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INULIN BASED NANO-ENCAPSULATED PROBIOTICS AS IMMUNE BOOSTING AGENT AGAINST GASTROINTESTINAL TRACT INFECTIONS: A CONTROLLED STUDY IN RAT MODEL

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ABSTRACT

Probiotics have been found to be effective in the treatment of many gastrointestinal diseases, they can be considered to be therapeutic agents. Reduction in diarrhea and improvement in growth of intestinal *Bifidobacteria* and *Lactobacilli* has been observed by using the combination of inulin and the probiotic microorganisms *Lactobacillus acidophilus* plus *Bifidobacterium bifidum*. This study designed to investigate the impact of nanoencapsulation of probiotics on microbiota and inflammatory markers in rat model. Sixty adult male albino rats were distributed into 10 groups (6 rats/group) for 2 rat's trial (5 groups/trial). The first trial group containing 5 groups (6 rats/group) that named as normal trial group that remained normal (not induce diarrhea). The second trial group also contained 5 groups (6 rats/group) that named as diseased trial group (diarrhea induce in all group). The study continued for 14 days and each group was treated according to the treatment plan i.e. lactobaccili, bifidobacteria, encapsulated lactobaccili and encapsulated bifidobacteria, after which the rats were decapitated under anesthesia to get blood samples. When comparisons were made between normal trial groups and diseased trial groups, inulin based nanoencapsulated probiotics significantly ($p \leq 0.05$) increase the levels for hemoglobin (12.81±0.23 g/dL), RBC (7.32±0.03 M/µL), MCH (21.11±0.26 pg), MCV (58.60±0.30 fL) and decrease for MCHC (35.55±0.35 g/dL), WBC (13.53±0.30 $\times 10^3$ /uL), and platelets count (948.67±3.50 $\times 10^3$ /uL). Inulin based nanoencapsulated probiotics also cause a significant ($p \leq 0.05$) decrease for urea (53.00±2.36 mg/dL), creatinine (0.51±0.01 mg/dL), ALT (42.33±2.58 U/L), AST (41.16±3.18 U/L), CRP (4.61±0.24 mg/L) and ESR (4.03±0.27 mm/h). The levels of microbiota also increased after treatment with probiotics. This study supported the prospective use of inulin based nanoencapsulated probiotics in prevention and management of diarrhea.

Keywords: Probiotics, Inulin, Nanoencapsulation, Microbiota, Lactobaccili, Bifidobacteria

1. INTRODUCTION

Developing and maintaining a healthy intestinal tract is a pre-requisite for general health and aiding disease prevention. The cells of epithelial lining of the gut are the first point of contact between intestinal contents and the rest of the body. It is at this edge point where nutrition, environment and genetics come together to determine gut health and overall wellbeing (Kanwar and Kanwar, 2009). The immune system of mucosal membrane plays a key role in host defense mechanism against pathogens because the most of antigens get enter into the body through the mucosa. Moreover, the microbes in the intestinal lumen act as prime means in the development of the immune system as well as oral tolerance and immunity. The maintenance of intestinal immune and metabolic homeostasis is strongly affected by the interactions between the mucosa and the intestinal microbiota. (Delcenserie *et al*., 2008).

Bacterial gastrointestinal infections remain a significant cause of illness and death, leading to economic losses in many regions worldwide, including high-income countries with established surveillance and control measures. While the symptoms of acute bacterial intestinal infections are typically mild to moderate and often resolve on their own, there are instances where the condition can quickly worsen, resulting in a rapid decline in the patient's health (Ternhag *et al*., 2008). Bacterial diarrhea poses a significant health challenge globally, with *Escherichia coli*, *Salmonella*, *Shigella*, and *Campylobacter* being the primary culprits (Lamps, 2007). Diarrheal diseases are particularly common and lead to higher rates of illness and death among children under five years old in low-income countries (LICs). This term encompasses a wide range of infectious agents, including viruses, bacteria, protozoa, and sometimes worms, each having unique impacts (Keusch *et al*., 2016). Gastrointestinal infections (including diarrhea and gut helminth infections) can significantly impact the integrity, structure, and function of the intestinal absorptive mucosa, potentially leading to malabsorption. It has been suggested that a substantial portion of childhood malnutrition stems from compromised intestinal absorptive function caused by multiple and repeated gastrointestinal infections (Guerrant *et al*., 2008).

Probiotic is a relatively new term that means 'for life' and refers to microorganisms known for their beneficial effects on humans and animals. These microorganisms help maintain a balanced intestinal microbiome and support overall health (Soccol *et al*., 2010). Probiotics have shown effectiveness in treating certain gastrointestinal diseases, positioning them as therapeutic agents (Marteau *et al*., 2001). The health benefits associated with consuming foods that contain *Lactobacillus acidophilus*, *Bifidobacterium*, and *L. casei* are well established (Shah, 2007). The approaches to improve the stability of probiotics in food products include product fortification with probiotic substrate (prebiotics as inulin), pH adjustment, aseptic packaging, freeze-drying and refrigerated storage (Lopez-Rubio *et al*., 2009).

