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Laxative Properties of Bacterial Cellulose Isolated from *Gluconacetobacter xylinum* sju-1 against Loperamide Induced Constipated Sprague-Dawley Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author GG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author RM managed the analyses and the literature searches of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the laxative properties of bacterial cellulose (BC) isolated from *Gluconacetobacter xylinum* sju-1 against loperamide induced constipated Sprague-Dawley rats.
Study Design: Completely Randomised Design (CRD).
Place and Duration of Study: Department of Agricultural Microbiology,Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu and Kovai Medical College Hospital, KMCH College of Pharmacy, Institute Animal House Facility, Coimbatore, Tamil Nadu between March 2013- April 2014.
Methodology: The efficacy of colloidal formulation of BC obtained from *Gluconacetobacter xylinum* sju-1 was studied against loperamide-induced constipation in Sprague-Dawley rats. The

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test laxative, namely colloidal bacterial cellulose was orally administered at levels based on body weight of animals. Isabgol, a commercial plant based natural laxative obtained from *Psyllium* husk was used to compare the laxative effect of colloidal bacterial cellulose laxative. The rats were randomly divided into 4 groups. Prior to the initialisation of the experiment, constipation was induced to all the 4 groups by administration of 0.15 mg of loperamide $100g^{-1}$ of body weight twice a day for 5 days. Then the laxatives were administered twice a day orally to the constipated rats for the period of 7 days. Feed and water intake were measured for each group for 24 h period. Faeces were collected from each groups daily at 9.00 h and measured for wet weight.

Results: Among the four groups tested, Group I and III had higher feed intake (20.21±3.39 g and 20.21±1.21 g) compared to Group II (19.99±1.26 g) and IV (19.58±1.52 g). Water intake was found to be higher in Group II and Group IV. Significantly lower water uptake was recorded in Group I followed by Group III. Number of faecal pellets, a most important parameter dealing with the laxative property of the bacterial cellulose was higher in group IV, which excreted 46.34±2.55 number of faecal pellets followed by Group II (44.89±2.36).The animals that fall in group IV recorded a faecal pellet weight of 16.87±54 g with the moisture percent of 2.94±1.24 followed by Group III, II and Group I recorded the least values for number of faecal pellet, moisture content of faecal pellet (%) and weight of faecal pellet of 29.66±1.28, 0.77±1.24 and 3.93±0.54 g of respectively.

Conclusion: Bacterial cellulose increased weight of faeces, water content of faeces, and promoted satiety feeling, and acted as laxative agent by holding water inside the bowel lumen, inhibition of water absorption in the colon and stimulating colonic motility.

Keywords: Bacterial cellulose; Gluconacetobacter xylinum; laxative effect; faecal properties; Sprague Dawley rats.

1. INTRODUCTION

The prevalence of constipation is a common problem and has been reported to be as high as 20%, especially in the elderly. Studies have shown that if people only have a bowel movement every 3-4 days, they are more at risk for having colon cancer, hemorrhoids and irritable bowel disease (IBS) [1]. Most remedies for constipation focus on modulating the motility of the gastrointestinal (GI) tract, which has been reported with severe side effects. For examples, cisapride, a first generation of promotility agent [2,3] was withdrawn because of safety concerns because it induced cardiac arrhythmias [4]. Tegaserod, a selective 5 HT_4 against, a most widely used medicine in the treatment of chronic constipation has been reported to cause coronary spasm, coronary artery contraction and even myocardial infarction [5,6]. Recent advances in research on the gastrointestinal pathology and physiology have paved the way for innovative new approaches to the treatment of patients with chronic constipation.

Cellulose is found in abundance in nature and it is virtually found in all plant tissues and is therefore a common component of our diet. Modified celluloses such as ethyl and carboxy methyl derivatives and methyl cellulose (which may be prescribed as a laxative or as an appetite suppressant), have very different chemical properties from pure cellulose. Their substituent groups disrupt the hydrogen bonding and the resulting compounds are more soluble. Dietary cellulose is thought not to be digested in the stomach and small intestine. But 85 per cent of cellulose was recovered from ileostomy contents of the test rats. In the large intestine however, it is fermented by the microflora with the ultimate production of short chain fatty acids, hydrogen, carbon dioxide and methane [7].

The use of various hygroscopic celluloses and hemicelluloses has gained a wide use in the treatment of constipation. Normally celluloses have stool bulking and laxative effect. Cellulose based laxatives act by release of lower volatile fatty acids and primarily based on a chemical and not by mechanical stimulation. Dietary fibre increases the faecal weight associated with the improvement of undesirable constipation, which can be mediated through the water-holding capacity of unfermented fibre [8]. Laxative effect of cellulose is due to the hygroscopic nature, which enables to imbibe and hold water in the intestinal tract and increases the quality and quantity of stools. In addition to the mechanical stimulus due to distention, the biochemical end product which arises from the decomposition of cellulose by intestinal microorganisms stimulates the evacuation of bowel. More the number of

pentose sugar in the laxative, the effect of bowel evacuation is more [9].

