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Research article

Leucaena leucocephala succinate based polyelectrolyte complexes for colon delivery of synbiotic in management of inflammatory bowel disease

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ABSTRACT

Polyelectrolyte complexes (PECs) formed by the interaction between oppositely charged polymers have emerged as promising carriers for accomplishing colon-specific release. In this study, we have explored the potential of polyelectrolyte complexes between a succinate derivative of *Leucaena leucocephala* galactomannan and cationic guar gum for colon delivery of synbiotic. The PECs were prepared using a polyelectrolyte complexation method and characterized. The PECs exhibited excellent stability, with high encapsulation efficiency for both probiotics (95.53 %) and prebiotics (83.33 %). *In vitro* studies demonstrated enhanced survivability and proliferation of the encapsulated probiotics in the presence of prebiotics (93.29 %). The SEM images revealed a smooth and firm structure with reduced number of pores when both prebiotic and probiotic were encapsulated together. The treatment with synbiotic PECs in acetic acid induced IBD rats significantly relieves colitis symptoms as was evident from colon/body ratio, DAI score and histopathology studies. An increase in the protein and reduced glutathione levels and reduction in superoxide dismutase activity was observed in colitic rats that received synbiotic treatment as compared to colitic rats. Overall, this study highlights the potential of *Leucaena leucocephala* succinate-cationic guar gum PECs as a promising system for colon-specific synbiotic delivery, with implications for improved gut health and the treatment of various gastrointestinal disorders.

1. Introduction

The gut microbiota, comprising of trillions of microorganisms, aboriginal to the human gastrointestinal (GI) tract, plays a pivotal role in regulating immune responses, facilitating the absorption of nutrients and maintaining the overall health. Any disruption or imbalance in the composition and function of the gut microbiota, often referred to as dysbiosis, manifests itself in the form of different

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diseases and conditions, including inflammatory bowel diseases (IBD), obesity, and metabolic syndromes [1]. IBD (Crohn's disease and Ulcerative colitis) is associated with the alterations in the gut microflora leading to dysbiosis. Studies have demonstrated an imbalance between commensal and pathogenic bacteria and reduced microbial diversity leading to inflammation and dysfunctional mucosal barrier [2]. Therefore, approaches of modulating the gut microbiota with interventions such as targeted delivery of probiotics, prebiotics and their combination (synbiotic) maybe explored for their therapeutic applications [3].

The colon is a major site of microbial fermentation and provides an ideal environment for the proliferation and activity of beneficial microflora. Probiotics refer to live microorganisms which when administered to the host in sufficient amount have a positive health impact. Probiotics exert their beneficial effects in IBD by upregulating the antioxidative defense system of the body, via immunomodulation and activating the intestinal barrier [3]. *Lactobacilli* and *Bifidobacterium* form the major class of probiotics explored in IBD. Various strains of *Lactobacillus fermentum* are reported to possess antioxidant and antimicrobial property which makes it a suitable candidate as a probiotic in treatment of IBD [4]. *L. fermentum* (MTCC9748) is gram positive, lactic acid producing bacteria and is one of the anti-oxidative strains of *lactobacilli* that are more tolerable to oxygen. Prebiotics are non-digestible carbohydrates that facilitate the growth of probiotics by acting as nutritive substrates. Lately, prebiotics have fascinated the scientists for their promising therapeutic role in IBD. Prebiotics present a distinct approach for modulating the gut microbiota and mitigating the dysbiosis associated to IBD [3,5]. Fructooligosaccharides (FOS) is one of the most extensively explored prebiotic along with inulin and other oligofructose. It is short chain polymer of fructose units that can resist hydrolysis in small intestine, but is fermented by resident flora in colon [6]. A combination of probiotic and prebiotic (synbiotic) offers a comprehensive strategy to regulate the gut microflora and potentially alleviate the symptoms associated with IBD. Synbiotics helps to enhance the survival, colonization, and metabolic activity of probiotics in the colon, thereby maximizing their therapeutic potential. The dual approach can positively impact both the function and composition of gut microbiota. Colon targeting of synbiotics is a very promising approach that delivers probiotics with prebiotics in a synergistic manner. However, a number of challenges are encountered during the sojourn of probiotics through the gastrointestinal tract (GIT) that result in considerable reduction of probiotic viable count before it reaches the colon. These include exposure to acidic pH, gastric and bile fluids during transit, oxygen intolerance, processing factors and storage stability. Some of these limitations can be overcome by microencapsulating the probiotics in polymers that can protect the microflora [7].

Polysaccharides are promising biocarriers that hold considerable potential for colon targeting. The functional properties of polysaccharides mainly depend upon the molecular structure, nature of the glycosidic bond, degree of branching and flexibility and confirmation of the chains [8]. Furthermore, several scientists have carried out molecular modification or derivatization of polysaccharides to confer them desirable functional properties [9,10].

Galactomannans are heterogeneous linearly branched polysaccharides that represent the second largest group of storage seed polysaccharide. Galactomannans such as fenugreek gum, guar gum, tara gum and locust bean gum have been widely used in colon targetd drug delivery since they are easily degraded by the microflora in the small intestine [8]. *Leucaena leucocephala* galactomannan (LLG) is a polysaccharide extracted from the seed endosperm of *L. leucocephala* and chemically composed of linear chain of mannose units substituted with single α-D-galactose units in a ratio of 1.19:1. Studies have demonstrated considerable potential of LLG as an excipient for drug delivery [11]. To our knowledge not much work has been done using LLG for colon targeting. LLG is soluble in water, thus it cannot provide protection in gastric environment. The incorporation of succinyl group introduces an anionic character to the galactomannan so that it can form ionic complex (polyelectrolyte complex) with cationic gums [12].

Cationic guar gum (CGG) is a cationic derivative of guar gum in which hydroxyl groups are substituted with trimethyl ammonium groups. The presence of ammonium groups imparts a net positive charge to CGG and thus it can easily cross-link with other anions. The pka of CGG as determined in our earlier studies lies in the range of 6–7 [13].

Various studies have demonstrated the potential of polyelectrolyte complexes (PECs) in colon targeting. The cross linking or complexation of two oppositely charged polymer alters the solubility and swelling degree of polymers and makes them potential carriers for controlled release applications [14].

The present study was envisaged to design colon targeted delivery of synbiotic (*L. fermentum* and FOS) by co-encapsulation in a polyelectrolyte complex formed between natural polymers. The first part of the study comprised of derivatization and characterization of succinyl derivative of LLG. In the next step polyelectrolyte complexes (PECs) using *Leucaena leucocephala* succinate (LLS) and cationic guar gum (CGG) were prepared and characterized. The complexes were loaded with probiotic bacteria and synbiotic system. *In-vitro* release studies were conducted to determine their suitability in delivering desired viable count of *L. fermentum* to colon. The pharmacodynamic studies were performed to evaluate the therapeutic efficacy of the synbiotic loaded PECs in alleviating symptoms in IBD induced rats.

2. Materials and methods

2.1. Materials

Leucaena leucocephala seeds were obtained from Green-field Agro Forestry Products, Madhya Pradesh. Fructooligosaccharide (FOS) was kindly gifted by Jay Chem Marketing, Mumbai. *Lactobacillus fermentum* (MTCC 9748), was acquired from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Sector 29, Chandigarh (India). Isopropyl alcohol was procured from Merck India Limited, Mumbai. DMAP and SA were procured from SD fine Chemicals Ltd., Mumbai. Cationic guar gum (CGG) (Mw 100000–500000Da) was a gift sample from Encore Pharmaceuticals, Gujarat.

2.2. Extraction of Leucaena leucocephala galactomannan (LLG)

The extraction of galactomannan (LLG) from *L. leucocephala* seeds was performed as per the method already reported in our earlier study [11]. The seeds were washed with water, air dried and then crushed. The crushed seeds were soaked overnight in double distilled water. Thereafter, swollen seeds were subjected to homogenization (Remi equipments, Mumbai) followed by the centrifugation at 4000 rpm. The supernatant was collected and the marc so obtained was further subjected to multiple extractions with water for complete extraction of galactomannan. The galactomannan was precipitated by treatment with isopropyl alcohol (double volume) and purified by dialysis against water using dialysis membrane 70 (M. W. cut off 12 kDa) for 48 h followed by lyophilization (Macflow Engineering Pvt. Ltd., Delhi). The dried galactomannan was characterized by method already reported by our group [11].

