CHAPTER 5

Induction of Gastro-intestinal Disease and application of Probiotic and Prebiotic on diseased animal model

5.1. INTRODUCTION:

Probiotic microbes are the live organisms which attribute beneficial health improving role in the intestinal environment. It can be included in our normal gut flora which also helps in improving the ecosystem balance of the host. Thus, any such organisms which were considered as probiotics must have one essential criteria that it should be non-pathogenic and should be safe [Zendeboodi *et al.* 2020].

The idea of the synbiotics has been proposed to characterize as an agent that influences the health promoting factors of the hosts. The development of synbiotics with increased importance has focused on the resistance to the different infections as well as the antimicrobial property [Gibson and Roberfroid, 1995].

There are several types of chronic inflammation which is categorized by different diseases namely; IBD, traveler's diarrhea etc. IBD is nothing but a severe inflammation of the digestive tract. It comprises of CD and UC that can be identified by the inflammation of the digestive tract and ulceration respectively. Indetreminate colitis is a condition in which all the symptoms of IBD is observed but the type of disease occurrence in dilemma [Torres *et al*, 2020].

The first line of drugs that is generally used for the treatment of ulcerative colitis are aminosalicylates, corticosteroid and some immunosuppressant which is reportedly not much effective in a large number of colitis induced host due to its side effects and many other damages [Deltenre et al., 1999; Ek and Rosenborg et al., 2017]. Thus, the concept of the disease induction by many chemical agents which induces the occurrence of disease in the host is important. The potentiality of the probiotics to treat against the colitis induced host is a very important strategy over the conventional modes of therapeutics [Jang *et al*., 2018].

Probiotic reduce the risk of disease by improving the defense mechanism and by changing the morphology of the gut tissue [Yang *et al*., 2009]. In mice reported different probiotic especially *Streptococcus thermophilus* showed increase in body weight as well as improved digestion via different mechanisms [Sanders 2000].

From the different reported study, it was suggested that the certain beneficial bacteria when administered on the hosts can successfully reduce the intestinal inflammation and injury, it can also improve intestinal ecosystem [Je *et al*.,2018]. Many a times the effect of probiotics on the colitis associated adeno-carcinoma was also reported [Talero *et al.*, 2015].

Different animal models were introduced in the research science world for the development of the probiotics. Disease induction in the mice model induced by DSS (Dextran Sodium Sulfate) was proved to be more advantageous in compare to that of the humans by their symptoms, which includes diarrhea, blood in feces, loss of body mass, ulceration of mucosa and shortening the large intestine [Korenaga *et al.*, 2002].

Now-a-days there are several studies which is going on to investigate the pathogenesis of the colitis. Ingestion of DSS through drinking water to induce colitis in mice is very common now-a-days. The colitis developed by the presence of the DSS varies on the basis of its concentration, dose, time span and the frequency of the DSS being administered. Generally mice show different vulnerability and responsiveness to the chemically induced colitis by DSS. Several external factors can also affect the induction of the colitis, such as, stress [Melgar *et al.,* 2018].

The histopathological study after the DSS applications appeared some chronic changes. The changes are as follows; infiltration of the mononuclear leucocytes, architectural disarrangement

of the crypts, increasing the distance between the crypts and the muscularis mucosa and transmural inflammation [Hall *et al.*, 2011].

In this sets of experiments, the first experiment was used to determine the most potent chemical inducer of the colitis in BALB/c mice. Followed by that, the colitis induced mice was then treated with different combination of treatment to find out the most effective dose and combination of probiotics and prebiotic as a therapeutic tool. To reconfirm the study, different parameters were considered such as, loss of body mass, consistency of stool, blood in feces and also histological changes in the intestinal tissues.

5.2. MATERIALS AND METHODS:

5.2.1: FINE CHEMICALS:

DSS (Dextran Sodium Sulfate), TNBS (Tri-nitro-benzene sulfonate) and acetic acid procured from Hi-Media, India. Acetic acid and rest of the chemicals like ethanol etc. required for histology study was purchased from the local pharmacy from Purba Bardhaman- 01. Hematoxylin and Eosin procured from Sigma-Aldrich, Mumbai, India.

5.2.2: INSTRUMENTATION:

The equipments used for the studies were: Bacteriological incubator (Remi, Mumbai), Centrifuge (REMI, Mumbai), Deep freezer (Blue Star, Mumbai), Electronic balance (Shimadzu, Kyoto, Japan), Laminar Air flow (Sanguine Bioinstruments, Hyderabad), Gavage feeder (Thermo Scientific) and Catheter (4 cm proximal to anus).

5.2.3: BACTERIAL CULTURES USED:

All the bacterial isolates already selected (PB1 – PB10) is used for this study. The conditions of growth and maintenance have been described under the section 3.2.5.2 and 3.2.5.3. The cultures were propagated twice before use.

5.2.4: ANIMAL MAINTENANCE AND DIET:

Thirty specific pathogen-free (SPF), BALB/c (an albino) mice were collected from Rita Animal House, Kolkata-52 which were nine weeks old and were female. The cage of the mice was maintained with a free space present at the top of the cage in specifically pathogen-free environment, under 50 to 60% humidity, and was maintained 12-hours dark and light cycles, in which food given were standard rat chow and potable sterile water filled in a bottle placed in the cage. The experimental procedures were followed as per the guidelines of the Animal Ethical Committee, Oriental Institute of Science and Technology, Vidyasagar University, Midnapore, West Bengal, India.