In 1972, the US Food and Drug Administration declared cellulose to be generally recognised as safe (GRAS). In animal studies conducted by Freeman et al. [10] cellulose was found to prevent colon cancer. Costerton et al. [11] has illustrated that the mode of action of cellulose in the large intestine is probably related to its digestibility, because the water holding capacity of the material is very limited. Breakdown of cellulose in the colon stimulates microbial growth, while any undigested cellulose provides a surface for bacteria, which may lead to the growth of specialised subpopulations and fermentation of cellulose, which requires a complex interaction of microorganisms that eventually produces short chain fatty acids. Montgomery [12] have indicated that cellulose is an inert material. In humans, bulk doses up to 30 g per day can be used as a laxative. In experimental animals, no reproduction toxicity was found in rats and mice after lifetime cellulose dosing in the diet.

Hillman et al. [13] have reported that doses of purified cellulose from 15 to 20 g day^{-1} given to volunteers in long term feeding studies lead to modest increase in stool output, shortening of transit time and a fall in stool pH. Cellulon^{TM} fibre is a cellulose produced by bacterial fermentation process employing a strain of *Acetobacter aceti* subsp. *xylinum* and it most closely resembles powdered and microcrystalline cellulose. Experiments revealed that Cellulon^{TM} was not a skin and eye irritant in rabbits [14]. Lee and Hwang [15] reported that cellulose increases faecal excretion by increasing water content and bulk, and elevating viscosity, and both water soluble and insoluble cellulose increases faecal egestion. Faecal water content and volume were increased by eating fibre that does not decompose and is not digested by coliform bacilli. Lee [16] reported that intestinal transit time was diminished following administration of food-mixed with cellulose and 5 per cent guar gum, compared with rats given normal food. Moreover, cellulose increases faecal egestion and reduces intestinal transit time, as well as reducing faecal sojourn time within intestines. Mesomya et al. [17] studied on the comparative effects of 6 different high dietary fibre diets and casein diet on protein bioavailability (protein efficiency ratio, net protein utilization, biological value and digestibility) in male Sprague-Dawley rats. The results indicated the highest dietary fibre diet (>10 per cent) showed the highest content of faeces and decreased the value of net protein utilization and digestibility.

Mesomya et al. [18] reported that feeding of *nata* decreases serum lipids in human and have indicated that 40 per cent of *nata-de-coco* effectively lowered serum triglycerides in human. Hagiwara et al. [19] conducted studies on the oral toxicity of fermentation derived cellulose to F344 rats and proved that bacterial cellulose did not show any adverse effect when both the sexes of rats were fed with dietary concentration upto 5 per cent level and no toxicity was observed in any of the organ of rats. Little medication for constipation is now available for promoting the secretary activity of the GI tract and colonic motility safely. Therefore the objective of the present study was to evaluate the laxative properties of bacterial cellulose isolated from *G. xylinum* sju-1 against loperamide induced constipated Sprague-Dawley rats model.

2. MATERIALS AND METHODS

2.1 Experimental Animals and Housing Conditions

Animal experiments were carried out based on the ethical guidelines laid down by the committee for the purpose of control and supervision of experiments on animals by the Government of India, Ministry of Social Justice and Empowerment.

Three weeks old weanling male Sprague-Dawley rats, with a mean initial weight of 50-60 g, mean body weight within a group not different than 10 g and among groups not more than 5 g, were obtained from the Kovai Medical College Hospital, (KMCH College of Pharmacy, Coimbatore, Tamilnadu and maintained in the Institute animal house facility. The Institute is recognized for animal studies by the ethical committee (IAEC No: 13621/2014). The animals were assigned to four groups, all consisting of 6 animals of each group. The animals were housed in polycarbonate cages (six rats per cage) on soft chip bedding, which was changed thrice per week. For drinking water, tap water was provided. Rats were given free access to diet and water. They were housed in a room maintained at 25 ± 2℃ with a relative humidity of 60 to 70 per cent and exposed to a light and dark cycle of 12 h duration. Rats were fed with commercial diet (M/s. Amrut – laboratory animal feed, Pranav Agro Industrial Ltd.) obtained from Bangalore.