More recent studies have focused on the effect of specific dietary carbohydrates. Prebiotic carbohydrates, such as inulin and oligofructose, stimulate growth of Bifido-bacteria that is essential for maintenance of a healthy gut microbiota (through daily ingestion of beneficial bacteria) and it is a key to a healthy long life. Furthermore, encapsulation technique has also been used to increase the delivery of probiotics into the intestines and it serves as a shield against processing conditions and gastrointestinal delivery to be released at targeted area such as the colon (Anal and Singh, 2007).

As nanoencapsulation with nano-fibers from inulin may be a promising and suitable capsule size for optimal cell loading without compromising the textural and organoleptic properties of food products and to enhance the delivery of probiotics into the intestines, improves immunity and combat the gastrointestinal tract infections so, the present study designed to assess the immune boosting effect in normal and gastrointestinal tract infectious rat models by inulin-based nanoencapsulated probiotics.

2. MATERIALS AND METHODS

2.1 Study Design

The present study was carried out using inulin-based nanoencapsulated probiotics in the Dept. of Nutritional Sciences, GCUF Pakistan. The trial was conducted to bioevaluate the nanoencapsulated probiotics and for the purpose, sixty adult male albino rats were distributed into 10 groups (6 rats/group) for 2 rat's trial (5 groups/trial).

2.2 Ethical Considerations

All the trial processes were in accordance with the principles and techniques of the laboratory animal i.e. ARRIVE guidelines and Principles for Experimental Rat Usage (McGrath et al., 2010). All the processes conducted in the trials were approved by the Ethical Review Board of University.

2.3 Animal Handling

Male albino rats served as subjects in the in vivo gastrointestinal tract infections (diarrhea) investigations model. Animals were allowed to acclimate for a week before the trial began. For the investigation, animal models showing no outward symptoms of disease were used. Every animal was kept in controlled conditions in cages of standard size with bedding made of wood shavings. A 12-hour light schedule was supplied along with a controlled temperature (25 °C) and relative humidity (55%). Both water and food were readily accessible.

2.4 Experimental Design

The present study included 2 rats' trials that run parallel. The first trial group containing 5 groups (6 rats/group) that named as normal trial group. They remained normal (not induce diarrhea) during the whole study. The second trial group also contained 5 groups (6 rats/group) that named as diseased trial group (diarrhea induce in all group). The rats in both trials were given with normal feed and water for the first 5 days. After that, all the groups of the second trial received intraperitoneal (ip) injections with *E. coli* O_{101} ($1x10^{11}$ colony-forming units/kg) for three consecutive days. The mode of administration is intragastric administration (Sun et al., 2019). After induction, the rats were feed with treatment lactobaccili, bifidobacteria, encapsulated lactobaccili and encapsulated bifidobacteria as shown in Table 1.

Table 1: Experimental grouping and Treatment plan for all groups

Trial 1			**Trial 2**		
Groups	**Condition**	**Treatment**	**Groups**	**Condition**	**Treatment**
NG1	Normal (negative control)	No treatment + Normal feed	**DG1**	Diarrheal (positive control)	No treatment + Normal feed
NG2	NLB	Normal feed + 0.5g/kg B.W./day	**DG2**	DLB	Diseased + 0.5g/kg B.W./day
NG3	NBB	Normal feed + 0.5g/kg B.W./day	**DG3**	DBB	Diseased + 0.5g/kg B.W./day
NG4	NELB	Normal feed + 0.02g/kg B.W./day	**DG4**	DELB	Diseased + 0.02g/kg B.W./day
NG5	NEBB	Normal feed + 0.02g/kg B.W./day	**DG5**	DEBB	Diseased + 0.02g/kg B.W./day

LA= *Lactobacillus acidophilus*, BB= *Bifidobacterium bifidum*, ELB= Encapsulate *Lactobacillus*, EBB= Encapsulated *Bifidobacterium bifidum*

NG1 Negative control
NG2 Normal with treatment lactobaccili
NG3 Normal with treatment bifidobacteria
NG4 Normal with treatment encapsulated lactobaccili
NG5 Normal with treatment encapsulated bifidobacteria
DG1 Positive control
DG2 Diseased with treatment lactobaccili
DG3 Diseased with treatment bifidobacteria
DG4 Diseased with treatment encapsulated lactobaccili
DG5 Diseased with treatment encapsulated bifidobacteria

2.5 Evaluation of physical parameters

Feed intake was measured daily, excluding any feed that was spilled or not consumed. Feed intake was tracked throughout the entire study period. Water was also provided in measured amounts according to guidelines, and water consumption was recorded on a daily basis. This approach is based on the research by Wolf & Weisbrode (2003). Weight of rats was measured on weekly basis and the changes in weight were noted to check the treatment effectiveness.