5.2.5: DESIGN OF THE EXPERIMENT:

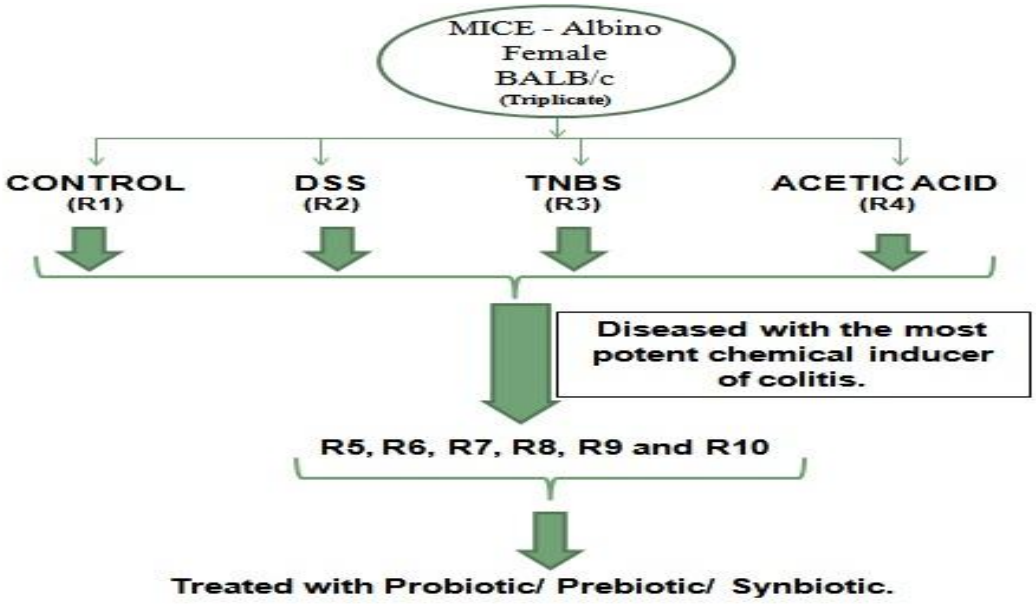


FIGURE 5.1: The flow chart diagram of the design of the study with the animal model.

The design of the study with the female BALB/c mice is as follows;

- At the first set, triplicate sets of the mice were arranged taking four animals in each set. This set of mice was taken to induce colitis in it with three different chemical inducers (such as; Dextran Sodium Sulfate, Tri-nitro-benzene sulfonate and Acetic acid).

- At the second set, again triplicate sets of mice were arranged taking six animals in each set. In this set of mice, the disease (i.e., colitis) was initially induced by the most potent chemical inducer of colitis among the three mentioned above. And then they were treated with different combination (such as; probiotic organisms, prebiotics and synbiotics too.)

5.2.6: INDUCTION OF COLITIS:

The induction of colitis on the animal model (BALB/c mice) was initiated by three chemical inducers; namely Dextran Sodium Sulfate (DSS), Tri-nitro-benzene sulfonate (TNBS) and Acetic Acid (AA). The dosage of the colitis induction by these three inducers is mentioned below;

[1] Dextran Sodium Sulfate - 5 % (w/v) DSS (mol. Wt. 40 K Da) per kg of mice along with water used for drinking purpose was given to the mice for fifteen days [Chassaing *et. al*. 2014].

[2] Tri-nitro-benzene sulfonate - TNBS of 200 mg per kg of mice (w/v) was dissolved in ethanol (30%) which can be instilled proximal to anus of the BALB/c mice by the help of a catheter approximately 3to4 cm long. It was administered in alternative days for 15 days [Almeida *et. al.* 2013].

[3] Acetic Acid - 1 ml of acetic acid (5%, v/v) was added to saline water, and then it was added to the mice of 100 gm that can be instilled into the cavity of the colon via catheter approximately of 4 cm long, that is, proximal to the anus and was administered for 15 days (in alternative manner) [Niu, Xiaofeng, *et al.* 2013].

5.2.7: POST-EXPERIMENT SAMPLE COLLECTION:

BALB/c mice were euthanized under isoflurane anesthesia upon completion of the entire experiment. And then, blood was collected in a sterile syringe by puncturing cardiac wall. The blood after withdrawing from heart is allowed to clot at 4°C for 2 hours following centrifugation

for 20 mins at 3000 rpm. Post centrifugation the supernatant was stored at -20°C. And for the others sets of experiments, the intestinal tissues were collected and stored until further use.

5.2.8: IDENTIFICATION OF THE MOST POTENT COLITIS INDUCER:

In the first triplicate sets of four BALB/c mice was taken, in which one was considered as control (i.e. no disease induction and no treatment is administered) and designated as R1. The colitis was induced to the rest of the three mice designated as R2, R3 and R4 (in three sets) by DSS, TNBS and AA respectively. The dosage of each colitis inducer is mentioned in the **Section 5.2.6**.

The clinical analysis of colitis can be evaluated by DAI which was measured daily on the basis of the loss of body mass, consistency of the stool, and also bleeding from rectum. It includes the following index;

The score of the **LBI** is as follows:

'**0** for no loss of body mass', '**1** for of 0 to 5% of body mass loss', '**2** for 5 to 10% of bady mass loss', '**3** for 10 to 20% of bady mass loss', and '**4** for >20% of body mass loss'.

The score of the **SCI** is:

'**0** for normal stool (pellet)', '**2** for pasty and non-formed stool', and '**4** for watery stool'.

The score of the **RBI** is:

'**0** indicates no loss of blood' and '**4** indicates gross bleeding from anus' [Yo Han Park *et al.*, 2000].

5.2.9: INDUCTION OF COLITIS WITH THE POTENT CHEMICAL INDUCER:

Considering the result of the Section 5.2.8 based on DAI, the second set of mice which contain six mice namely R5, R6, R7, R8, R9 and R10 present in each set (triplicate sets) was induced to colitis by DSS. Following the same index (DAI), the development of colitis is confirmed to BALB/c mice by the most effective chemical inducer of colitis.

5.2.10: PREPERATION OF THE PROBIOTIC AND PREBIOTIC DOSE FOR TREATMENT:

The overnight grown isolated bacterial strains (PB1 – PB10) belonging to exponential growth phase at A_{600} was taken and was subjected to 3,000 *rpm* spinning for 10 min. The pellet obtained was washed with PBS buffer, and was re-suspended at $NaHCO_3$ buffer with 2% glucose. Microbial suspensions were freshly prepared at every alternative day for the dose of treatment. Mice received 10^9 CFU via oral administration of each isolates which is re-suspended in bicarbonate buffer. The dose of probiotic that was administered to the mice through mouth via gavage is 25 µl. And the dose is given to the diseased mice in alternate days that were freshly prepared [Pavan *et al.* 2003].