2.3. Synthesis of Leucaena leucocephala succinate (LLS) and characterization

The succinylation of LLG was carried as per earlier reported method [15]. Briefly, the required quantity of LLG was dissolved in DMSO. 4-Dimethylaminopyridine (DMAP) was then added to this solution followed by the addition of succinic anhydride (SA) and stirring was continued at room temperature for 24 h. The molar ratio of LLG and SA was varied (1:1 to 1:15) to assess the impact of esterifying agent on degree of succinylation. The mixture was neutralized with sodium hydroxide (2 N) and the LLS was precipitated with double volume of isopropyl alcohol. The product was separated by centrifugation and purified against water using dialysis membrane 70 (M. W. cutoff, 12 kDa) for 72 h. The purified derivative (LLS) was then lyophilized and stored for further use.

The degree of succinylation (DS) was determined according to procedure reported by Seeli and Prabaharan, 2016 with slight modifications [16]. 0.1 g of LLS was kept overnight at room temperature in 25 mL of double distilled water for complete swelling. To this solution 15 mL of sodium hydroxide solution (0.1 N) was added and refluxed for 3 h. The reaction mixture was cooled and excess of alkali was titrated with 0.1 N HCl using phenolphthalein as an indicator. The DS was calculated using following equation:

$$\%\ \text{Succinyl substitution} = \frac{(V1 - V2)XMXN}{WX1000} X100$$

where, V_1 is the volume of HCl used for blank titration, V_2 is the volume of HCl required for sample titration, W is the weight of sample in grams, M is the molecular weight of SA, and N is the normality of HCl.

$$DS = \frac{162X\%\text{succinylsubstitution}}{100XM - \{(M-1)XW\}}$$

where, 162 = molecular weight of anhydroglucose unit.

Fourier Transform Infrared (FTIR) spectroscopic analysis of LLG and LLS was performed on PerkinElmer-Spectrum RX-I FTIR. The spectrum was recorded in a mid IR range of 4000–400 cm^{-1} with a resolution of 1 cm^{-1}. The thermal properties of LLG and LLS were studied by differential scanning calorimeter (EVO131, SETARAM Instrumentation France). Powdered LLG and LLS samples were heated in the range of 30–450 °C at a heating rate of 10 °C/min. The water uptake by LLG and LLS was measured gravimetrically by monitoring swelling LLG and LLS in buffers of pH 1.2 and 7.4 and recording their weight changes during swelling. The experiment was repeated thrice and the degree of swelling was calculated using the following equation:

$$\text{Degree of swelling} = \frac{\text{Mt-Mo}}{\text{Mo}}$$

where M_t denotes the weight of the swollen sample at time t and M_0 denotes the initial weight of the sample before swelling [17].

2.4. Preparation of bacterial growth curve

The growth curve of *L. fermentum* was prepared in De Man, Rogosa and Sharpe (MRS) broth. An aliquot of 1 mL overnight grown culture of *L. fermentum* was added to MRS broth and incubated at 37 °C for 48 h. The samples were collected at predefined intervals and optical density (600 nm) was determined. The growth curve was constructed by plotting a graph between OD and time.

To check the ability of *L fermentum* to utilize FOS as carbohydrate source, carbohydrate-free MRS was used as a basal growth medium. An aliquot of 1 mL overnight grown culture of *L. fermentum* was added to basal MRS-media supplemented with 1 %, 2 %, 3 % and 4 % w/v solution of sterilized FOS. A positive control i.e. glucose (2 % w/v) and a negative control (water) was used. The culture was incubated for 48 h at 37 °C. The samples were collected at predefined intervals and optical density (600 nm) and cell density (log CFU/mL) were determined. For determination of cell density, the sample of the culture was serially diluted, plated on MRS agar plates and incubated for 48 h. The number of colony forming units (CFU) was counted from each plate and cell density (log CFU/mL) at any given dilution was calculated. The specific growth rate (h^{-1}) at a particular time point was calculated using the following formula:

$$\log_{10}N_2 - \log_{10}N_1 = k\ (t_2 - t_1) / 2.303$$

where N_1 and N_2 are bacterial cell concentrations at different time point, t_1 and t_2 are the time points (h) and k is the specific growth rate (h^{-1}) [18].

2.5. Determination of antioxidant property of L. fermentum

The antioxidant property of *L. fermentum* was determined in terms of free radical scavenging activity using 2,2-diphenyl-1-picryl hydrazyl (DPPH). Ascorbic acid was used as standard antioxidant. 0.1 mL ascorbic acid solution (150–800 μg/mL) was mixed with methanol (1.9 mL) and 2 mL of DPPH (6mg/100 mL methanol. The blank solution comprised of methanol while the mixture of 2 mL methanol and 2 mL DPPH was used as control. All the tubes were vortexed and incubated for 30 min in dark. The absorbance of the sample was determined at 517 nm [19] and a standard plot was constructed by plotting ascorbic acid concentration against % DPPH scavenging activity. The overnight grown bacterial cultures were centrifuged at 5000–8000 rpm for 10 min at 4 °C and supernatant was used for the activity. The antioxidative activity of cell free extract was determined using the above procedure. The percentage free radical scavenging activity was measured using the following equation

$$\text{DPPH radical scavenging activity } \% = (\text{Acontrol} - \text{Atest})/\text{Acontrol X100}$$

2.6. Preparation and characterization of polyelectrolyte complexes (PECs)

Appropriate volumes of aqueous CGG dispersion were added to aqueous LLS dispersion to form PECs. The total polymer concentration was maintained at 1.5 % w/v. The PECs were separated after 24 h by centrifugation for 20 min at 6000 rpm. The precipitates were dried (45 °C), and the percentage yield was determined. The PECs were prepared at different ratios ranging from 10:90 to 90:10 CGG: LLS (Batch P1–P9) (Table 1). The viscosity of the supernatant obtained after removal of PECs was determined employing Brookfield viscometer LVDV-I Prime, spindleS18. The swelling studies of dried PECs particles were carried out in a sequential manner in buffers of pH 1.2 for 2 h followed by pH 7.4 for a defined period of time and the degree of swelling was calculated as discussed above.

2.7. Preparation of probiotic and synbiotic loaded PECs

L. fermentum was grown in MRS media broth at 37 °C for18 h followed by cell harvesting by centrifugation (Remi, C-24BL) under cold conditions. The supernatant was decanted, and the pellet was washed thrice with 0.9 % w/v sterilized saline followed by centrifugation [20]. The washed pellet was used for encapsulation in PECs. The cell suspension containing 10^9–10^{10} CFU/mL was prepared by adjusting the optical density to 1 at 600 nm. The probiotic loaded PECs were prepared by dropwise addition of sterilized LLS dispersion containing 10^9–10^{10} CFU/mL of *L. fermentum* cells to sterilized CGG solutions in different ratios under gentle stirring. The total polymer concentration was kept constant (1.5 % w/v). The cell loaded PECs so formed were stirred for 1 h. The different probiotic loaded PECs were designated as batch PF1–PF9 (Table 2).

For preparation of synbiotic loaded PECs, *L. fermentum* cells (10^9 CFU/mL) and 3 % or 4 % w/v FOS (Batch S1 and S2, respectively) were added to LLS solution followed by dropwise addition into CGG solution under gentle stirring. The synbiotic PECs so formed were stirred for 1 h.

All the above procedures were carried out under aseptic conditions. Subsequently, the PECs were separated by filtration. The PECs were washed twice with sterilized normal saline, centrifuged at 8000 rpm for 10 min at 4 °C for removal of any unentrapped *L. fermentum* cells and FOS [21].

2.8. Encapsulation efficiency of L. fermentum in probiotic and synbiotic loaded PECs

The viable cells after disintegration of the PECs were grown on MRS media and counted using spread-plate technique. The encapsulation efficiency was calculated using the following equation.

$$\text{Encapsulation efficiency } (\%\text{EE}) = (\text{Log}_{10}\ \text{N} / \text{Log}_{10}\text{No}) \times 100$$

where, N is the number of viable encapsulated bacteria cells and N_0 is the number of viable free bacteria cells added initially [22].