2.6 Blood and Serum Collection

The blood was collected in specialized vials after the dissection of rats under chloroform anesthesia. It was allowed to clot and centrifuged at 3000 rpm for 10 minutes to achieve serum (lsmail & El-Gawad, 2010). The serum was carefully separated in clean Eppendorf tubes and stored properly for further use.

2.7 Complete Blood Count Examination

After the in vivo experiment, hematological study of blood parameters of the treated rats was carried out. Blood sample (2ml) was collected from their jugular vein with a disposable syringe and needle and immediately transferred into sterile Ethylene Diamine Tetra-acetic Acid (EDTA) embedded vials for hematological study of total erythrocyte (RBC), leukocyte (WBC) counts, Hematocrits, Hemoglobin (Hb) content and Platelets (PLT) count. Various hematological indices were calculated from the results obtained. These include Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) (Falana et al., 2016).

2.8 Safety Analysis

It includes the Liver function tests (LFTs) and Renal function tests (RFTs). The second sample put in plain tube without anticoagulant for collecting serum after centrifugation of the sample for 10 min at 3000 rpm, serum samples were collected and then kept frozen at − 20 °C until further analysis. Serum samples were used for analysis of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline Phosphatase (ALP), and concentrations of urea, serum creatinine and uric acid (Shehta et al., 2022).

2.9 Inflammatory Markers

Blood inflammatory biomarkers including highly sensitive C-reactive protein and erythrocyte sedimentation rate (ESR). CRP as a diagnostic marker of inflammatory diarrhea was superior to the other inflammatory markers and clinical characteristics (Kim *et al*., 2013). The micro-ESR is a method of obtaining the ESR using capillary tubes and quicker testing times. This method uses four drops of capillary blood drawn via fingerstick, then mixed in a 4:1 ratio on a slide with a 3.8 percent sodium citrate solution. The sample is then drawn into a 7.5-centimeter heparin-free microhematocrit capillary tube. The results are measured at just 20 minutes and then adjusted to predict conventional ESR values from the micro-ESR value (Hashemi *et al*., 2015).

2.10 Microbiota Analysis

In order to check the amount of Lactobaccilus, Bifidobacterium and *E. coli* in the feces, we performed Reverse Transcriptase PCR (RT-PCR). For this purpose, the fecal sample was taken from ileocecal junction. First, we isolate genomic DNA. To extract and isolate genomic DNA, chromosomal DNA must be dislodged from the cell matrix. Fecal DNA was extracted by boiling the sample, followed by centrifugation, protein digestion with Proteinase K, phase separation using chloroform and isopropanol, and final purification in buffer. The extracted DNA was stored at –20 °C. For RT-PCR 0.5µl dNTPs were mixed with 2.5µl F101 buffer. Forward and reverse (0.5 µl each) and amplified cDNA in a concentration of 100ngµl were used. After adding 25 µl ddH_2O and 0.25µl Taq-polymerase the PCR was run with the following program (Köchl et al., 2005).

2.11 Statistical Analysis

The data was analyzed through completely randomized design (CRD). The results are mentioned as mean of three with mean ± standard error of the mean (SEM.). To compare the different experimental groups following ANOVA, the least significant difference (LSD) test was used. Statistical analysis was performed using Statistics 8.1 software. Values with $p < 0.05$ were considered statistically significant (Fathy *et al*., 2019).

3. RESULTS

3.1 Evaluation of Physical Parameters

The experimental animals were monitored daily to measure their food and fluid intake. All rats were given with *iso-caloric* and *iso-nitrogenous* diet and plenty of water.

3.1.1 Body Weight Changes and Feed and Water Intake

Mean body weight changes between the groups and weeks for both the trials (Normal and Diseased) are illustrated in Figure 1. For the first trial, the body weight was observed to be significantly different ($p \leq 0.05$) only between the weeks ($p=0.00$) while there was no significant difference between the groups ($p=0.91$) and between weeks and groups ($p=0.51$). For the second trials, the weight was observed to be significantly different ($p \leq 0.05$) between the groups (0.00), between the weeks (0.00) but there was no significant difference between weeks and groups ($p=0.41$). Box plot comparing weight in different groups among weeks are provided in Figure 1. The box shows the median value and the 25^{th} and 75^{th} percentiles while the whisker shows the min and max values. Box plot shows significant results between different groups. It was observed that the highest body weight was seen in diseased with treatment encapsulated bifidobacteria (DG5). Positive control group (DG1) shows the lowest weight mean compared to all others groups of the study. Likewise, there was an increasing trend in mean body weight changes between the weeks in both trials. The weight significantly increased in 2^{nd} week as compared to 1^{st} week. The variation in feed and water intake of the different groups of both trial (normal and diseased) during the whole study have been shown in Figure 2. The Figure 2(a) shows the scattered graph between days and average intake in normal trial which was observe to be the highest 23 g/day (feed) on day 9 and 18 ml/day (water) on day 13 while, the lowest was 19 g/day (feed) on day 5 and 15 ml/day (water) on day 14 irrespective of the groups. Similarly, the Figure 2(b) shows the scattered graph between days and average intake in diseased trial which was observe to be the highest 23.5 g/day (feed) on day 13 and 17 ml/day (water) on day 3 while, the lowest was 17 g/day (feed) on day 8 and 12 ml/day (water) on day 7 irrespective of the groups. In second trial, the decrease in feed and water intake during the mid of the study was due to the induction of diarrhea disease.