The dose of the prebiotic (inulin and FOS) was also prepared freshly before administrating it to the mice. While treatment, 2.5 gm of inulin and Fructose-oligosaccharide was given to per kg of the diseased mice. The treatment with prebiotic alone and together with probiotic follows the same dose as mentioned [Zhu *et al.*, 2017].

5.2.11. PROPOSED TREATMENT COMBINATION TO THE DISEASED MICE:

TABLE 5.1: The diseased combination for the treatment of diseased mice.

Animal Model	TREATMENT COMBINATION
R5	PB1+PB6 (*Bacillus cereus*)
R6	PB7 *(Lactobacillus fermentum*)
R7	PB2+PB3+PB4+PB5+PB8+PB9+PB10 *Streptococcus thermophilus*)
R8	PB1 to PB10
R9	PB1 to PB10 + INULIN + FOS
R10	INULIN + FOS

From the **TABLE 5.1**, the dose and combinations referred for the treatment of colitis was much diversified. One set of mice R5 was treated with two new strain of *Bacillus cereus*, then the other mice (R6) was treated with a new strain of Lactobacillus *fermentum* and R7 was treated with the new strains of *Sterptococcus thermophilus*. Whereas, R8 was treated with all the ten new strains isolated (PB1 – PB10), R9 had been given combination treatment with all the isolates and prebiotic. And finally, R10 was treated only with prebiotics. Thus, it can be also said in this way that; R8 received the treatment with probiotic, R9 with symbiotic and R10 with prebiotic.

5.2.12. DETERMINATION OF GAIN IN BODY MASS POST-TREATMENT:

Based on the same indexing protocol, post-treatment the gain percentage of the body mass of the mice was measured daily along with stool consistency and rectal bleeding as mentioned in the **Section 5.2.8.**

5.2.13. HISTOPATHOLOGICAL ASSAY:

The tissue samples (small intestine) were fixed in 4% formalin and routinely evaluated before embedding in paraffin. Several transverse 4 µm of slices were stained with hematoxylin–eosin, and observed under oil immersion of the light microscopy [Kruschewski et. at. 2001].

5.2.14. FECAL MICROBIOTA ASSAY:

Feces of mice (1 g) were collected at an interval of 5days of experiment. This study was used to evaluate the fecal microbial count. At first, feces were homogenized in 9mL of anaerobic dilution buffer (which contains cysteine, resazurin and gelatin). After defecation, the isolates were re-inoculated on Eosin methylene blue (EMB) agar media by spread plate technique for plate counting. The fecal samples were serial diluted upto 10^{-6} were prepared in sterile saline buffer (9gm/L) from which 50µL was used for the inoculation purpose [Yang *et al.*, 2005].

5.2.15. SERUM BIOCHEMICAL ANALYSIS:

Levels of total Bilirubin, Conjugated bilirubin, Unconjugated bilirubin, Serum SGPT, SGOT, ALP and Hemoglobin were analyzed in the serum using standard analytical kits [Jendrassik and Groff, 1938; Reitman and Frankel, 1957; Ochei & Kolhatkar, 2000].

5.3. RESULT AND DISCUSSION:

Chemical induction of intestinal inflammation on murine models is one of the popular models used now-a-days. It is because chemical induction protocols are simple to induce inflammation are under control. In well-established animal models, both DSS and TNBS are potent inducer of colitis. Along with that acetic acid is also widely used for colitis induction [Neurath *et al.,* 2000 & Wirtz *et al*, 2007].

In this research, the *in vivo* effect of the probiotics, prebiotics and synbiotics was analyzed. It was observed that in the BALB/c mice, disease can be successfully induced by the help of different chemical agent, where the most effective colitis inducer was proved to be Dextran Sodium Sulfate (DSS) in compare to TNBS and acetic acid. These diseased mice were then treated with different combination of probiotic organisms along with few prebiotics. Among the different combinations, the treatment with synbiotic gave the best result based on some specific significant parameters; DAI (which includes LBI, SCI and RBI), histological assay, liver enzyme assay and by total fecal anaerobic count.

5.3.1. DAI OF THE DISEASED MICE (R1 to R4):

After seven days of acclimatization with normal diet, four animals were taken (in three sets), where one was kept as control and colitis was induced to the rest of the mice by three different chemical colitis inducer namely, DSS, TNBS and Acetic acid.

As per mentioned in the **SECTION 5.2.6,** the dose of the colitis inducers was administered for 15days. Throughout these days of disease induction the mass of body as well as their stool consistency was tracked and tabulated in the **TABLE 5.2 (a, b)** in the form of different indexing score mentioned in the **SECTION 5.2.8.**

At the recent time, no. of studies suggested that colitis induced model by DSS is very effective to investigate disease causing ability on mice. Colitis can be introduced on an animal model with DSS dissolved in potable water. On the basis of the concentration, the time span, and frequency of the DSS administration, the animal model develops either acute or chronic colitis [Okayasu et al., 1990].

TABLE 5.2(a): The average loss of body mass of the BALB/c mice during colitis induction by different chemical inducer.

Animal Model	Average loss of body mass ± S.D.				Loss % of Body mass	LBI
	0^{th} Day	5^{th} Day	10^{th} Day	15^{th} Day		
R1 (Control)	55 ±1	56.67±1.52	58.67±2.08	60.67±2.08	-	0
R2 (DSS)	55.67±3.05	56±3.60	53.33±3.21	49.33±1.52	11.38	3
R3 (TNBS)	56 ±2	56.33±1.52	55.33±0.57	52.33±1.52	6.55	2
R4 (Acetic Acid)	55.33±2.08	55.67±2.51	55±2	53.67±1.5	3.0	1

TABLE 5.2(b): The SCI(stool consistency index) and RBI(rectal bleeding index) of the colitis induced BALB/c mice.