Table 1
Effect of different ratios of CGG-LLS on physical characteristics of PECs.

PEC code	CGG:LLS	Percentage yield	Viscosity		Swelling Index	
			cP	Spindle speed (rpm)	pH 1.2	pH 7.4
P1	10:90	57.31 ± 0.63	–	100	1.75 ± 0.08	0.79 ± 0.04
P2	20:80	57.92 ± 0.86	21.5 ± 1.25	100	1.63 ± 0.05	0.67 ± 0.06
P3	30:70	61.03 ± 1.22	15.5 ± 0.71	100	1.47 ± 0.06	0.58 ± 0.06
P4	40:60	66.98 ± 0.93	13.5 ± 1.75	100	1.27 ± 0.09	0.42 ± 0.04
P5	50:50	89.15 ± 0.45	10.0 ± 1.02	100	1.11 ± 0.05	0.34 ± 0.05
P6	60:40	65.57 ± 0.33	23.0 ± 0.76	100	2.75 ± 0.15	0.80 ± 0.04
P7	70:30	54.85 ± 1.53	36.5 ± 1.26	100	3.32 ± 0.10	0.96 ± 0.12
P8	80:20	42.63 ± 2.51	49.0 ± 1.06	100	4.40 ± 0.13	1.49 ± 0.33
P9	90:10	40.22 ± 2.83	55.0 ± 1.76	100	5.05 ± 0.11	2.05 ± 0.10

Table 2
Encapsulation efficiency of probiotic and prebiotic in different batches.

Formulation code	CGG: LLS	Encapsulation efficiency (%)	
		FOS	*L. fermentum*
PF1	10:90	–	78.07 ± 0.95
PF2	20:80	–	81.04 ± 0.90
PF3	30:70	–	80.93 ± 1.72
PF4	40:60	–	83.14 ± 1.04
PF5	50:50	–	91.11 ± 1.35
PF6	60:40	–	84.65 ± 1.52
PF7	70:30	–	73.29 ± 0.94
PF8	80:20	–	68.83 ± 1.75
PF9	90:10	–	58.29 ± 1.76
S1	50:50	68.55 ± 0.65	92.53 ± 1.54
S2	50:50	83.33 ± 0.97	95.53 ± 1.35

2.9. Encapsulation efficiency of prebiotic in synbiotic PECs

An indirect method was used to measure the percentage encapsulation efficiency of prebiotic (FOS) by analyzing the unentrapped FOS in the supernatant based on Selinwanoff's reaction of ketoses with resorcinol [23]. A standard plot of FOS was constructed in phosphate buffer. To 2 mL of each dilution 1 mL resorcinol reagent and 7 mL dilute hydrochloric acid were added. All the test tubes were then heated in boiling water bath at 80 °C for10 min. The test tubes were cooled under running water and the absorbance was checked at 407 nm within 30 min. Similar procedure was followed for test samples. A mixture of 2 mL water, 1 mL resorcinol reagent and 7 mL dilute hydrochloric acid, was given same treatment and used as blank [24]. The concentration of FOS present in supernatants of each formulation was determined from the standard plot of FOS ($R^2 = 0.9976$).

2.10. In-vitro release studies

2.10.1. In vitro release of L. fermentum from probiotic and synbiotic loaded PECs

The *in vitro* release studies were carried out by incubating free *L. fermentum* cells (10^9–10^{10} CFU/mL) and PECs loaded with probiotic and synbiotic (weight equivalent to10^9-10^{10} CFU) in a shaker incubator (50 rpm) at 37 °C. The release media comprised of 20 mL buffer pH 1.2 (0.2 M hydrochloric acid buffer) for 2 h followed by sequential transfer to buffer pH 7.4 (0.2 M phosphate buffer) for 3 h and then to buffer pH 6.8 (0.2 M mixed phosphate buffer) for next 19 h [25]. At predetermined intervals, 1 mL supernatant was removed and replaced with equal volume of respective buffer. The sample withdrawn at each time point was analyzed for viable *L. fermentum* cell count by serial dilutions of the supernatant in sterile 0.9 % saline and growth on MRS agar for 48 h at 37 °C [26].

2.10.2. In-vitro release studies of prebiotic from synbiotic PECs

In-vitro release of prebiotic (FOS) from synbiotic PECs was determined using dialysis bag method [27]. The synbiotic loaded PECs were placed in dialysis bags (MW cut off 12,000 Da) and suspended in a beaker containing 20 mL of buffer pH 1.2 for 2 h, the samples were then sequentially transferred to buffer pH 7.4 for 3 h and then to buffer pH 6.8 for next 19 h and stirred at 50 rpm using magnetic stirrer. At predetermined time, 1 mL sample was withdrawn from the release medium which was replaced with equal volume of respective buffer. Samples were filtered and assayed for the released FOS employing resorcinol method. Blank PECs were also treated similarly and analyzed to ensure no interference in the quantification method.

2.11. Scanning electron microscopy (SEM)

The surface morphology of the probiotic and synbiotic PECs particles (optimized batch) were examined using SEM (Jeol JSM-6610 LV). The fine PECs were placed on the sample holder using double adhesive tape. The powders were coated with gold for 70s under argon environment. The samples on the SEM grid were air dried for 10 min and images were recorded at acceleration voltage of 10 kv electron beam.

2.12. Stability of PECs

The probiotic and synbiotic-loaded PECs were kept in closed sample vials at 25 ± 2 °C and at 5 ± 2 °C for 2 months. The bacterial count during this period was determined and the viability (%) was calculated as follows:

$$\text{Viability } (\%) = \frac{(\log CFU/gXti)}{\log CFU/gXt0} X100$$

here, t_i is the time point of the evaluation, t_0 is the starting time point and CFU/g is Colony Forming Unit per g of the dry PECs.

2.13. In vivo studies

2.13.1. Animal groups

Sprague-Dawley rats (200–250 g), 8–12 weeks age were housed under ambient conditions (25 °C and a 12: 12 light-dark cycle) with free access to food and water. The experimental protocol was approved by IAEC of Punjabi University, Patiala vide protocol approval no. 107/99/CPCSEA/2017-34) and the handling and animal care was done in compliance with the National Institute of Health guidelines. The animals were divided into different groups and the treatment protocol is summarized in Table 3.

2.13.2. Induction of colonic inflammation

A 24 h fasting period was observed before induction of ulcerative colitis. Acetic acid (4 % v/v) was administered into the anus of anesthetized rat through a Teflon cannula (6 cm from the anus). After 15s, the colon was flushed thrice with normal saline (1 mL). The rats of control group received 1 mL normal saline via same method. The rats were observed for three days for the induction of ulcerative colitis [28]. The rats that gained 80–100 % of their initial weight (before induction of colitis) were chosen for subsequent studies. The rats were administered the formulations for 5 and 10 days via oral tubing and were sacrificed on 6th and 11th day of treatment and the following tests were carried out.

2.13.3. Evaluation of colonic inflammation

2.13.3.1. Disease activity index (DAI). A qualitative evaluation of the colonic damage and inflammation was assessed by disease activity index (DAI) scoring procedure starting from the first day of administering acetic acid solution till the end of the study. An average of the combined scores of bleeding, consistency of stool (diarrhea), and body weight (% difference between the body weight before and after acetic acid treatment) was used to determine DAI. A daily evaluation of all the three parameters was carried out during the induction of disease and period of treatment and scoring was carried [29].

2.13.3.2. Colon: body weight ratio. The rats were sacrificed on sixth and eleventh day of treatment. The distal colon segments (6 cm) were removed and cut longitudinally. The body weight and wet weight of the colon were recorded and colon: body weight ratio was calculated.

2.13.3.3. Histopathological studies. The mid sections of the colon were used to prepare the histopathology samples. Hematoxylin and eosin (H and E staining) of 5 μm thick longitudinal sections of colon was carried out and the stained sections were visualized under light microscope.