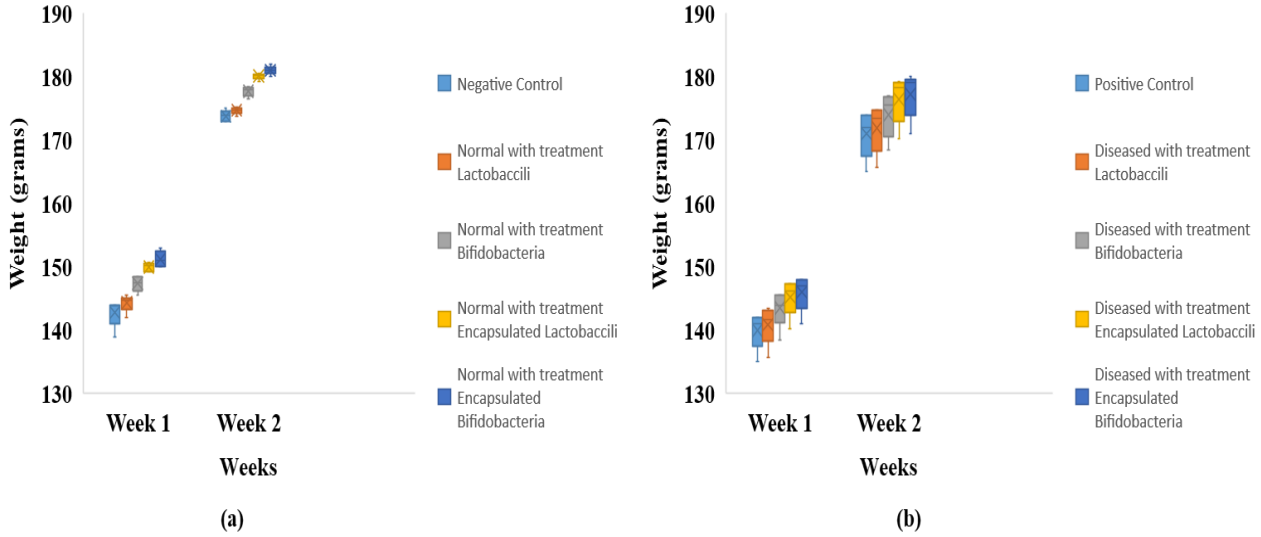

Figure 1: Box plot showing the differences in body weight among various study groups in weeks. The line in the center of each box indicates the median of the groups analyzed, while the edges of the box represent the first and third quartiles (a) Groups of Normal trial, (b) Groups of Diseased trial

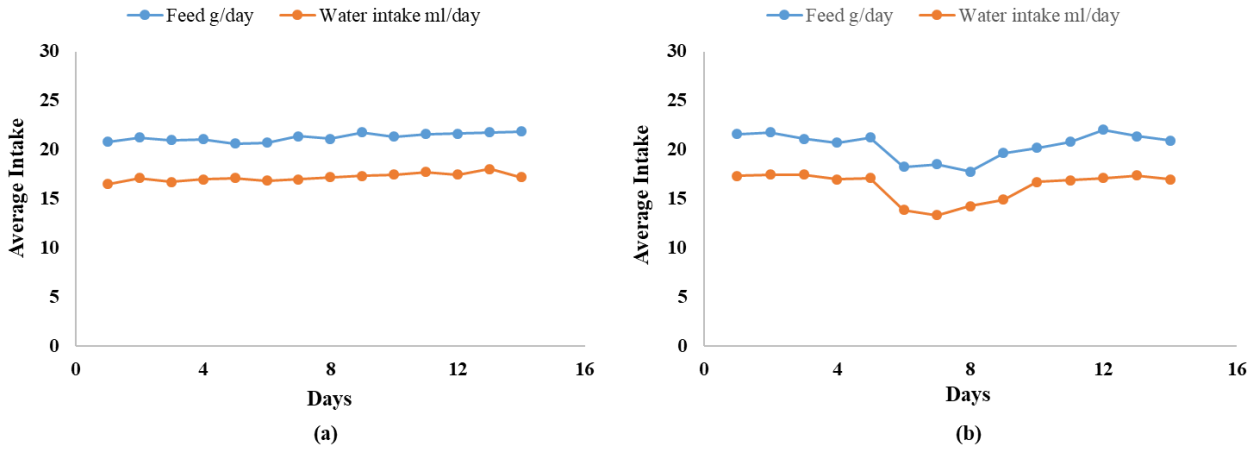

Figure 2: Mean changes in feed (g/day) and water (ml/day) between days (a) Average intake of normal trial rats (b) Average intake of diseased trial rats

3.2 Biochemical Parameters

The serum samples of the male albino rats were s]ubjected to biochemical analysis in relation to CBC test, inflammatory markers and safety profile (including liver function and kidney function tests).