Animal Model	0 day		5^{th} day		10^{th} day		15^{th} day	
	RBI	SCI	RBI	SCI	RBI	SCI	RBI	SCI
R1	**0**	**1**	**0**	**1**	**0**	**1**	**0**	**1**
R2	**0**	**1**	**0**	**1**	**0**	**2**	**4**	**2**
R3	**0**	**1**	**0**	**1**	**0**	**2**	**0**	**2**
R4	**0**	**1**	**0**	**1**	**0**	**2**	**0**	**2**

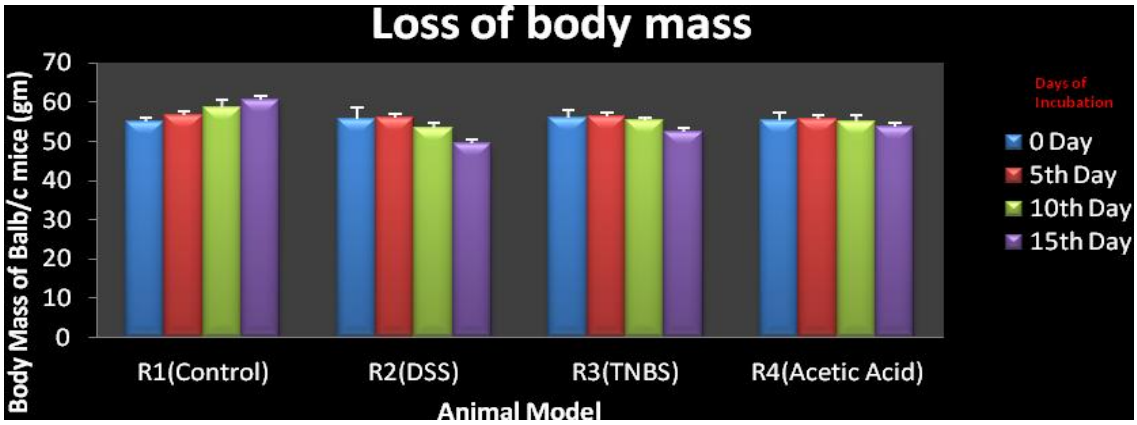


FIGURE 5.2: A comparitive study of loss of body mass of BALB/c mice during the colitis induction.

From the **FIGURE 5.2** it is evident that, all the chemical colitis inducer namely, DSS, TNBS and AA showed a sharp declination in the body mass parameter confirming the disease to be activated in all the animal models (i.e., R2, R3 and R4). But, from the DAI score metioned in the TABLE 5.1(a) it was confirmed that the maximum percentage of loss of body mass was observed in R2 having DAI 3 in compare to the other disease model. Again,in the respect of SCI and RBI it was observed R2, R3 and R4 started forming pasty pellets after 10 days of disease induction, whereas the rectal bleeding is observed only in R2 model at end of 20 days of disease induction. The colitis was induced to this R2 model is by Dextran sodium sulfate (DSS). Thereby, it can be concluded that in the present study, DSS is the most effective colitis inducer in compare to the TNBS or AA.

5.3.2. DAI OF THE DISEASED MICE (R5 to R10):

The DSS-induced colitis model is advantageous over other animal models as because it initiates an relapsing, acute and chronic disease model just by changing the concentration of

DSS administrated on the mice. DSS-induced model is also a pioneer tool to investigate the colitis related cancers [De *et al.*, 2011].

It is a pre-established fact that DSS is lethal to intestinal cells. It affects the epithelial cells, which allows large molecules (DSS) to permeate across the membrane barrier. The exact mode of action of DSS is still unclear [Tomikawa *et al.*, 2011].

From the previous set of experiment, it was established that in the present study DSS is the most potent colitis inducer among the three used. Thus, in the next set of experiment initially DSS was used to induce colitis on six BALB/c mice (in three sets). To reconfirm the development of colitis on R5, R6, R7, R8, R9 and R10 the scoring indexes were evaluated such as, LBI, SCI and RBI.

TABLE 5.3(a): The average loss of body mass of the BALB/c mice during colitis induction by DSS.

Animal Model	Average Loss of Body Mass ± S.D.				Loss % of Body mass	LBI
	0^{th} Day	5^{th} Day	10^{th} Day	15^{th} Day		
R5(Control)	54 ± 1	54.33 ± 1.2	56 ± 1	56.33 ± 2.1	-	0
R6 (DSS)	53.33 ± 2.5	53.33 ± 2.0	50.33 ± 1.5	48.33 ± 1.5	9.37	2
R7(DSS)	56.33 ± 1.5	56 ± 1	51 ± 1	48.67 ± 0.6	13.59	3
R8(DSS)	53.33 ± 2.5	53.33 ± 2.5	51 ± 1	49.67 ± 0.6	6.86	2
R9(DSS)	52.33 ± 1.5	52 ± 1	50.33 ± 1.5	48 ± 1	8.27	2
R10(DSS)	53.67 ± 1.2	53.67 ± 1.2	51.33 ± 1.5	49 ± 1	8.70	2
R11(DSS)	54.33 ± 2.08	54 ± 1.7	5.33 ± 0.5	49.67 ± 1.5	8.57	2

TABLE 5.3(b): The SCI (Stool Consistency Index) and RBI (Rectal Bleeding Index) of the DSS induced BALB/c mice.