2.1.1 Experimental design

The studies on laxative properties were designed as per the methods prescribed by Wintola et al. [20] with little bit modification as follows

2.1.1.1 Colloidal bacterial cellulose laxative preparation

The test laxative, namely colloidal bacterial cellulose was orally administered at levels based on body weight of animals. To prepare BC in colloidal form, the cellulose obtained from *Gluconacetobacter xylinum* sju-1 was freeze dried and made into a fine powder. To 100 ml of sterile water, 9:1 ratio of fine powder of freeze dried BC and sterilized guar gum was added to make into a colloidal form. The prepared formulation was stored in sterilized vials at room temperature. Isabgol, a commercial plant based natural laxative obtained from *Psyllium* husk was used to compare the laxative effect of colloidal bacterial cellulose laxative.

2.2 Development of Constipation and Grouping of Animals

The efficacy of colloidal formulation of bacterial cellulose obtained from *Gluconacetobacter xylinum* sju-1 was studied against loperamide lab-induced constipation in Sprague-Dawley rats. The rats were randomly divided into 4 groups. Prior to the initialisation of the experiment, constipation was induced to all the 4 groups by administration of 0.15 mg of loperamide $100g^{-1}$ of body weight twice a day for 5 days. During the development of constipation, parameters such as feed intake, water intake, faecal numbers, water content of faecal pellet and weight of faecal pellet was quantified for the normal control fed with 0.9 per cent saline and constipation induced rats for 5 days. The passage of reduced, hard and dry faecal pellets indicated constipation in the rats. Then the laxatives were administered twice a day orally to the constipated rats for the period of 7 days.

Group-I: Constipation induced rats without any laxative treatment (negative control).

Group-II: Constipation induced rats fed with Isabgol laxative twice a day at the rate of 2.5 ml per dose (positive control).

Group-III: Constipation induced rats fed with colloidal bacterial cellulose laxative twice a day at the rate of 2.5 ml per dose.

Group-IV: Constipation induced rats fed with colloidal bacterial cellulose laxative twice a day at the rate of 5.0 ml per dose. Feed and water intake were measured for each group for 24 h period. Faeces were collected from each groups daily at 9.00 h and measured for wet weight. The dry weight was recorded after keeping it at 60℃ overnight, and the moisture per cent of the faeces were calculated.

The experimental design used was Completely Randomized Design (CRD) and the data was analyzed using Agres, Agdata software.

3. RESULTS AND DISCUSSION

3.1 Testing of Laxative Properties of Bacterial Cellulose by Animal Studies

The effect of loperamide on feed, water intake and faecal properties of constipated Sprague-Dawley rats were studied and the results are presented in the Table 1. The constipated rats had more feed intake of 21.24±0.87 g compared to the normal control (21.24±0.87 g). Whereas water intake was significantly higher in the normal control (24.00±1.47 ml) than the constipated rats.Number of faecal pellets increased twice (70.77±3.00) in normal control producing a faecal weight of 7.12±0.63 g than the constipated rats which recorded 30.55±4.00 pellets with the faecal weight of 3.19±0.44 g. Similarly the moisture content of the faecal material was higher in the normal rats compared to the constipated ones. The effect of colloidal bacterial cellulose laxative on feed intake, water intake and faecal properties of constipated rats were carried out and were presented in Table 2.

Fig. 1. shows the oral administration of colloidal bacterial cellulose laxative to a Sprague-Dawley rat. Among the four groups tested, Group I and III had higher feed intake (20.21±3.39 g and 20.21±1.21 g) compared to Group II (19.99±1.26 g) and IV (19.58±1.52 g). Water intake was found to be higher in Group II and Group IV. Significantly lower water uptake was recorded in Group I followed by Group III. Number of faecal pellets, a most important parameter dealing with the laxative property of the bacterial cellulose was higher in group IV, which excreted 46.34±2.55 number of faecal pellets followed by Group II (44.89±2.36). Similar results were obtained with respect to the moisture content and faecal weight. The animals that falls in group IV recorded a faecal pellet weight of 16.87±54 g with the moisture percent of 2.94±1.24 followed by group III rats which recorded 15.23±1.14 g

with the moisture per cent of 2.15±1.11. Group II also recorded a very closer faecal weight of 15.21±0.65 g with a moisture per cent of 2.00±1.23. Group I recorded the least values for number of faecal pellet, moisture content of faecal pellet (%) and weight of faecal pellet of 29.66±1.28, 0.77±1.24 and 3.93±0.54 g of respectively. Fig. 2. shows the faecal differences between the different treatment groups.

Fig. 1. Oral administration of colloidal laxative bacterial cellulose in Sprague-Dawley rats

The results showed that loperamide administration induced severe experimental constipation in rats. The faeces content was decreased by loperamide. The water content in faeces as a constipation parameter was also decreased by loperamide. On the other hand, the dry weight of faeces was not significantly different among the groups. And also the feed intake was not significantly different between the control and constipated animals. This represents that the amount of food consumption might have been similar among the groups, and constipation was caused by delayed intestinal movement. Both the Isabgol and the colloidal laxative of BC treated rats for 7 days recovered from the loperamide induced constipation. This was evidenced by the faecal weight of the treated rats.