3.2.1 Hematological Parameters

The mean concentrations for Hb (g/dL), RBCs (M/µL), HCT (%), MCV (fL), MCH (pg), MCHC (g/dL), WBCs ($\times 10^3$ /uL) and PLT ($\times 10^3$ /uL) for rats in various groups of normal trial are illustrated in Table 2. The results indicated a significant difference ($p \leq 0.05$) between the groups. From the table, it is obvious that all the group shows normal values closely related to each other. The groups (NG2, NG3, NG4, and NG5) that were given with treatments (i.e. LB BB, ELB, EBB) showed high values as compared to negative control group (NG1). Normal with treatment encapsulated lactobaccili (NG4) showed highest values for Hb, RBCs, HCT, MCV and WBCs while, remaining hematological parameters showed highest values in negative control group (NG1). Similarly, the mean concentrations for all these hematological parameters for rats in different groups of diseased trial are illustrated in Table 3. The results indicated a significant difference ($p \leq 0.05$) between the groups. The

highest values for Hb, RBCs, HCT, MCV, and MCH were noticed in diseased with treatment encapsulated bifidobacteria (DG5) while MCHC, WBCs and PLT showed highest values in positive control group (DG1). All the treatment groups (DG2, DG3, DG4, DG5) showed effective results for all the hematological parameters.

3.2.2 Determination of Inflammatory Markers

The mean concentrations of CRP (mg/L) and ESR (mm/h) for rats in various study groups of normal trial are illustrated in Table 2. The results showed a significant difference ($p \leq 0.05$) among all study groups of normal trial. The highest mean concentration for CRP was seen in normal with treatment encapsulated lactobaccili (NG4) while for ESR, it was found in normal with treatment lactobaccili (NG2). Moreover, the mean concentrations of these inflammatory markers for rats in different study groups of diseased trial are illustrated in Table 3.

It is obvious from the table the results showed a significant difference ($p \leq 0.05$) among diseased study groups. The highest and lowest value of both CRP and ESR in diseased groups was seen in positive control group (DG1) and diseased with treatment encapsulated bifidobacteria (DG5), respectively.

3.2.3 Determination of Liver and Renal Function Tests

The mean concentrations for rats in groups of normal trial has shown a significant difference ($p \leq 0.05$) for Alkaline phosphate (ALP, U/L), Alanine aminotransferase (ALT, U/L), Aspartate aminotransferase (AST, U/L), urea (mg/dL), creatinine (mg/dL) and uric acid (mg/dL) among different study groups (Table 2). All these safety analysis parameters shows highest value in normal control group (NG1).

Moreover, all the other groups (NG2, NG3, NG4, NG5) shows results closely related to the normal control group (NG1). Likewise, the mean concentrations of LFTs and RFTs for rats in various groups of diseased trial are illustrated in Table 3. The results indicated a significant difference ($p \leq 0.05$) between the groups. In diseased trial, the highest and lowest value for all the parameters was seen in positive control group (DG1) and diseased with treatment encapsulated bifidobacteria (DG5), respectively.

3.2.4 Microbiota Analysis

The gene expression of Lactobaccili, Bifidobacteria and *E. coli* among rats of different study groups in normal trial are illustrated in Table 2. The results indicated a significant difference ($p \leq 0.05$) between the groups. From the table, it is obvious that the gene expression of lactobaccilus increase and highest is expressed by normal with treatment encapsulated bifidobacteria (NG5). Similarly, the microbiota bifidobacterium shows the same pattern with highest gene expression in normal with treatment encapsulated bifidobacteria (NG5).

In contrast, *E. coli* shows highest gene expression in negative control group (NG1). The gene expression among rats of different study groups in diseased trial has shown a significant difference ($p \leq 0.05$) for Lactobaccili, Bifidobacteria and *E. coli* (Table 3). Both microbiota (Lactobaccili and Bifidobacteria) shows the same gene expression with highest value expressed by diseased with treatment encapsulated lactobaccili (DG4).

E. coli shows the highest gene expression in positive control group (DG2) with no treatment and disease diarrhea that induced through microbiota *E. coli*.