Animal Model	0 Day		5th Day		10th Day		15th Day	
	SCI	RBI	SCI	RBI	SCI	RBI	SCI	RBI
R1	1	0	1	0	1	0	1	0
R5	1	0	1	0	2	0	2	4
R6	1	0	1	0	2	0	2	4
R7	1	0	1	0	2	0	2	4
R8	1	0	1	0	2	0	2	4
R9	1	0	1	0	2	0	2	4
R10	1	0	1	0	2	0	2	4

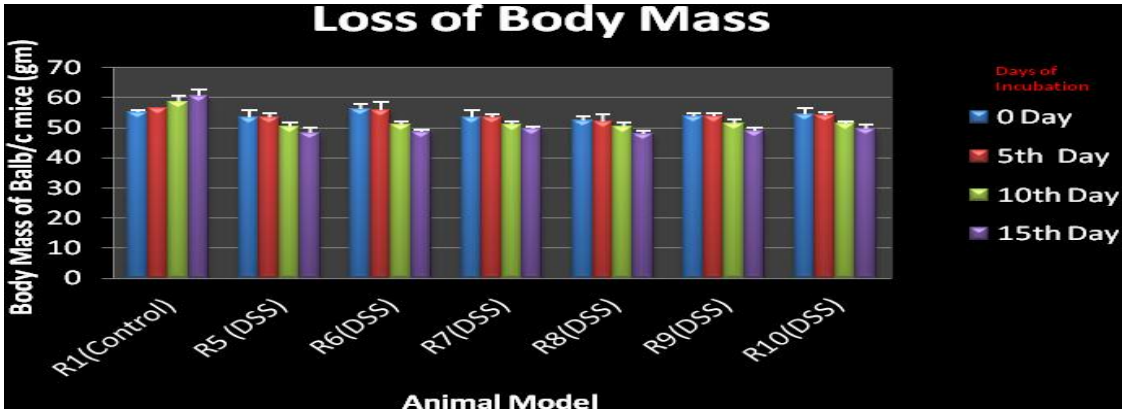


FIGURE 5.3: A comparitive study of loss of body mass of DSS induced BALB/c mice.

In the present study, it was observed from **TABLE 5.3 (a,b)** that all the colitis induced mice had shown a stiff declination in the body mass during the developent of the diseases. Though the loss of body mass index (LBI) is not same for all the model but the percentage of loss of body mass was more or less showed same trend of declination after 20 days of disease

induction period. Again, another very important parameter that showed successful colitis development in R5 to R10 mice is SCI and RBI. All the animal models showed semi-solid pellet formation after 10 days of disease induction along with rectal bleeding the end of of the disease induction process. Both these parameters reconfirmed the active disease induction in all the animal models.

5.3.3. GAIN IN BODY MASS POST TREATMENT:

The combination of probiotics and prebiotics formulate a synbiotic which can enhances growth rate of probiotics in the GI tract [Henriksson and Mitchell, 2007]. Human breast milk is a very good example of symbiotic which contains oligosaccharides and LAB, which together have the capability to elevate the activity of probiotics [Collado *et al.*, 2009].

In this study indicated that, colitis was induced on the BALB/c mice with the aim to investigate the most effective mode or combination of treatment on it. Thus, to fulfill the aim of the study, different combinations of treatment were administered to the colitis induced mice which is already discussed in the **SECTION 5.2.11.**

The combinations of treatment include; treatment with probiotics maintaining the individual genus specifications, with all the probiotic strains isolated, with probiotic strains (PB1-PB10) and prebiotics (inulin and FOS) and also only with prebiotic.

TABLE 5.4 (a,b): The LBI, SCI and RBI of the colitis induced BALB/c mice during the course of treatment with different combinations.

Animal Model	Average Gain in Body Mass (gm) ± S.D.					Gain%In Body mass
	20th day	25th day	30th day	35th day	40th day	
R1 (Control)	57±1.7	58.3±1.5	59.7±1.5	59.7±1.5	61±1	7.01
R5 (PB1 + PB6)	48.3±1.5	48.3±1.1	48.3±1.5	49.3±0.6	49.7±0.6	2.77
R6 (PB7)	48.7±0.6	49.7±1.5	50±1	51±1	52.3±1.5	7.52
R7(PB2,PB3,PB4,PB5.PB8,PB9,PB10)	49.7±0.6	50.3±0.6	50.7±0.6	51.7±0.6	53±1.7	6.07
R8 (PB1 – PB10)	48±1	48.7±0.6	49.3±0.6	50.7±0.6	51.7±0.6	7.64
R9 (PB1-PB10 + Inulin+FOS)	49±1	49.3±0.6	49.7±0.6	53±1	53±1	8.1
R10 (Inulin+FOS)	49.7±1.5	49.7±0.6	49.7±1.5	50±1	50±1	0.66

(a)

Animal Model	20th day		25th day		30th day		35th day		40th day	
	SCI	RBI	SCI	RBI	SCI	RBI	SCI	RBI	SCI	RBI
R1	1	0	1	0	1	0	1	0	1	0
R5	2	4	2	4	2	0	2	0	1	0
R6	2	4	2	4	2	0	2	0	1	0
R7	2	4	2	4	2	0	2	0	1	0
R8	2	4	2	4	2	0	2	0	1	0
R9	2	4	2	4	2	0	2	0	1	0
R10	2	4	2	4	2	0	2	0	1	0

(b)

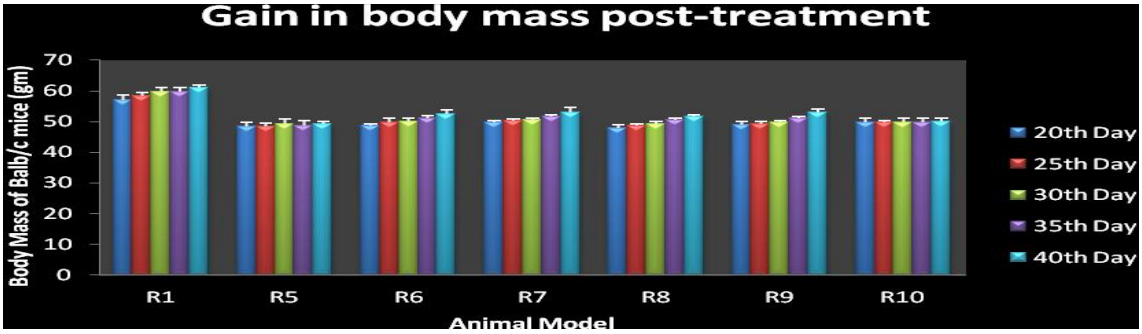


FIGURE 5.4: A comparitive study of gain of body mass for DSS induced BALB/c mice during treatment with different combinations of probiotics and prebiotics.