2.13.4. Biochemical parameters

The colon tissue was minced and homogenized in cold phosphate buffer (pH 7.4) using Teflon homogenizer. It was then centrifuged at 3000–4000 rpm for 15 min at 4 °C. The clear supernatant obtained after centrifugation was used to estimate total protein concentration [30]. The reduced glutathione (GSH) level was determined using the earlier reported method [31]. The supernatant obtained after homogenization and centrifugation of colonic mucosa and trichloroacetic acid (TCA) (10 % w/v) were mixed in equal proportions (1:1) and centrifuged at 1000 rpm for 10 min at 4 °C. The supernatant was mixed with 0.3 M disodium hydrogen phosphate and 0.001 M 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) dissolved in sodium citrate reagent (1 % w/v). The absorbance was read at 412 nm against reagent blank. The concentration of GSH in colon samples of different groups was estimated from the standard plot of reduced glutathione.

The superoxide dismutase (SOD) activity was determined employing the supernatant of the colon tissue homogenate. The reaction mixture comprised of sodium carbonate buffer, nitro-blue tetrazolium, Triton X-1100 and hydroxylamine. The absorbance was read at 540 nm and the percentage inhibition of nitro-blue tetrazolium was calculated [30].

The colon myeloperoxidase (MPO) activity was determined by a method as previously described in literature [32]. The colon mucosa scrapings were homogenized in 50 mM potassium phosphate buffer (pH 6) and sonicated in ice bath for 10s. The homogenized samples were centrifuged to obtain pellets. The pellets were mixed with solution containing 0.5 % hexadecyl trimethyl ammonium bromide (HETAB), 10 mM ethylenediaminetetraacetic acid in an ice-cold potassium phosphate buffer. The above mixtures were subjected to three cycles of freeze-thawing followed by centrifugation for 15 min at 20,000×*g*. The supernatant (100 μL) was collected and mixed with 2.9 mL of phosphate buffer containing 0.167 mg/mL *o*-dianisidine dihydrochloride and 0.0005 % hydrogen peroxide.

Table 3
Animal groups for *in vivo* pharmacodynamic studies.

Group No.	Treatment
I	Normal control
II	Colitis control
III	Probiotic solution (1 mL of 10^9 CFU/mL of *L. fermentum* cells)
IV	Synbiotic solution (1 mL containing 10^9 CFU/mL of *L. fermentum* cells and 4 % FOS)
V	PF5 (Probiotic loaded PECs)
VI	S2 (Synbiotic loaded PECs)

The activity was measured spectrophotometrically (Beckman DU 640B, California, USA) by measuring the change in absorbance at 460 nm. One unit of MPO activity is defined as that degrading 1 μL of peroxide per minute at 25 °C.

2.14. Statistical analysis

The *in vitro* data is represented as mean ± SD and *in vivo* data as mean ± SEM. Statistical analysis was done by employing one–way ANOVA followed by Tukey's (Post hoc multiple comparison) test using Graph pad prism7 software**.**

3. Results and discussion

3.1. Extraction of Leucaena leucocephala galactomannan (LLG)

The yield of LLG from the seeds of *L. leucocephala* employing aqueous extraction technique was 21.35 % w/w, which was almost equal to reported method [11]. The mannose to galactose ratio 1.19:1 and the average molecular weight 6.3×10^5 g/mol have already been reported by our group [11].

3.2. Synthesis and characterization of LLS

LLS was synthesized by esterification reaction of LLG with succinic anhydride (SA) (esterifying agent) in presence of catalyst, 4-dimethylaminopyridine (DMAP). A schematic representation of reaction for synthesis of *L. leucocephala* succinate (LLS) is illustrated in Fig. 1a.

The DS of LLS was determined by saponification of the ester linkages of LLS with sodium hydroxide and back titrating the excess sodium hydroxide with hydrochloric acid. An initial increase in esterification (DS) was observed with an increase in mole ratio of SA to LLG (from 1:1 to 10:1), which may be ascribed to an increased availability of esterifying agent. However, no significant increase in DS was observed beyond 10:1 (SA to LLG mole ratio). The percentage yield of the product (LLS) also followed a similar pattern (Fig. 2). Similar results have been observed during succinylation of wheat straw hemicellulose using succinic anhydride and DMAP [15].

The chemical structure of LLS was evaluated by FTIR spectra. The FTIR spectra of LLG (Fig. 3a) showed a broad stretching vibration corresponding to hydroxyl groups (–OH) of aliphatic alcohols at 3271.04 cm^{-1} and a peak at 2927.16 cm^{-1} indicating the C–H stretching vibration of alkane. The peaks conforming to C–O–C bending vibration of glycosidic linkages in LLG were observed at 874.91 cm^{-1} and 1027.59 cm^{-1}. All the peaks detected in the spectrum of LLG were in consonance with the literature [11]. The FTIR spectra of LLS (Fig. 3a) demonstrated a stretching vibration peak at 1734.49 cm^{-1} which may be ascribed to carbonyl group (-C═O). The presence of another peak at 1170 cm^{-1} corresponding to C–O stretching indicated the ester linkage. The FTIR spectra of LLS revealed an additional peak at 1553.64 cm^{-1} corresponding to asymmetrical stretching of carboxyl anion (COO^-) confirming the derivatization [16].

The DSC thermograms of both LLG and LLS showed a broad endotherm at 90.0 °C and 80.25 °C, respectively, indicating the loss of moisture associated with the samples. Both the samples (LLG and LLS) displayed an exothermic peak at 305.30 °C and 337.53 °C, which was attributed to the thermal decomposition of the polymers. However, in case of LLS an additional endotherm appeared at 213.04 °C, confirming the derivatization of LLG (Fig. 3b). The results obtained were found to be in concordance with the findings of Sav et al., 2013 [33].

The drug release behavior and degradation of polymer-based formulations are controlled by its swelling character to a great extent. Therefore, to study the swelling behavior of LLG and LLS (DS = 0.853), swelling studies were carried out in buffers of pH 1.2 and pH

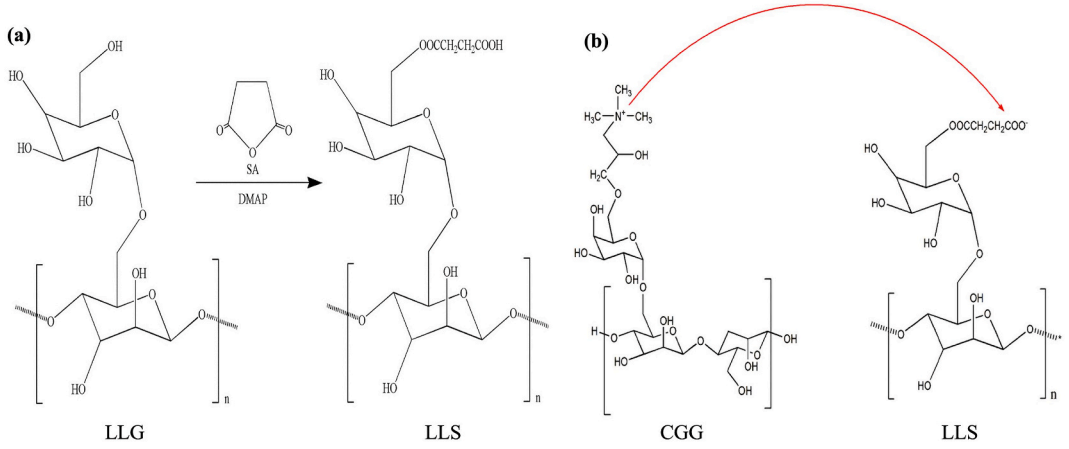

Fig. 1. Schematic reaction scheme for synthesis of *Leucaena leucocephala* succinate (LLS) (a), Polyelectrolyte complex formation between cationic guar gum (CGG) and *Leucaena leucocephala* succinate (LLS) (b).

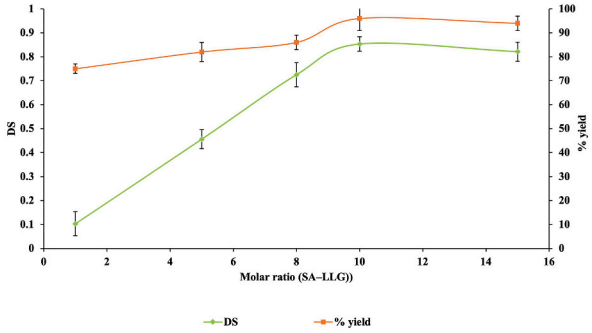

Fig. 2. Effect of molar ratio of SA-LLG on degree of succinylation and % yield of *Leucaena leucocephala* succinate.