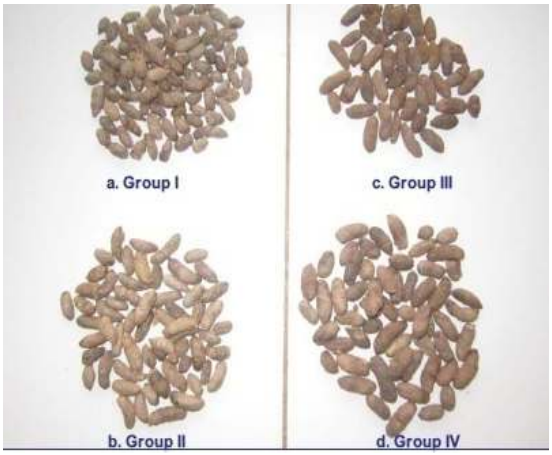

Fig. 2. Fecal pellets of Sprague-Dawley rats fed with colloidal bacterial cellulose laxative

Table 1. Effect of loperamide on feed intake, water intake and faecal properties of constipated rats

S. No	Parameters	Normal control	Constipated rats
1.	Feed intake (g)	20.21± 1.23	21.24±0.87
2.	Water intake (ml)	24.00±1.47	13.21±1.21
3.	Number of faecal pellet	70.77±3.00	30.55±4.00
4.	Moisture content of faecal pellet (%)	1.99±0.45	0.68±0.15
5.	Weight of faecal pellet (g)	7.12±0.63	3.19±0.44
	SD	0.56	0.35
	CD (0.05)	1.33	0.72

Data are mean ± SD of three measurements

Table 2. Effect of colloidal bacterial cellulose laxative on feed and water intake and faecal properties of constipated rats

S. No	Parameters	Group I	Group II	Group III	Group IV
1.	Feed intake (g)	20.21±3.39	19.99±1.26	20.21±1.21	19.58±1.52
2.	Water intake (ml)	12.38±1.11	19.99±2.65	17.57±1.77	18.54±2.34
3.	Number of faecal pellet	29.66±1.28	44.89±2.36	40.63±2.00	46.34±2.55
4.	Moisture content of faecal pellet (%)	0.77±1.24	2.00±1.23	2.15±1.11	2.94±1.24
5.	Weight of faecal pellet (g)	3.93±0.54	15.21±0.65	15.23±1.14	16.87±54
SEd		0.27	0.39	0.37	0.41
CD (0.05)		0.61	0.88	0.82	0.91

Data are mean ± SD of three measurements

Group I: Constipation induced rats without any laxative treatment (negative control).

Group II: Constipation induced rats fed with Isabgol laxative twice a day at the rate of 2.5 ml per dose (positive control).

Group III: Constipation induced rats fed with colloidal bacterial cellulose laxative twice a day at the rate of 2.5 ml per dose.

Group IV: Constipation induced rats fed with colloidal bacterial cellulose laxative twice a day at the rate of 5 ml per dose.

In the present study, faecal content and moisture percent was found to be greater compared to the constipated rats. Basically, BC laxative increased the weight of the stools and also the moisture content. The present findings on the effect of colloidal laxative of bacterial origin correlates with the studies of Kim et al. [21] who have established that probiotic cultures at 1×10^6 Colony Forming Units per milliliter administered for 5 days partially recovered or prevented constipation in the loperamide induced constipated rats. The laxative studies of bacterial cellulose conducted in the present study highly correlated with the findings of Kakino et al. [22] who have demonstrated that ethanol extract of *Aquilaria sinensis* (EEA) increases both the intestinal tension of the small intestine, stool frequency and weight of stools, and the rate of gastrointestinal transit.

The present study was in agreement with Hong-Geun et al. [23] that constipation can be relieved by the intake of fibre rich foods and natural laxatives like fig which contains more amount of cellulose that relieves chronic constipation. Mikkelsen et al. [24] have evaluated the use of methyl bacterial cellulose as a colloid laxative and evidenced that at the dose of 10 g day^{-1}, methyl cellulose doubled the volume of the stools.

4. CONCLUSION

In the present study, laxative formulation prepared using bacterial cellulose obtained from *G. xylinum* was found to have stimulatory effect on bowel evacuation and increased the stool weight in the experimental rats. Xanthum gum, a polysaccharide of bacterial origin namely *Xanthomonas campestris* has been used in the food and pharmaceutical industry for over years. Likewise the pure form of cellulose obtained from the *Gluconacetobacter xylinum* sju–1 will certainly find its application as pharmaceutical incipients or directly as high dietary fibre food.

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. (IAEC NO: 13621/2014).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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