The comparative study represented in **TABLE 5.4 (a,b)** and **FIGURE 5.4**, it was clearly evident that all the colitis induced mice gained body mass after different combinations of treatment. Besides that, they also showed significant improvement in fecal consistency as well as prevent rectal bleeding in the course of treatment.

In this study, the degree of improvement in the colitis induced mice was represented in the terms of gain in body mass, SCI and RBI. The study suggests that, although all the combinations of treatment gave a positive result of improvement but the best result was observed in case of R9 which was treated with synbiotic (i.e., combination of PB1 to PB10 along with inulin and FOS). According to the value of SCI and RBI they are more or less same effective towards the colitis. But the significant gain in body mass in case of R9 in compare to the others was maximum.

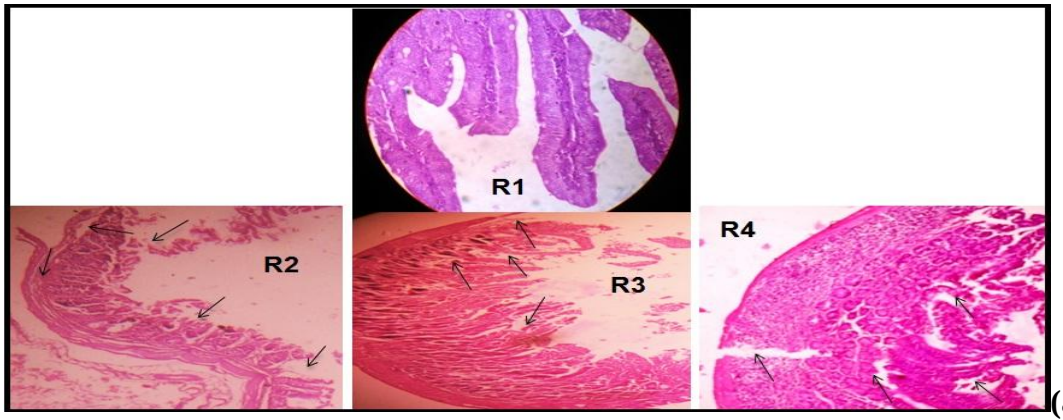
5.3.4. HISTOLOGICAL STUDY OF EACH SAMPLE:

Hematoxylin and eosin staining of intestine micrographs showed diminished inflammation in therapeutic groups as well as in the control group in compare to the diseased group. Diseased mice showed disintegration in the intestinal linnings showing damage of mucosal crypts and narrowing of the muscle lining is indicated in the **FIGURE 5.5(a,b)** by different arrows. However, synbiotic treatment normalised the tightness of the intestinal mucosa [Zarfeshani et al. 2011].

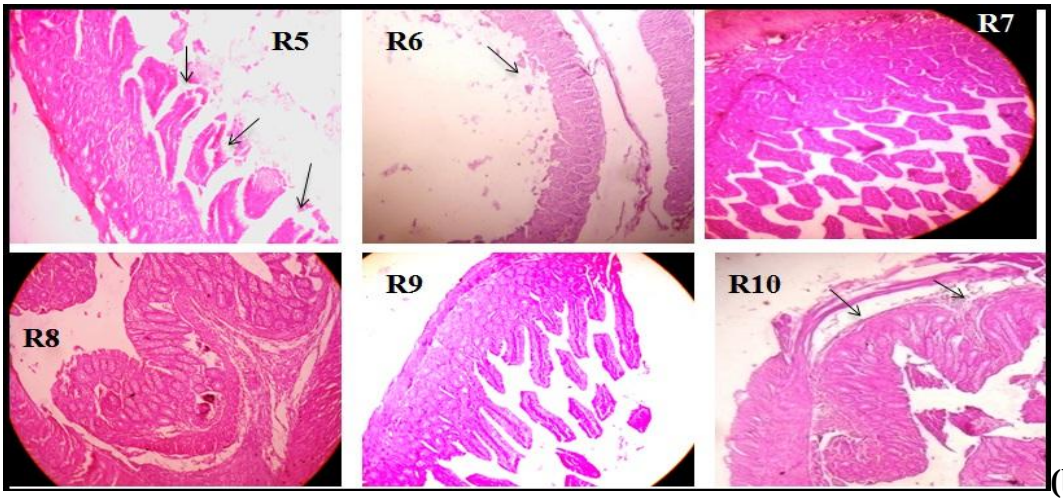
Intestinal damage could be due to dysbiosis of the intestinal environment [Tian et al. 2013].

Histological abnormalities observed in colitis (DSS-induced) can be classified as severe and/or preliminary phase. The changes in histological section includes mucin depletion, degeneration of epithelial tissue, and necrosis which leads to absence of proper linings of epithelial cells along with infiltration of neutrophils present in lamina propria and sub

mucosa, cryptitis, which is a common histological criteria of IBD that is generally reported in colitis induced by DSS models and studied by haematoxylin-eosin staining method.[Melgar *et al.*, 2005]



(a)



(b)

FIGURE5.5(a,b): The arrows indicates the irregular crypts, damage of epithelial surface, inflammations and shedding of the smooth muscle linings, infiltrations of inflammatory cells in sub mucosa, edema in sub mucosa.

In this study, it is clearly evident that the damage observed in the epithelial lining along with irregular crypt of R2 (diseased with DSS) is much more than that of R3 (diseased with TNBS) and R4 (diseased with AA). Again, in case of treatment the best damage control of the epithelial

tissue was observed in R9 (treated with synbiotics) in compare to the others. Although, R7 also showed a proper lining of the epithelial tissue but the improper lining of crypt is disqualifies it's mode of treatment to be considered as the most potent treatment.

