The synergistic effects of dietary fibers and probiotics on gut bacteria, microbial balance, and inflammatory markers against castor oil–induced diarrhea in mice

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Abstract

This study was conducted in the postgraduate laboratories of the College of Agriculture, University of Baghdad, and in the animal house of the College of Veterinary Medicine, University of Tikrit, to determine the synergistic effect of dietary fiber and probiotics on intestinal bacteria, microbial balance, and inflammatory markers in mice with diarrhea treated with castor oil.The study results showed that administering castor oil to albino mice led to a significant increase ($p \leq 0.05$) in the levels of the inflammatory markers TNF-α and IL-6, and also caused a significant increase in the number of intestinal bacteria, while the number of lactic acid bacteria decreased.The results indicated positive effects represented by a decrease in the level of inflammatory factors and an increase in the total number of lactic acid bacteria in the intestines when oral administration of the probiotic *Lb. acidophilus* alone or with feeding with carrot powder or brown rice or both, and the total number of colonic bacteria decreased as a result of the increase in the number of lactic acid bacteria.We conclude that oral administration of the probiotic and feeding with dietary fiber powder improved parameters of inflammatory factors and microbial balance in male rats with diarrhea.

Keywords: Carrot powder - Brown rice powder - Castor oil - Diarrhea - Probiotic - Mice - Inflammatory factors - Microbial balance

Introduction

The digestive system operates within a precise physiological mechanism aimed at regulating fluid volume within the digestive tract through a balance between secretion, absorption, and intestinal motility. Any disruption to these mechanisms directly affects the fluid balance within the intestinal lumen.A normal intestine receives 8 to 10 liters of fluid daily from ingested food and intestinal secretions. Under the influence of intestinal motility, most of this fluid is reabsorbed by the small intestine. The colon absorbs 1.5 to 2.0 liters, and approximately 200 ml is excreted in the faeces. Therefore, decreased absorption, increased motility, increased secretion of the small intestine, or decreased absorptive capacity of the colon can lead to diarrhea [17].The World Health Organization defines diarrhea as having at least three loose or watery stools per day (or more frequent bowel movements than usual for the affected person). It is an abnormal excretion of stool, especially watery stools, exceeding 300 grams per day or 350 ml/24 hours.Diarrhea is characterized by an increase in stool water content, volume, or frequency [37]. It can be non-infectious (inflammatory, neoplastic, or drug-induced) or infectious (bacterial, viral, parasitic, or fungal). In the latter case, 70% of diarrhea is a symptom of an intestinal infection, which can be caused by one of the major pathogens implicated in humans: *Shigella, Staphylococcus aureus, Escherichia coli, or Salmonella Typhi* [2].Diarrheal diseases are a leading cause of illness and death worldwide and constitute a significant public health problem. According to the World Health Organization [36], it is estimated that there are approximately 3 to 5 billion cases of diarrhea each year, resulting in about 5 million deaths.Diarrheal diseases are the second leading cause of death in children under five years of age, affecting 1 billion cases and 2.5 million deaths each year [41-7].Treatment of diarrheal diseases generally aims to correct their pathophysiological characteristics, as diarrhea is usually accompanied by symptoms. Fluid and electrolyte loss is controlled using oral rehydration salts (ORS), vomiting is controlled using antiemetics (metoclopramide), and weight loss is achieved through dietary measures such as protein and calorie intake.Treatment can also be specific, using transit slowers (Loperamide) and antispasmodics (Phloroglucinol) for motility diarrhea, antisecretory drugs (Tiorphan) for secretory diarrhea, while intestinal dressings (Diosmectite) are prescribed for inflammatory or infectious diarrhea.Macrolides, quinolones, and nitroimidazole are also used. Treatment for the cause of diarrhea depends on the type of microbe, the antibiotic, and contraindications 10-22].Despite numerous advances in conventional medicine, the treatment of diarrhea remains unsatisfactory and urgent therapeutic challenges, exacerbated not only by the side effects of chemical drugs such as causing bronchospasm, vomiting (racicadotril), intestinal obstruction, constipation (Loperamide), and addiction, but also by bacterial resistance to commonly used antibiotics, due to their often inappropriate and indiscriminate use [20] and the rapid spread of resistant pathogens within communities. In addition, the high cost of pharmaceutical products and their unaffordability [21].All these reasons prompted researchers to return to nature again, despite the great strides they made in the sciences of chemistry and drugs and the development in these fields. Due to the growing public health awareness in recent years, there has been an increased demand for natural sources of treatment for diseases as an alternative to chemical treatments. Among these treatments are medicinal plants and herbs. Every plant found in nature contains substances and elements that represent a complete pharmacy, and the body can easily and naturally interact with its contents to rid itself of diseases and their causes, some of which have been described as resistant to chemical drugs [12].The use of medicinal plants and herbs has been widespread throughout the ages as a means of treating many diseases without knowing their contents or the nature of the interactions that occur or cause them for treatment. These prompted humans over the years to conduct research and studies and use medicinal and herbal plants in the manufacture of medicines due to their medical and physiological effects [40]. Subsequently, the science of human nutrition developed and became based on research and studies that showed the negative and positive effects of each element in any substance, whether natural or manufactured. This was accompanied by the progress in the field of functional and therapeutic foods, which have a major and effective role in the field of human health and have proven their ability to reduce the risk of many diseases and enhance the health of the human body, especially when adding probiotics, which are distinguished from other microorganisms by many therapeutic properties such as their resistance to organisms that cause food poisoning, enhancing the body's protective and immune status, improving the state of the intestinal flora in humans, restoring balance within the intestines, and ridding the body of fats through multiple mechanisms, which increased their use in many medical conditions [11]. and quality of two grapevine cultivars (*Vitis vinifera* L.) [4].