Table 2: Concentration of hematological parameters, safety analysis, and inflammatory markers in blood and microbiota in feces (Mean± SD) for different study groups in normal trial

Parameters	NG1	NG2	NG3	NG4	NG5
Hematological Parameters					
Hb (g/dL)	12.45±0.24^{c}	13.34±0.38^{a}	12.83±0.21^{b}	13.43±0.28^{a}	12.68±0.29^{bc}
RBCs (M/µL)	6.43±0.035^{d}	6.57±0.17^{c}	6.91±0.06^{b}	7.11±0.07^{a}	6.83±0.03^{b}
HCT (%)	36.40±0.30^{d}	37.66±0.29^{c}	38.45±0.35^{b}	40.55±0.28^{a}	38.30±0.23^{b}
MCH (pg)	19.53±0.21^{a}	18.73±0.21^{b}	18.66±0.30^{b}	18.80±0.26^{b}	18.30±0.23^{c}
MCV (fL)	53.31±0.23^{d}	54.30±0.35^{c}	55.56±0.30^{b}	56.46±0.31^{a}	55.60±0.32^{b}
MCHC (g/dL)	36.43±0.28^{a}	32.41±0.28^{e}	33.30±0.26^{d}	34.56±0.30^{b}	33.63±0.24^{c}
WBCs (×10^{3} /uL)	12.35±0.27^{b}	14.21±0.26^{a}	11.56±0.42^{c}	14.38±0.33^{a}	11.48±0.33^{c}
PLT (×10^{3} /uL)	1044.5±2.42^{a}	1023.2±2.63^{c}	1012.5±3.08^{d}	1035.8±2.78^{b}	1025.0±2.60^{c}
Liver Function Tests					
ALP (U/L)	531.83±2.31^{a}	525.33±2.80^{b}	514.67±3.77^{c}	496.67±3.98^{e}	505.83±3.18^{d}
ALT (U/L)	34.83±2.31^{a}	30.83±2.31^{b}	28.16±2.31^{c}	26.50±1.87^{c}	34.00±1.78^{a}
AST (U/L)	44.83±2.31^{a}	43.00±2.82^{ab}	41.66±2.16^{bc}	39.00±2.36^{c}	39.83±2.31^{c}
Renal Function Tests					
Creatinine (mg/dL)	0.64±0.04^{a}	0.56±0.05^{b}	0.48±0.04^{c}	0.56±0.04^{b}	0.57±0.04^{b}
Urea (mg/dL)	49.50±1.87^{a}	49.00±2.60^{a}	47.00±2.36^{a}	43.33±2.16^{b}	47.66±1.86^{a}
Uric Acid (mg/dL)	2.33±0.29^{a}	2.25±0.28^{a}	1.93±0.21^{bc}	1.83±0.21^{c}	2.20±0.23^{ab}
Inflammatory Markers					
CRP (mg/L)	4.19±0.13^{c}	4.43±0.12^{b}	4.28±0.08^{bc}	4.79±0.12^{a}	4.69±0.14^{a}
ESR (mm/h)	2.26±0.32^{d}	4.25±0.32^{a}	3.56±0.25^{b}	3.11±0.21^{c}	2.31±0.26^{d}
Microbiota Analysis					
Lactobaccilus	0.95±0.02^{a}	0.89±0.03^{b}	0.89±0.03^{b}	0.97±0.03^{a}	0.98±0.01^{a}
Bifidobacterium	0.97±0.02^{a}	0.87±0.02^{b}	0.82±0.03^{c}	0.98±0.02^{a}	1±0.03^{a}
E. coli	0.98±0.02^{a}	0.84±0.03^{b}	0.96±0.02^{a}	0.98±0.03^{a}	0.95±0.03^{a}

$^{a-e}$ Different letters in a row show significant difference at $p \leq 0.05$, Hb= Hemoglobin; RBCs= Red blood cells; HCT= Hematocrit; MCH= Mean corpuscular hemoglobin; MCV= mean corpuscular volume; MCHC= Mean corpuscular hemoglobin concentration; WBCs= White blood cells; PLT= Platelets count; ALP= Alkaline phosphate; ALT= Alanine aminotransferase; AST= Aspartate aminotransferase; CRP= C-Reactive proteins; ESR= Erythrocyte sedimentation rate

Table 3: Concentration of hematological parameters, safety analysis and inflammatory markers in blood and microbiota in feces (Mean± SD) for different study groups in diseased trial