5.3.5. SERUM BIOCHEMICAL ASSAY OF EACH SAMPLE:

Hepato-biliary disease is very common in extra-intestinal problems of IBD, where elevation of LFT (Liver Function Test) is very common in 11 to 49% of IBD patients. Abnormal LFT is defined as the increased level of serum amino-transferase (ALT, AST) and ALP. Bilirubin is itself a potent endogenous anti-oxidant that protects against lipid peroxidation. Thus, in case of reduction of bilirubin level suggests the induction Crohn's disease [Hirofumi, et al., 2007].

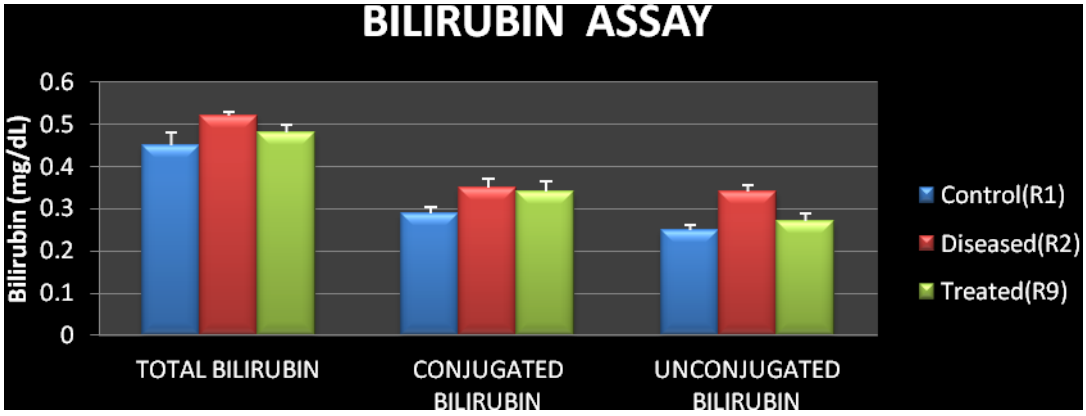


FIGURE 5.6: The evaluation of the level of conjugated bilirubin, unconjugated bilirubin and total bilirubin.

Conjugated bilirubin (CB), also known as direct bilirubin are polar and water soluble due to presence of glucoronic acid moiety. It can react with reagent without accelerator. Whereas, uncojugated bilirubin (UB) also known as indirect bilirubin is non polar and non water soluble that can react with reagent but in the presence of the accelerator.

From the study it is observed that, the bilirubin level is more or less elevated in case of diseased serum sample and decreased in the treated sample. Thus, controlling the level of bilirubin to its normal range is initiated by the treatment with synbiotics.

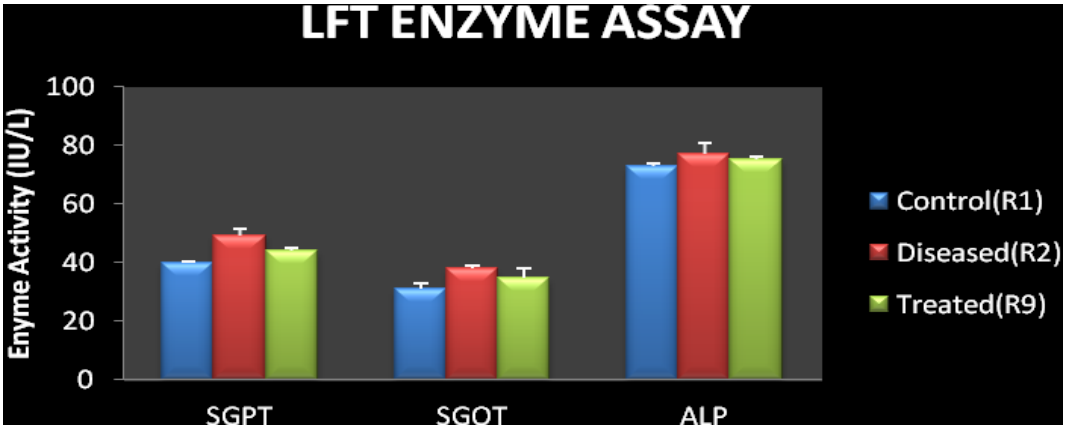


FIGURE 5.7: The comparitive study of LFT enzymes in R1, R2 and R9.

Transaminase is an enzyme that transfers the a.mino group from the amino acid to the keto acid. The two transaminases are SGOT and SGPT which is generally found in liver and kindney along with ALP. But from the research article which was alreay been published said that the abnormal in the level of these enzymes indicates the development of the disease and simultaneous treatment of the disease.

All the levels of SGOT, SGPT and ALP had shown very less increase post- disease induction and decrease post treatment administration.

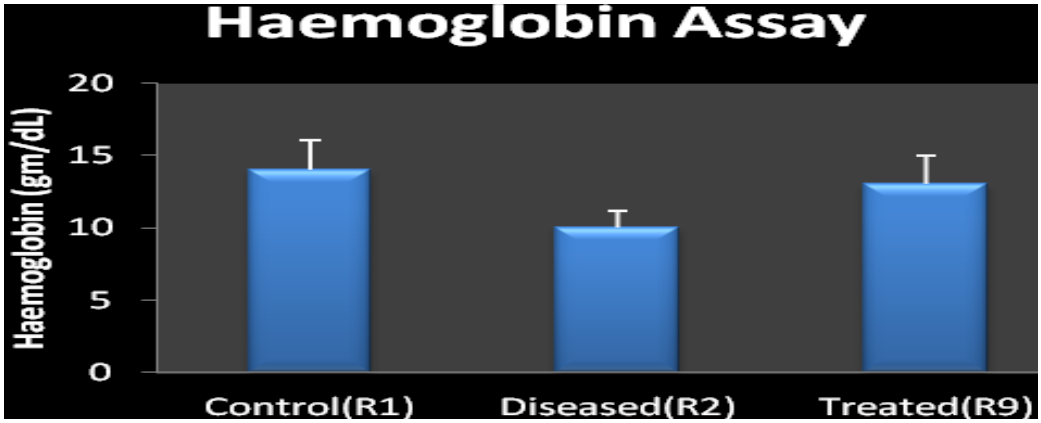


FIGURE 5.8: The haemoglogin assay of the R1, R2 and R9.

