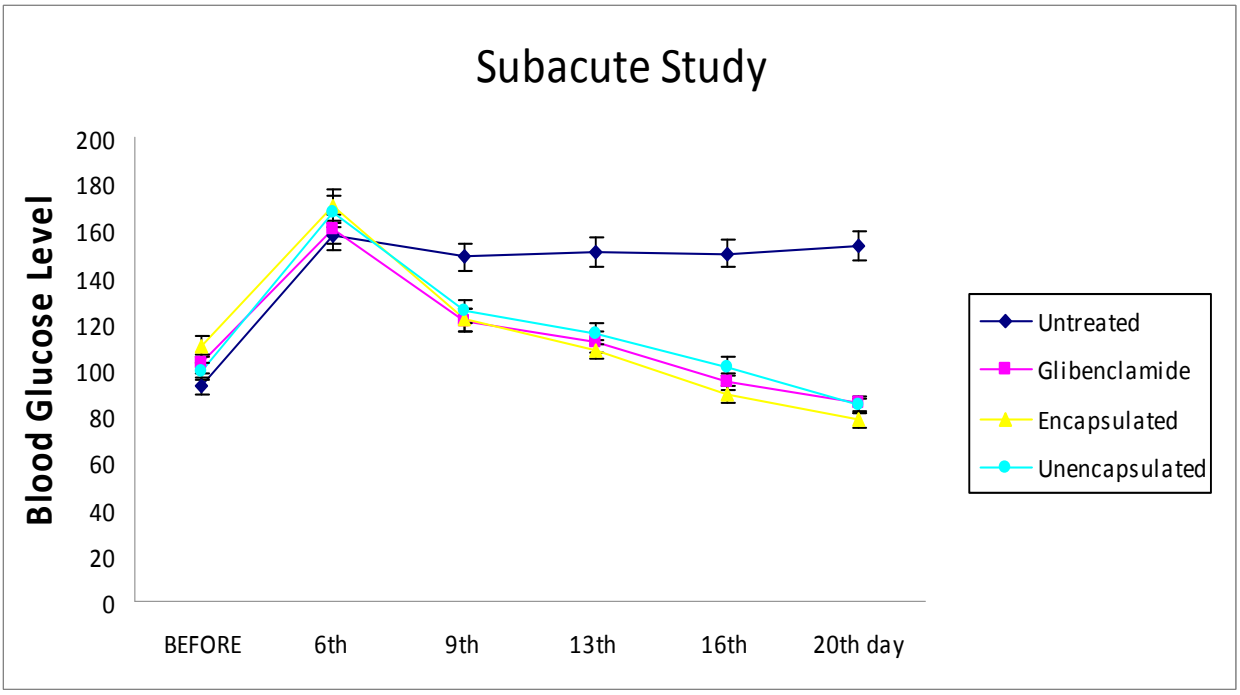


Figure 1(b): Subacute Study

DISCUSSION

Diabetes is a fatal disease which results from increased blood glucose levels beyond the normal range. There occur millions of premature deaths worldwide caused by diabetes and is the 3rd leading cause of death after cancer and cardiovascular diseases[11,12,13].

Diabetes was induced by using alloxan in the animals. Alloxan (1,3-Diazinane-2,4,5,6-tetrone) is an oxygenated pyrimidine derivative. It is a glucose analogue, which selectively destroys insulin-producing beta cells in the pancreas. This causes an insulin-dependent diabetes mellitus; called Alloxan Diabetes which is similar to type 1 diabetes in humans. Alloxan accumulates in beta cells through uptake via the GLUT2 glucose transporter. In the presence of intracellular thiols, it generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid. The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction. One study suggests that alloxan does not cause diabetes in humans. Others found a significant difference in alloxan plasma levels in children with and without diabetes Type 1.

Diabetes could be produced in the animals only when the blood GSH level could be lowered. When alloxan is administered it undergoes destruction partly by the glutathione of the blood and partly by other mechanisms. When the dose is high, some of it reaches the P cells of the pancreas and reacts with the glutathione of these cells and even causes cell death by reacting with the sulphydryl groups of the protein and the enzymes. It is, therefore, expected that any substance which would decrease GSH in the body would also increase the susceptibility to alloxan [14].

The exact mechanism of synbiotic action in altering gut or gastric microflora is not known. Although prebiotics which are non-digestible food ingredients, stimulate the growth and activity of bacteria in the digestive system which are intended to colonize the gut and large intestine and confer physiological health benefits to the host [15].

Hence, the current study conducted lead to results showing 54% decrease in blood glucose levels by the encapsulated synbiotics which is higher than the unencapsulated form (51%). Also encapsulated form shows a slightly greater potential in lowering blood glucose levels than glibenclamide (46%) which otherwise can have side effects too.

This effect shown by encapsulated synbiotics is due to the fact that these are more target oriented and safer as compared to commercial drugs available and also they have a positive effect on the immune system of an individual.

CONCLUSION

It is concluded that coencapsulated synbiotics are more potent in decreasing the blood glucose levels of induced diabetic mice as compared to unencapsulated form and Glibenclamide. So, prebiotics in combination with probiotics can prove to be very effective to help fight various disorders when used in food applications or neutraceuticals formulations and therefore people can shift to these more natural products which have less or no side effects as compared to commercially available drugs.

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