Parameters	DG1	DG2	DG3	DG4	DG5
Hematological Parameters					
Hb (g/dL)	9.35±0.30^{e}	10.53±0.25^{d}	11.60±0.33^{c}	12.40±0.28^{b}	12.81±0.23^{a}
RBCs (M/µL)	6.44±0.03^{e}	7.15±0.03^{c}	6.71±0.04^{d}	7.21±0.03^{b}	7.32±0.03^{a}
HCT (%)	36.33±0.28^{d}	37.20±0.26^{c}	38.13±0.20^{b}	38.26±0.25^{b}	40.68±0.26^{a}
MCH (pg)	18.65±0.28^{d}	18.83±0.21^{d}	19.76±0.19^{c}	20.20±0.14^{b}	21.11±0.26^{a}
MCV (fL)	53.31±0.26^{e}	53.96±1.00^{d}	55.33±0.31^{c}	56.40±0.26^{b}	58.60±0.30^{a}
MCHC (g/dL)	38.50±0.30^{a}	37.40±0.35^{b}	36.30±0.23^{c}	36.31±0.31^{c}	35.55±0.35^{d}
WBCs (×10^{3} /uL)	20.66±0.43^{a}	16.21±0.23^{b}	15.26±0.32^{c}	14.68±0.29^{d}	13.53±0.30^{e}

PLT (×10³ /uL)	1042.8±3.31[a]	1036.0±2.19[b]	1019.3±2.58[c]	992.33±4.32[d]	948.67±3.50[e]
Liver Function Tests					
ALP (U/L)	904.83±3.71[a]	805.17±3.71[b]	627.00±4.38[c]	553.83±3.92[d]	496.50±4.76[e]
ALT (U/L)	63.66±2.80[a]	62.16±2.63[a]	54.99±2.60[b]	50.50±1.87[c]	42.33±2.58[d]
AST (U/L)	56.83±2.31[a]	54.66±3.55[a]	54.50±2.42[a]	45.00±2.89[b]	41.16±3.18[c]
Renal Function Tests					
Creatinine (mg/dL)	0.84±0.02[a]	0.74±0.02[b]	0.63±0.02[c]	0.55±0.02[d]	0.51±0.01[e]
Urea (mg/dL)	59.16±2.85[a]	56.00±2.60[b]	53.66±2.58[b]	42.83±2.31[c]	53.00±2.36[b]
Uric Acid (mg/dL)	4.60±0.26[a]	4.28±0.23[b]	3.33±0.28[c]	2.70±0.23[d]	2.26±0.21[e]
Inflammatory Markers					
CRP (mg/L)	5.73±0.21[a]	5.26±0.21[b]	5.06±0.27[b]	5.18±0.19[b]	4.61±0.24[c]
ESR (mm/h)	9.43±0.39[a]	6.95±0.27[b]	5.85±0.36[c]	5.15±0.18[d]	4.03±0.27[e]
Microbiota Analysis					
Lactobaccilus	0.46±0.03[d]	0.62±0.03[c]	0.68±0.03[b]	0.74±0.02[a]	0.69±0.02[ab]
Bifidobacterium	0.35±0.03[c]	0.54±0.03[b]	0.64±0.04[a]	0.66±0.03[a]	0.63±0.03[a]
E. coli	2.78±0.03[a]	1.65±0.03[d]	2.12±0.05[b]	1.48±0.03[e]	1.74±0.03[c]

[a-e] Different letters in a row show significant difference at $p \leq 0.05$, Hb= Hemoglobin; RBCs= Red blood cells; HCT= Hematocrit; MCH= Mean corpuscular hemoglobin; MCV= mean corpuscular volume; MCHC= Mean corpuscular hemoglobin concentration; WBCs= White blood cells; PLT= Platelets count; ALP= Alkaline phosphate; ALT= Alanine aminotransferase; AST= Aspartate aminotransferase; CRP= C-Reactive proteins; ESR= Erythrocyte sedimentation rate

4. DISCUSSION

Probiotics have shown effectiveness in treating certain gastrointestinal diseases, positioning them as therapeutic agents (Marteau *et al*., 2001). The health benefits associated with consuming foods that contain *Lactobacillus acidophilus*, *Bifidobacterium*, and *L. casei* are well established (Shah, 2007). In accordance with the present study, Nasri et al. (2022) studied that the weight of rats in group 1 (negative control) decreased on the day 14th of the experiment because the group was only induced with *E.coli*. However, groups 3, 4, and 5 given *Lactobacillus fermentum* showed an increase in their weight. The results of the homogeneity test indicated that the data were homogeneous (p=0.378). Meanwhile, there was a significant difference in term of weight gain of rats between the 0th and the 14th day of observations (p=0.001). There was also a significant difference in term of weight gain of rats between the 0th and the 21st day of observations (p=0.000). The decrease in weight of the rats in the control group was caused by *E.coli* that induced digestive tract infections which consequently disrupted the absorption of nutrients in the feed in the rat's intestines. In contrast, increase in weight was observed in the groups treated with *Lactobacillus fermentum*. This fact indicated that the intestinal mucosas of the rats were protected by the presence of these bacteria. Diarrhea can also result in fever, abdominal pain, decreased appetite, fatigue, and weight loss (Luthfiana & Utami, 2016). Diarrhea is usually associated with weight loss which ultimately leads to malnutrition. This clinical condition and requires nutrition intake to improve nutritional status (Giannattasio et al., 2016). Hemoglobin (HB) is a protein located in red blood cells that transports oxygen from the lungs to the body's tissues and returns carbon dioxide back from the tissues to the lungs. It is essential for maintaining physiological homeostasis and supporting metabolic needs (Ahmed et al., 2020). Hematocrit provides important information about the overall blood volume; in addition, it tended to be higher at birth and then decreased with age. Furthermore, hemoglobin is related to the rate of