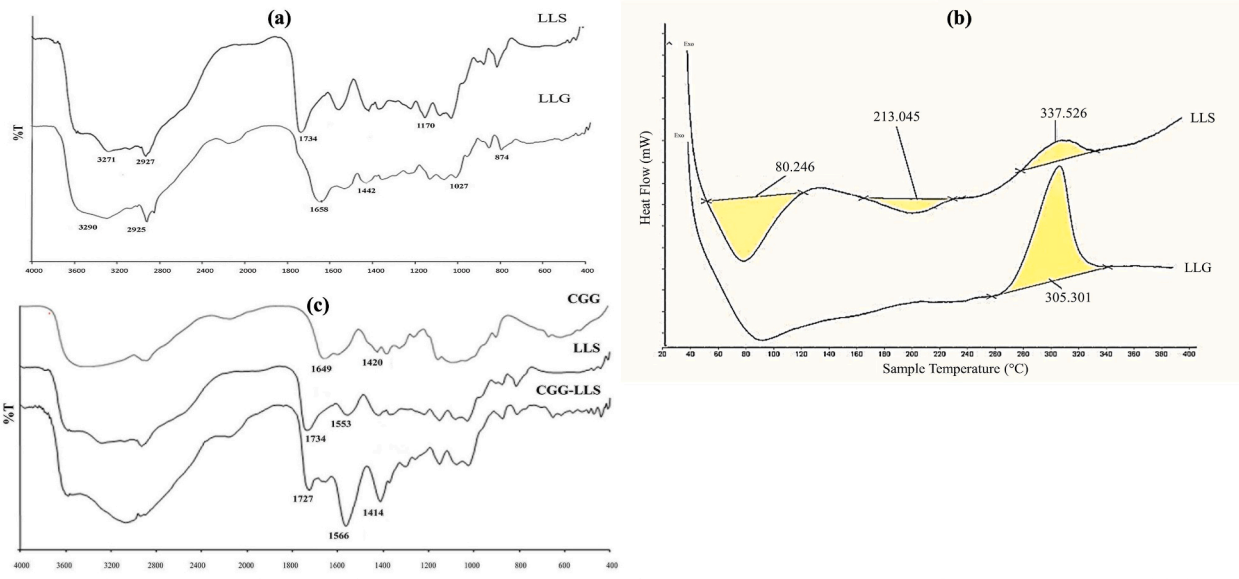

Fig. 3. FTIR spectra of *Leucaena leucocephala* galactomannan (LLG) and *Leucaena leucocephala* succinate (LLS) (a), DSC spectra of *Leucaena leucocephala* galactomannan (LLG) and *Leucaena leucocephala* succinate (LLS) (b), FTIR spectra of cationic guar gum (CGG), *Leucaena leucocephala* succinate (LLS) and PECs of CGG-LLS (c).

7.4, separately. The swelling studies revealed higher swelling of LLS as compared to LLG which may be attributed to the addition of hydrophilic groups in LLS. It is noteworthy that LLG didn't show any significant difference ($p > 0.05$) in swelling in buffers, pH 1.2 (6.25 ± 0.13) and 7.4 (7.14 ± 0.15), owing to its nonionic character. However, LLS demonstrated a pH dependent swelling. The swelling of LLS was found to be less under acidic conditions (13.59 ± 0.17) as compared to pH 7.4 (20.25 ± 0.13). The reduced swelling at pH 1.2 may be due to protonation of carboxyl groups of succinate moiety, which facilitated the formation of intra-molecular hydrogen bonding. The ionization of carboxyl groups at pH 7.4 augmented the repulsive force leading to long intermolecular distance that led to a higher swelling index. Similar observations have earlier been reported in our study with LLG and carboxymethyl LLG [11].

3.3. Preparation and characterization of PECs

The polyelectrolyte complexes between CGG-LLS were prepared by mixing them in different ratios. LLS is a negatively charged derivative of LLG, and consists of $–COO^-$group whereas CGG possess a net positive charge due to presence of $–N^+(CH_3)_3$ group. As a result of these groups, when LLS and CGG are mixed, the oppositely charged polymers undergo spontaneous reaction (Fig. 1b) to yield PECs in the form of gel like mass.

The percentage yield of PECs, and viscosity measurements of supernatant were employed to confirm the stoichiometric ratios of the PECs formed. As is evident from Table 1 an initial decrease in the viscosity of supernatant followed by sharp increase was noticed, when the ratio of CGG in the mixture was increased. In our earlier studies with chitosan and carboxymethyl tamarind kernel powder, we observed a similar trend in the viscosity results [34]. In the present study, minimum viscosity of supernatant was observed at 50:50, CGG: LLS, suggesting maximum interaction at this ratio. This observation was supported by the results of percentage yield (maximum,

89.15 %) at 50:50 of CGG: LLS. The higher yield of PECs resulted in a decreased concentration of unreacted polymer in the supernatant, leading to reduced viscosity.

Table 1 suggests that CGG-LLS PECs demonstrated pH dependent swelling. The amount of cationic polymer (CGG) had a significant impact on the swelling index of PECs under acidic conditions. The neutralization of carboxyl groups on LLS under acidic condition results in generation of excess of positive charge due to free ammonium groups of CGG inside the PECs. The mutual repulsions and the entry of counter ions along with water, to neutralize this charge leads to higher swelling. However, the shrinkage of the polymers at pH 7.4 resulted in a decreased swelling of PECs [25].

Fig. 3c represents the FTIR spectra of CGG, LLS and PECs formed by the interaction of CGG: LLS at 50:50 ratio. The spectra of CGG shows sharp characteristic peaks at 1649.98 cm^{-1} and 1418.54 cm^{-1} indicating the presence of–$N^{+}(CH_3)_3$ ions [35]. The FTIR spectra of LLS shows peak at 1734.49 cm^{-1} representing carbonyl group in ester linkage and a peak at 1553.64 cm^{-1} representing–COO^{-} ion. The PECs formed by interacting CGG and LLS showed a peak at 1557.89 cm^{-1} indicating the presence of –COO^{-} ion in the PECs. Another peak at 1415.55 cm^{-1} was observed which may be attributed to presence of –$N^{+}(CH_3)_3$ ions from CGG. The presence of peak at 1729.8 cm^{-1}in the spectra of PECs also indicated the presence of carbonyl group LLS. Therefore, the presence of –COO^{-}group and –$N^{+}(CH_3)_3$ group in the FTIR spectra of PECs strongly suggests interaction between CGG and LLS molecules.

3.4. Studies on L. fermentum

Fig. 4 depicts the growth curve of *L. fermentum*. The growth of the *L. fermentum* in MRS basal media supplemented with 3 % and 4 % w/v FOS was found to significantly high ($p < 0.05$) as compared to 1 %, 2 % w/v FOS, PC and MRS media. The value of specific growth rate (μ_{max}) was found higher in 3 % and 4 % FOS (1.101 ± 0.011/h and 1.173 ± 0.075/h, respectively) which was considerably greater than positive control containing 2 % w/v glucose (0.901 ± 0.007/h). Therefore, further studies were carried with 3 % and 4 % w/v FOS.

3.5. Antioxidant property of cell free extract of L. fermentum

Lactobacillus spp. is reported to possess antioxidant activity and therefore are beneficial in the management of IBD. Their ability to secrete superoxide dismutase (SOD) and other antioxidant compounds helps to ameliorate oxidative stress in the inflamed colon [36]. The antioxidant nature of probiotic bacteria was determined using the DPPH radical-scavenging assay. The equivalent antioxidant activity of the bacterial (*L. fermentum*) cell filtrate (CF) was calculated using the standard plot of ascorbic acid ($R^2 = 0.9938$). The percentage DPPH scavenging activity of CF was found to be 74.82 %, which corresponded to antioxidant activity of 551.57 μg/mL of ascorbic acid.

3.6. Encapsulation efficiency of probiotic and synbiotic loaded PECs

Table 2 depicts the percent encapsulation efficiency of probiotic and synbiotic loaded PECs. The maximum encapsulation of probiotics (91.11 %) was observed in formulation PF5, CGG-LLS, (50:50). The encapsulation of this formulation was significantly ($p < 0.05$) higher than all other probiotic-loaded PECs. The results of percentage yield and viscosity of blank PECs suggested maximum polymer interaction at this ratio. The encapsulation of FOS in synbiotic formulations (S1 and S2) was found to be 68.55 % and 83.33 %, respectively. The corresponding percentage encapsulation of *L. fermentum* in synbiotic loaded PECs was found to be 92.53 % and 95.53 %, respectively.