Material and Methods

The carrots were obtained from the local market, while the brown rice was obtained from a supermarket in Al-Muqdadiyah (Indian made), and the castor oil was obtained from a pharmacy in Diyala.

Preparing High-Fiber Plants:

First: Food Preparation

1- Carrot Powder Preparation: Carrots were obtained from the Al-Muqdadiyah market. Carrot powder was prepared by slicing the carrots after cleaning and washing them according to [32], then boiling them for 5 minutes. They were then dried in an oven at 40-50°C for 24 hours, ground in a Willy grinder, and stored until needed.

2- Brown Rice Powder Preparation: As mentioned in[9], the rice was boiled and then dried at 120°C in an electric oven for 5-6 hours, or until dry. It was then ground and stored until needed.

Second: Preparation of the Bacterial Suspension

The bacterial suspension was prepared from the probiotic *Lactobacillus acidophilus* by culturing the isolate in 100 ml of liquid skim milk in volumetric flasks and incubating anaerobically at 37°C for 48 hours. Then, 1 ml of the bacterial suspension was withdrawn and transferred to 9 ml of liquid skim milk at 37°C for 24 hours. It was then compared with McFarland's solution at a concentration of 0.5 to obtain (1.5×10^8 cells/ml). The animals were given 1 ml daily.

Chemical composition of carrot and brown rice powder

Chemical analysis tests were carried out on carrot and brown rice powder (moisture, ash, protein, fat, fiber, and carbohydrates).

Animals and Groups

Preparation of Laboratory Animals:

The laboratory animals (male Albino mice) were obtained from Tikrit University / College of Veterinary Medicine, numbering 42 mice, with ages ranging between (3-4) weeks and weighing 20-25 grams. They were confirmed to be free of diseases through examination by the veterinarian present in the animal house, in addition to monitoring them for a week in terms of health indicators and motor activity. The animals were placed in controlled conditions in terms of ventilation and temperatures, which ranged between 25 ± 2 °C, with humidity up to 50%, and a lighting period of (12:12 hours) inside the animal house at Tikrit University, in special plastic cages with metal mesh covers designed for this purpose. These cages are cleaned and disinfected with 70% ethanol alcohol, and the floor is covered with wooden shavings, which are changed twice a week regularly to avoid disease and minimize losses as much as possible. The animals are supplied with filtered water through the container attached to the cage and provided with the feed allocated to each group continuously throughout the actual biological experiment period, which lasted for 14 days, starting from (May 1, 2025, until May 15, 2025). Identification cards were placed on each cage indicating the name of the experiment, the researcher, the type of treatment, and its number. The animals were placed inside the cages 10 days before the actual experiment period in order for them to adapt and get used to the place. Six animals were left to eat the standard feed, and 30 animals were made to develop diarrhea by replacing 3% corn oil with castor oil, so that the ratio became 3% castor oil and 7% corn oil. Wire mesh was placed about 2 cm above the paper to prevent the rats from eating it. In all experiments, the animals were fasted for 18 hours before treatment after it was confirmed that they had diarrhea within 2-3 hours.

Feed preparation:

The ready-made feed available in the animal house at Tikrit University consists of the materials below, according to what was stated in accordance with [4]. The feed is prepared every three days to avoid spoilage of the feed.

Table (1) shows the contents of a

Ingredients	ratio
Cellulose	%5
Casein	%10
Corn Oil	%10
Vitamins	%1
Minerals	%4
Starch	%70

standard ration

Carrot powder and brown rice powder were added in a specific ratio per kg of feed according to the groups and proportions. Then the feed components were mixed well in order to obtain a homogeneous mass in terms of components. Sterile distilled water was used to facilitate the kneading process. Then the dough was transferred to a meat grinder (without the blade) sterilized with alcohol to enhance homogeneity and obtain a cylindrical shape of the feed. Then the electric oven was used to dry the sample, but at a temperature not exceeding 40 degrees to preserve the sensitive feed components from spoilage, for a period ranging from one to one and a half hours (depending on the amount of water). After that, the cylindrical feed was cut into pieces in the form of pellets called pellets in order to consume them by the experimental animal and reduce losses and waste of them during the experimental period. Then each prepared sample was stored in special plastic containers labeled with the name and number of the treatment and the type of feed.