The clinicial decrease of haemogobin in the diseased mice was obseeved along with the significant rise in the level of the sample that is already treated with synbiotics.

5.3.6. FECAL MICROBIOTA ASSAY:

Fecal microbial analysis revealed some significant changes in the microbial count in different experimental model, i.e.; in the control group (R1), the diseased mice (R2) and the treated with synbiotics group (R9). However, the treatment with synbiotics seemed to decrease the count of the Gram negative anaerobic bacteria in the treated group of mice. Thus, this study suggests that the application of the synbiotics affect its gut microbiota modulation.

Generally both beneficial bacteria and commensal bacteria are present in the gut and are found to maintain equilibrium. However, due to different external factors the (i.e., dietary habits, disease conditions, etc.) the intestinal microbial balance is altered which results in increased pathogenic bacteria and decreased in beneficial microbiota in the gut [Yadav et al., 2018].

TABLE 5.5: The CFU count of the anaerobes present in the faecal matter of the animal model such as; R1, R2 and R9.

Animal Model	CFU/gm of anaerobic bacteria ± S.D.							
	5th Day	10th Day	15th Day	20th Day	25th Day	30th Day	35th Day	40th Day
R1	9.25±0.21	9.1±0.27	8.8±0.28	9.03±0.33	9.12±0.32	8.86±0.39	9.24±0.31	8.89±0.29
R2	9.51±0.19	9.22±0.18	9.21±0.17	9.29±0.18	9.25±0.16	9.08±0.18	9.55±0.15	9.11±0.20
R9	8.8±0.17	8.85±0.22	8.81±0.21	8.67±0.19	8.59±0.20	8.17±0.24	8.39±0.25	8.76±0.23

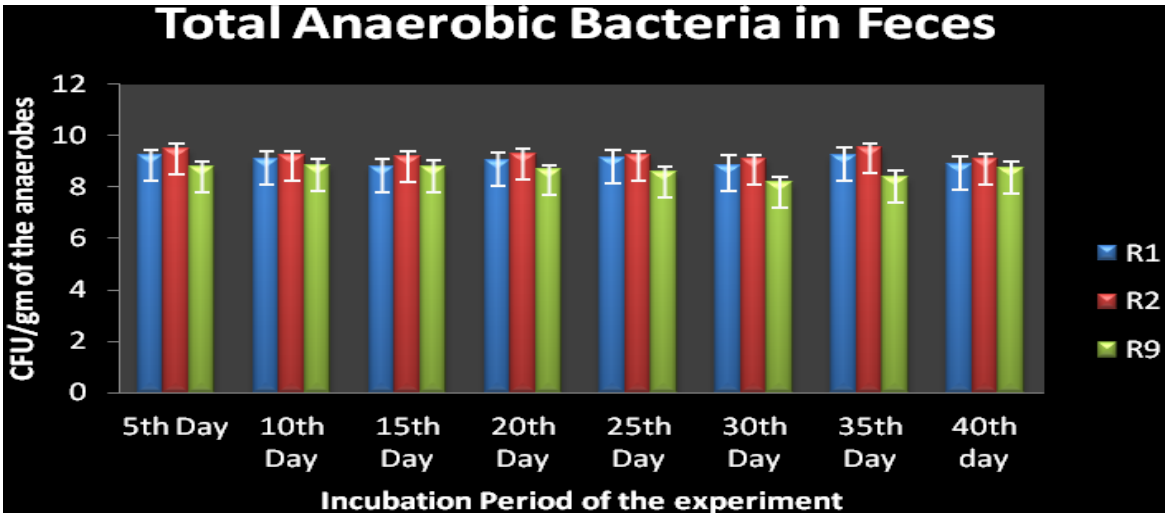


FIGURE 5.9: The comparitive study of the count of Gram negative anaerobes in the faecal matter of the animal models that is either diseased or treated with respect to the control.

It was observed that the presence of the anaerobic bacteria flactuates in the feces of the diseased animal with the treated one. The study also suggests that the observed elevation of the count of

the anaerobic bacteria in the diseased model are all Gram negative in nature as they were grown in a selective media, EMB agar. Thus, we can infer that the increased range of the anaerobes present in the diseased model are anaerobic bacteria excluding LAB group of bacteria which is Gram positive in nature. Moreover, the most of the Gram negative anaerobes includes the pathogenic bacteria which imparts health hazard to the host.

5.3: CONCLUSION:

According to the guidelines of FAO/WHO, 2002 probiotic strains should be fit for surviving in the gastro-intestinal tract which confers health benefit. Therefore, these are the properties help in selection of the probiotic strains which can withstand different adverse condition such as, high acidic environment in the stomach and extensive presence of bile salts in the gut [Tuomola *et al*., 2001]. In addition to it, probiotic strains must be capable to adhere and colonize in the GI tract. The adherent quality of probiotic strains has facilitates its ability to colonize on the gut [Smits *et al.*, 2005]. The role of mechanism of the probiotics in establishing the beneficial role which are - exclusion of pathogenic bacteria via competitive adherence, the production of antibacterial components, increased intestinal barrier function and certain defined immune-modulation, etc [Borchers *et al.*, 2009]. Probiotics play a indispensible role as preventive measure for many abnormalities present in the intestine which includes IBD. [Wollowski *et al.*, 2001].

Thus, the present study can conclude the following facts which includes that one of the best chemical inducer of the colitis in BALB/c mice is DSS; the colitis induced mice gave a very good response when treated with different combinations of probiotics and prebiotics. Based on the different parameter such as, DAI, histological assay of the intestine and the enzyme assay of liver attribute the conceptualization of the synbiotics as a very potent therapeutics on colitis.

Though, the treatment with different combination of genus specific LAB group showed better result on the basis of the gain ass but the treatment with all the selected new bacterial strain and prebiotics.