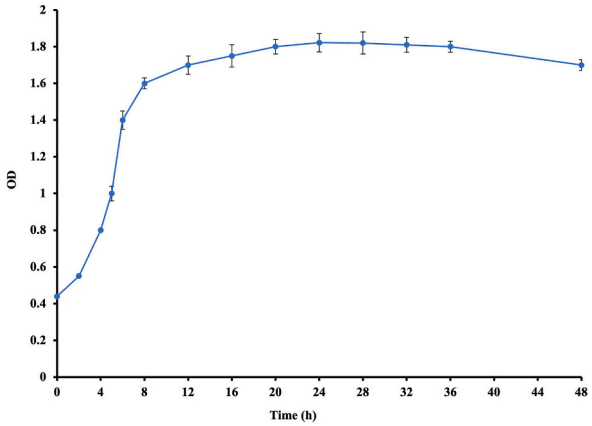

Fig. 4. Growth curve of *Lactobacillus fermentum*.

3.7. In-vitro release studies from probiotic and synbiotic PECs

The pH in the GIT changes from pH 1.2 in stomach to pH 7.4 in small intestine and finally to pH 6.8 in colon [37]. To simulate the GIT environment, *in-vitro* release studies of *L. fermentum* from probiotic and synbiotic PECs were performed by sequentially exposing the PECs to pH 1.2 for 2 h, pH 7.4 for 3 h and pH 6.8 for 19 h [25]. The viable number of (log CFU/100 mg) released from probiotic-loaded PECs at each time point were determined and percentage viability after 24 h as compared to initial cell load was calculated. It was observed that the unencapsulated cells lose their viability completely after 0.5 h in pH 1.2, indicating the need to encapsulate the cells so as to maintain their *L. fermentum* cells viability between therapeutic ranges. Fig. 5 depicts the number of bacterial cells released over a period of time. The cells encapsulated within different ratios of CGG:LLS shows significantly higher viability as compared to free cells. Among the CGG: LLS PECs, formulation PF5 (CGG: LLS; 50:50) shows highest viability of 85.62 % after 24 h.

In case of synbiotic-PECs, viability (%) of 89.21 % and 93.29 % were observed in S1 and S2 batch, respectively, which was significantly ($p < 0.05$) higher than viability to the PF5 (82.62 %). Studies have depicted that co-encapsulation of probiotic with prebiotics (synbiotic system) leads to increase in probiotic survival [38]. The probiotic batch PF5 and synbiotic batch S2 were selected for further studies.

3.8. Scanning electron microscopy (SEM)

The surface characteristics of probiotic loaded PECs (PF5) and synbiotic loaded PECs (S2) was analyzed using SEM (Fig. 6). A marked difference was found between the morphology of probiotic and synbiotic loaded PECs. The probiotic loaded PECs (PF5) depicted rough and porous structure (Fig. 6a). The SEM images of synbiotic loaded PECs (S2) depict a smooth and firm structure with reduced number of pores, suggesting a decrease in number of pores due to addition of FOS (Fig. 6b). Atia et al., 2016 has reported similar changes in the structure of alginate beads after addition of inulin (prebiotic) to the alginate beads [39].

3.9. Stability of probiotic-loaded PECs

One of the major challenges related to the development of probiotic and synbiotic loaded formulations is their stability during storage. In present study the stability of *L. fermentum* in unencapsulated forms and encapsulated in PECs was investigated over 2 months during their storage under both refrigerator (5 ± 2 °C) and room temperature (25 ± 2 °C). The results demonstrated that the free cells become non-viable after 45 days under refrigerated conditions whereas at room temperature they lost their viability completely after 30 days. The encapsulated cells (PF5) retain a viability of 89.79 % in refrigerator and 83.54 % at room temperature after the period of 2 months. The percentage viability of *L. fermentum* in S2 formulations at 5 ± 2 °C was significantly higher (93.95 %) as compared to PF5 (89.79 %) over 2 month's storage. Similarly, the viability of encapsulated *L. fermentum* cells (S2) at room temperature was 85.91 % after 60 days. The results indicated increased storage viability of *L. fermentum* in case of CGG: LLS PECs after addition of FOS. This may be attributed to decreased number of pores and hence, increased physical barrier, which maintains the viability of cells for longer period of time even at room temperature [40].

3.10. In-vivo studies

3.10.1. Evaluation of colonic inflammation

3.10.1.1. Disease activity index (DAI) scoring. The DAI score was calculated as mean of total sum of the weight loss score, the diarrheal score and the hematochezia score [29]. The results of daily DAI score are represented in Fig. 7a. The control group had a DAI score of 0; however, the administration of acetic acid (4.0 % v/v) resulted in a significant weight loss and bloody diarrhea. After the induction of

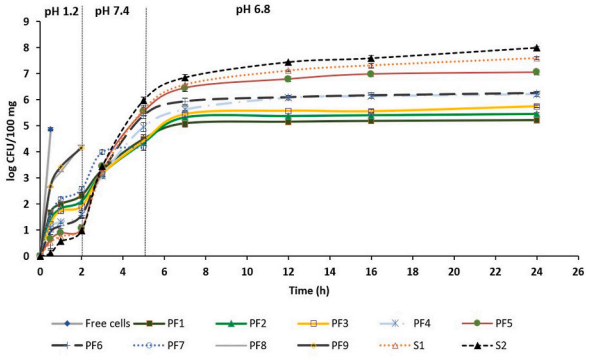

Fig. 5. *In vitro* release of unencapsulated and encapsulated *L. fermentum* cells in pH progression media.

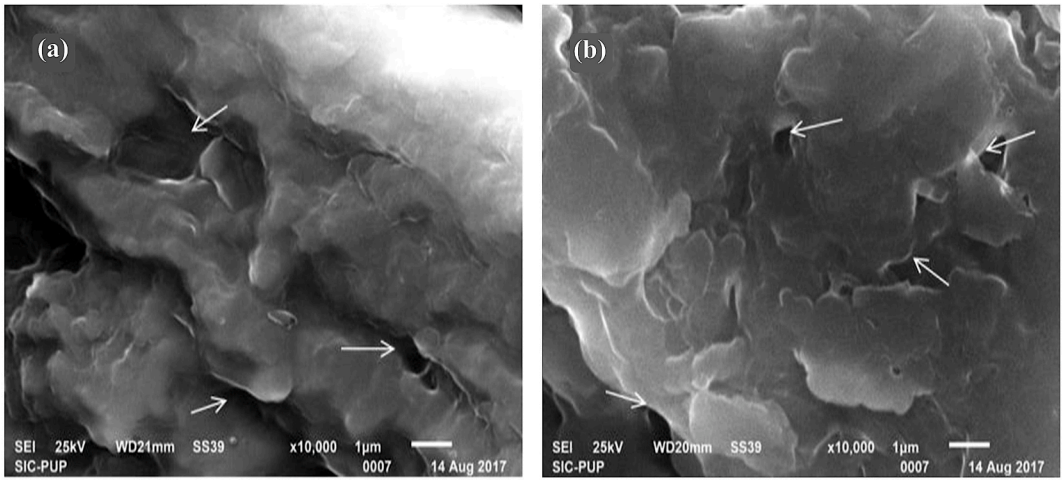

Fig. 6. SEM images of probiotic (a) and synbiotic (b) loaded PECs.