oxygen transported in the bloodstream (Malheu, 2007). Shehta et al. (2022) concluded that bacterial diarrhea has a negative impact on the clinical and hemato-biochemical profile of the neonatal calves with diarrhea. Bibi et al. (2024) found that there was a substantial increase in hemoglobin and RBCs related to the control in male and female rats. Compared with the control group results, the standard group and other treatment groups displayed significant variation (P=0.001) in WBC count and lymphocyte levels. Among the groups of male rats, HCT, MCHC, platelets, and neutrophil values showed significant differences. MCH exhibited no significant differences compared to NC and 0-day. However, in female rat groups, values of Hb, MCH, WBC, basophils, MCHC, RBC, MCV, platelets, neutrophils, and lymphocytes indicated significant differences. Aboderin et al. (2006) investigated the effect of oral administration of different doses of probiotic, *Lactobacillus plantarum*. There was a significant increase in hemoglobin and red blood cell when compared with the control in rat. Shehta et al. (2022) also studied that the mean values of hematological indices in diarrheic calves showed significant reduction in total erythrocyte count and hemoglobin.

While, there was a significant increase in the total white blood cell count, neutrophils and HCT compared to the results of the control group. The increase in WBCs and neutrophilia in calves suffered from diarrhea than apparently healthy group is due to the infection by pathogenic *E. coli* and *Salmonella* spp. According to Khan and Zafar (2005), haemtological studies are useful in the diagnosis of many diseases as well as investigation of the extent of damage to blood. Khan and Zafar (2005) also stated that hematological parameters are good indicators of the physiological status of animals.

To assess the damage on liver tissues, hepatic enzymes concentrations are investigated since high levels of these enzymes indicate damage to mitochondria and cell membranes. Shehta et al. (2022) found a significant ($p < 0.05$) increase in blood urea nitrogen, creatinine, ALT and AST levels, while total protein, serum albumin and glucose concentrations showed significant ($p < 0.05$) decreases in the diarrheic calves compared to healthy calves. Blood urea nitrogen and creatinine concentrations were elevated in the diarrheic calves that might be due to deficit in renal blood perfusion thus reducing urine formation and alteration in renal function as hyponatremia, hypochloremia and hyperkalemia as previously reported by Singh et al. (2014). Inflammation of GIT and subsequently pathological affection reflected on the liver might be the cause of elevation of serum AST and ALT.

On the other hand, serum glucose, Albumin and total protein levels were diminished in the diarrheic calves due to the excretion of those parameters in the intestinal lumen with diarrhea, this was in agreement with Constable et al. (2016) and Choi et al. (2021). Park et al. (2019) studied that CRP at a cut-off value of 13.7 mg/L showed moderate diagnostic sensitivity (83.3%) and specificity (68.2%) with a 29% prevalence of bacterial diarrhea in this study.

These results were similar to a prior study reported by Berger et al. (1996) in which a CRP cut-off value of 20 mg/L showed a sensitivity of 83% and a specificity of 67% at a 24% prevalence of serious bacterial infections (Sanders et al., 2008).

The findings are also in line with previous reports indicating that CRP has moderate sensitivity and specificity to detect bacterial infection in children with fever and can discriminate inflammatory from non-inflammatory diarrhea in young adults without underlying gastrointestinal diseases (Kim et al., 2013). Shehta et al. (2022) revealed that 76% of the diarrheic calves had pathogenic *E. coli* and a 24% had *Salmonella species* isolated from the fecal samples. *Salmonella species* and *E. coli* are known as the most common pathogens identified in diarrheic calves. These results were consistent with previous findings reported by and El-Seadawy et al. (2020).

5. CONCLUSION

In patients with gastrointestinal tract infections (diarrhea), probiotics and prebiotics may be linked to improvements in hematology, inflammatory biomarkers and microbiota.

The present research has demonstrated the use of inulin based nanoencapsulated probiotics for the administration of GIT tract infections. It has been demonstrated to correct hematological parameters, liver biomarker levels, kidney function tests, and inflammatory indicators. It is concluded that bacterial diarrhea has a negative impact on the clinical and hemato-biochemical profile but with the use of probiotics along with prebiotics shows positive impact on clinical and biochemical profile. This study will helpful to control the issues related to gastrointestinal tract infections. Future research can be done to extend the use of probiotics and nanoencapsulation of others commonly consumed food.

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