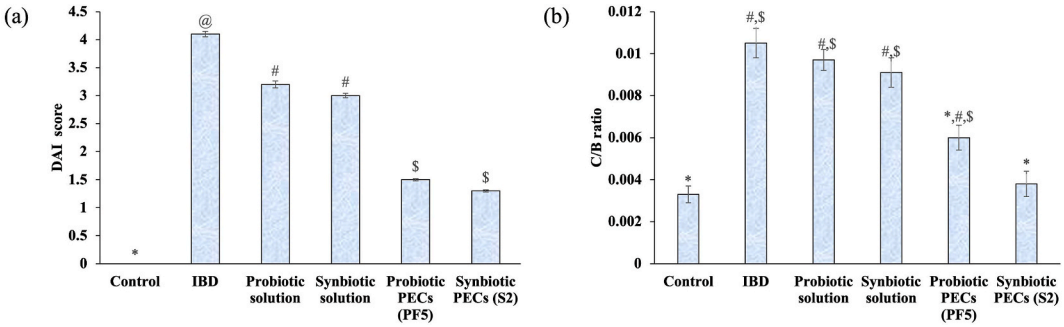

Fig. 7. DAI scores of animals treated with different formulations (a), Effect of various formulations on colon/body weight (C/B) ratio (b).

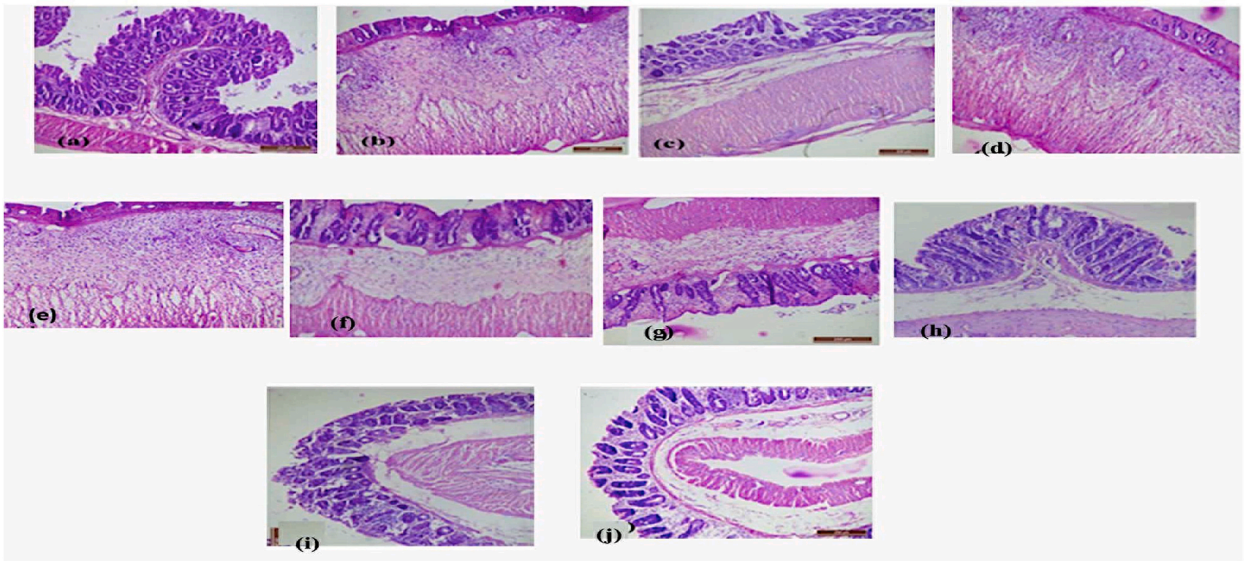

Fig. 8. Histopathological changes in colon of rats treated with different formulations. Control (a), IBD induced rats (b), probiotic solution after 5 days (c) and 10 days (d), synbiotic solution after 5 days (e) and 10 days (f), probiotic loaded PECs (PF5) after 5 days (g), and 10 days (h), synbiotic loaded PECs (S2) after 5 days (i) and 10 days (j).

IBD, nearly all animals lost around 20 % of their initial body weight and after 10 days the DAI scores were around 4-fold higher as compared to control group. (Fig. 7a). The animals in treatment Group III and IV (probiotic and synbiotic solution) showed a reduction in DAI. However, the animals were still weighing 10 % less than their original weights. Furthermore, the stool was soft till the end of the treatment period. The treatment of colitic rats with probiotic (PF5) or synbiotic (S2) loaded formulations resulted in significant reduction in colitis symptoms such as diarrhea, hemoccult resulting in a reduced DAI score after 10 days of treatment.

3.10.1.2. Colon: body weight ratio. For the purpose of quantitative evaluation of the inflammatory colitis of different groups, the colon: body weight (C/B) ratio of rats in different groups was calculated and is depicted in Fig. 7b. The animals from different groups were sacrificed on two time points i.e., after 5 and 10 days of treatment and their colon: body weight (C/B) was calculated [29]. The C/B ratio in colitis induced rats (Group II) was significantly high ($p < 0.05$) as compared with normal control (Group I). After 10 days of treatment with solution of probiotic or synbiotic (Group III and IV, respectively), there was no significant difference in C/B ratio compared to colitis control (Group II). The administration of PEC encapsulated probiotic (PF5) or synbiotic (S2) resulted in a significant reduction ($p < 0.001$) in C/B ratio. The reduction in C/B was significant ($p < 0.05$) in case of Group VI (treated with S2) as compared to all other groups, indicating higher therapeutic effect of synbiotic loaded PECs. After 10 days of administration of synbiotic loaded PECs, the difference in the C/B ratio became insignificant as compared to normal control. This suggested a greater therapeutic efficacy of probiotic in the presence of prebiotic.

3.10.1.3. Histopathology. Fig. 8 shows the histology of colon of different treatment groups after 5 and 10 days of treatment. The normal colon (Group I) shows the presence of the lamina propria, crypts, muscularis mucosae, and submucosa (Fig. 8a). The colon of colitis induced rats (Group II) present a high damage score with widely, eroded mucosa, massive neutrophil infiltration and crypt distortion (Fig. 8b). After 5 days of treatment with probiotic solution and synbiotic solution (Group III and IV), no effect on the severity of colonic damage was noticed as depicted in Fig. 8 c and e). The group treated with PF5 (Group V) shows healing in epithelia with a little change in inflammatory cells infiltrates (Fig. 8 g) whereas in the histology of group treated with S2 (Group VI) noticeable reparative epithelial changes were observed indicating the correction of morphological disturbances associated with acetic-acid induced colitis (Fig. 8 i).

After 10 days, no significant improvement in the treatment group III and IV was observed (Fig. 8 d and f). This indicates the ineffectiveness of solutions in treating ulcerative colitis. The groups receiving PF5 and S2 (Group V and VI) showed marked improvement in the severity of colitis (Fig. 8 h and j). The groups receiving synbiotic loaded PECs for 10 days, showed signs of attenuated cell damage with almost complete ulcer healing. This shows a greater effectiveness of synbiotic loaded PECs in treating acetic acid induced colitis.

3.10.2. Estimation of biochemical parameters

The acetic acid model for inducing colitis leads to an accumulation of white blood cells and vascular dilation. The increased blood flow and oxygen production results in the generation of free radicals and reactive oxygen species (ROS). The enzymatic (SOD) and nonenzymatic antioxidants (GSH)form the oxidative defense system of the body. The biochemical parameters were assessed to monitor inflammation in colitis induced rats.

3.10.2.1. Total protein levels in colon. The total protein content from the colon samples of different treatment groups was estimated by employing Lowry method. A standard plot of bovine serum albumin (BSA) was constructed to calculate the protein concentrations in the samples ($R^2 = 0.992$). The results of total proteins present in the colon tissue samples obtained from different groups are represented in Fig. 9a.

The results suggested a significant decrease ($p < 0.001$) in levels of total protein in colon of colitis induced group as compared to

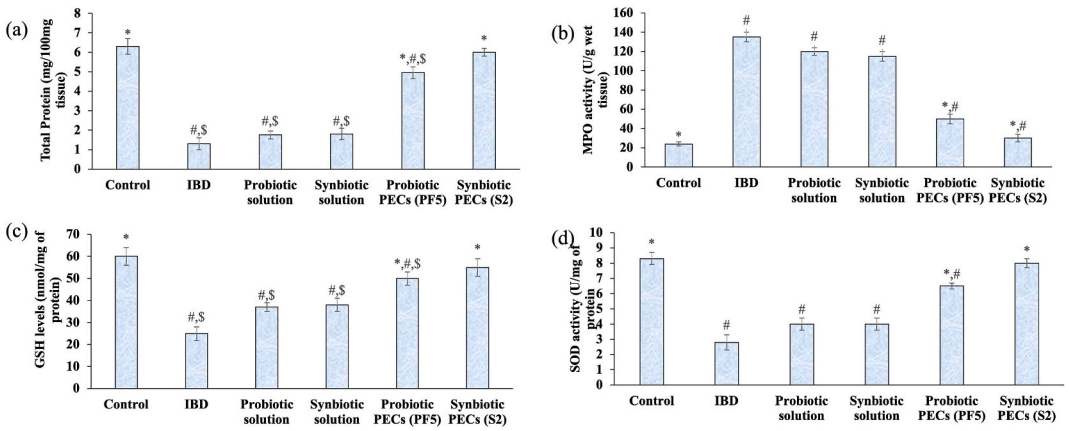

Fig. 9. Effect of various formulations on total protein (a), MPO activity (b), reduced glutathione levels (c), SOD activity (d).

control group. A decrease in protein content indicates increased generation of reactive-oxygen species (ROS) in IBD, which induce protein damage by protein oxidation [30]. The treatment with a solution of probiotic or synbiotic did not result in a significant increase in the protein levels as compared to colitis control after 5 or 10 days of treatment. However, the groups that received PF5 or S2 loaded PECs for 5 days showed significant ($p < 0.001$) improvement in colon protein levels with significantly ($p < 0.05$) higher protein levels in synbiotic loaded PECs group as compared to all other treatment groups. However, even after 5 days of treatment, no group showed signs of complete recovery, since the colon protein level in all the treatment groups were significantly compared to control group.

After10 days of treatment, the groups receiving PF5 or S2 showed higher levels of protein as compared to 5 days treatment groups. The synbiotic loaded PECs showed highest levels of protein which were comparable to the control group, indicating better effectiveness of synbiotic PECs in treating acetic-induced colitis. Similar results showing protective effect of naringenin on acetic acid-induced ulcerative colitis in rats have been reported [30].

3.10.2.2. Colon myeloperoxidase (MPO) activity. Myeloperoxidase is an enzyme present in high concentration in neutrophils and at a much lower concentration in the macrophages and monocytes. MPO activity acts as a marker of neutrophil infiltration, which is reported to be up-regulated in inflamed tissues [32]. This suggests that a reduction in the MPO activity can be interpreted as the expression of anti-inflammatory activity of the compound.

Fig. 9b represents the MPO activity of colon samples from different groups after administration of different treatments for 5 and 10 days, respectively. A marked significant increase in MPO activity was observed in animals of colitis control groups as compared to control group ($p < 0.001$), indicating increased neutrophil infiltration after induction of colitis. The groups III and IV also demonstrated enhanced MPO activity. However, a significant decrease in the MPO activity was observed in Group V and VI. After 10 days of treatment, the Group V and VI further showed a significant ($p < 0.001$) decrease in the MPO activity as compared to colitis control, and Group III and IV. The rats treated with synbiotic loaded PECs showed highest decrease in MPO activity after 10 days, indicating a decrease in the infiltration of inflammatory cells that are responsible for the progression of colitis. A similar result has been reported by Peran et al., 2005, where significant preventive effects of *Lactobacillus salivarius* ssp. *Salivarius* (probiotic) were observed in the TNBS model of rat colitis [41].

3.10.2.3. Reduced glutathione (GSH) in colon. Increased oxidative stress is known to be one of the major causes of initiation and progression of IBD. In experimental models of induced colitis, an imbalance between antioxidant and prooxidant, and damage due to oxidative stress has been reported. A number of studies have suggested the role of free radicals in the pathogenesis of mucosal injuries. In biological systems, reduced glutathione (GSH) is known to be the first line defense system against free radicals due to the presence of sulphdryl group in it. GSH works as non-enzymatic antioxidant and is distributed widely in all the biological tissues. In case of inflammation or increased oxidative stress, it is reported that the antioxidant defense of this glutathione system is decreased [42]. The depletion of GSH promotes the generation of ROS resulting in the improper functioning and integrity of the cell. In this study, the level of reduced glutathione (GSH) in the colon tissue samples from different treatment groups was determined. A standard plot of reduced glutathione was prepared for calculating the concentration of GSH in the colon samples ($R^2 = 0.9961$).

The results indicated that there was a significant decrease ($p < 0.001$) in the levels of GSH in colitis control group as compared to control group. Treatment with probiotic or synbiotic solution did not show any significant improvement in the GSH levels after 5 days as compared to colitis control. However, the groups that received PF5 or S2 for 5 days showed significant ($p < 0.001$) increase in colon GSH levels with significantly ($p < 0.05$) higher levels with synbiotic loaded PECs group as compared to all other treatment groups. However, after 5 days of treatment, no group showed signs of complete recovery, since the colon GSH level in all the treatment groups were significantly lower from the control group.

After 10 days of treatment, the groups receiving PF5 and S2 showed higher level of GSH as compared to 5 days treatment groups. The rats treated with synbiotic loaded PECs showed highest levels of GSH which were comparable to the control group, indicating the better effectiveness of synbiotic PECs in treating acetic-induced colitis (Fig. 9c).

2.10.2.4. Colon superoxide dismutase (SOD) activity. Superoxide dismutase (SOD) is a primary antioxidant enzyme that forms an important part of oxidative defense systems in body. SOD regulates the levels of reactive oxygen species in body by converting superoxide anion to hydrogen peroxide (H_2O_2), which is converted to water by the action of catalase. SOD activity was significantly ($p < 0.001$) reduced in the colons of animals in colitis induced groups as compared to the control group. After 5 days of treatment with probiotic or synbiotic solution, no significant increase in the SOD activity was observed in groups III and IV as compared to colitis group. The groups receiving probiotic or synbiotic PECs, showed significantly ($p < 0.05$) high SOD activity as compared to the colitis group, probiotic solution and synbiotic solution groups. After 10 days of treatment, a significantly high ($p < 0.001$) SOD activity was observed in the groups V and VI as compared to groups II, III and IV. The synbiotic loaded PECs (S2) showed highest SOD activity which was comparable to the control group, indicating the high effectiveness of synbiotic PECs in treating acetic-induced colitis (Fig. 9d).

The analysis of biochemical parameters revealed an increase in the levels of protein, reduction in the MPO activity (neutrophil infiltration marker), increased SOD activity and GSH levels in the rats that received synbiotic treatment as compared to colic rats. These results indicated the protective effects of synbiotic treatment.

4. Conclusion

A succinyl derivative of *Leucaena leucocephala* galactomannan was synthesized and characterized. The polyelectrolyte complex between *Leucaena leucocephala* succinate and cationic guar gum formed by electrostatic interaction showed maximum interaction at 50:50 ratio. Both probiotic and synbiotic loaded PECs were prepared and showed a cell viability of 80.62 % and 93.29 % after 24 h in *in vitro* release studies. The formulations were found to stable under refrigerated conditions and at room temperature. The *in vivo* studies demonstrated significant improvement in DAI score and colon/body ratio. The biochemical parameters also demonstrated an alleviation of colitic symptoms. Although, the study demonstrated that synbiotic loaded PECs could be employed to protect and deliver the probiotics to colon for the treatment of dysbiosis associated with IBD, the long-term stability studies and the stringent conditions required during the production of PECs need to be further addressed.

Ethics statement

The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) of Punjabi University, Patiala vide protocol approval no. 107/99/CPCSEA/2017-34).

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Data availability statement section

The data has been submitted in the library of Punjabi University, Patiala in the form of M Pharm dissertation.

CRediT authorship contribution statement

Gaganpreet Kaur: Investigation. **Aman Kumar:** Methodology. **Samridhi Kurl:** Writing – review & editing, Methodology. **Neeraj Mittal:** Writing – review & editing. **Deepinder Singh Malik:** Formal analysis, Data curation. **Pallavi Bassi:** Writing – review & editing, Software. **Tanveer Singh:** Writing – review & editing. **Azmat Ali Khan:** Funding acquisition. **Amer M. Alanazi:** Funding acquisition. **Gurpreet Kaur:** Writing – original draft, Conceptualization.

Declaration of competing interest

We hereby declare that there is no conflict of interest with this manuscript entitled"*Leucaena leucocephala* Succinate based Polyelectrolyte complexes for Colon Delivery of Synbiotic in Management of Inflammatory Bowel Disease." The authors have approved the order of authors listed in the manuscript.

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