Experimental Design

The mice were divided into seven groups, each containing six mice, according to the following plan:

Group 1:This was the healthy control group, consisting of six healthy mice fed the standard diet (the composition of which is described above) without additives and provided with naturally filtered fresh water.

Group 2:This was the positive control group, infected with induced diarrhea and fed the standard diet.

Group 3:This was the group of animals infected with induced diarrhea and fed the probiotic *Lactobacillus acidophilus* (live-cell count) via oral administration at a volume of 1 ml.

Group 4: This was the group of animals infected with induced diarrhea and fed the probiotic *Lactobacillus acidophilus* (live-cell count) via oral administration at a volume of 1 ml, in addition to a 20% carrot powder diet.

Group 5:This group consists of animals with induced diarrhea who were fed the *probiotic Lactobacillus acidophilus* (live-cell count) administered orally at a dose of 1 ml, in addition to a 20% brown rice meal diet.

Group 6:This group consists of animals with induced diarrhea who were fed a 20% mixture of carrot meal and brown rice meal in a 10:10 ratio.

Group 7:This group consists of animals with induced diarrhea who were fed the probiotic *Lactobacillus acidophilus* (live-cell count) administered orally at a dose of 1 ml, in addition to a 10% mixture of carrot meal and brown rice meal.

Blood sample collection: After the end of the specified period of the experiment (14) days, the animals were fasted for 20 hours and then anesthetized with ether. Blood samples were then drawn directly from the heart by cardiac puncture, as approximately (2) ml of blood was drawn and placed in test tubes (Gel tubes) free of coagulation material. They were left for about a quarter of an hour at room temperature, and then the serum was separated by centrifugaging at a speed of 3000 rpm for 15 minutes. The serum was kept at (-20) degrees Celsius in new and clean plastic tubes until the biochemical tests were carried out.

Biochemical tests

The IL-6 and TNF-α factors were estimated in the blood serum of male laboratory rats using an ELISA device (tech-Bio of Korean origin) using the estimation kit for each factor supplied by Shenzhen Lvshiyuan Biotechnology Co. Ltd., China, according to the instructions of the supplying company.

Determining the microbial balance in the intestine

After completing the dissection, a 25 g section of the small intestine was placed in 225 ml of normal saline. After stirring and mixing, the samples were diluted appropriately. Bacterial cultures were then performed on MRS agar and MacConkey agar to determine the effect of the treatments on the microbial balance in the intestines of laboratory animals, as follows:

Estimation of Total Enteric Bacterial Counts

10 g of animal intestines were collected using a sterile scalpel to obtain the intestinal section. The intestinal pieces were cleaned by rinsing with distilled water, then added to 90 ml of physiological saline. The necessary dilutions were carried out up to the sixth dilution, then 0.1 ml of the last dilution was withdrawn and spread on MacConkey agar medium. The plates were incubated upside down at 37°C for 24 hours, after which the colonies on the plate were counted for each sample [14].

Estimation of the total lactic acid bacteria count:

0.1ml of the dilution prepared in the physiological saline solution in the previous step was withdrawn and spread onto MRS agar. It was then incubated anaerobically at 35°C for 24–48 hours. The number of cream-colored,halo-enclosed colonies was then counted [13].

Statistical Analysis

The experiment was conducted using a Complete Randomized Design (CRD). Analysis of variance (ANOVA) was performed using the Linear Model General within the SAS statistical software (2012). Duncan's test (1955) was used to determine the significance of any differences between the means at a probability level of 0.05[55].

Results and Discussion

Efficacy of oral administration of a probiotic and dietary fiber supplementation with carrot powder and milk rice on inflammatory markers in diarrhea-induced rats

Table (2) shows the effect of the probiotic and dietary fiber supplementation with carrot powder and brown rice on the levels of inflammatory factors in mice induced with castor oil-induced diarrhea. A significant decrease in the levels of inflammatory factors was observed in all studied treatments compared to the infected control group.

Table 2 : Effect of oral administration of probiotic bacteria and diet with carrot powder and brown rice on the levels of inflammatory factors in diarrhea-induced mice

Treatment	TNF-α	IL-6
T1	179.5±1.9f	96.17±3.54f
T2	230.8±2.7a	126.15±0.74a
T3	205.3±7.20 c	115.82±0.04c
T4	195.7±1.5e	110.63±0.06d
T5	200.5±0.15 d	111.17±0.06d
T6	217.5 ±1.05b	119.50±1.14b
T7	176.2 ±1.05f	105.54±0.06f

Different letters on the rates in a single column indicate significant differences at a probability level of 0.05.

T1: Healthy Control T2: Infected Control T3: Infected + Lb.a T4: Infected + Lb.a + 20% Carrots T5: Infected + Lb.a + 20% Brown Rice T6: Infected + 10% Carrots + 10% Brown Rice T7: Infected + Lb.a + 10% Carrots + 10% Brown Rice.

Table2 Effect of oral administration of probiotic bacteria and diet with carrot powder and brown rice on the levels of inflammatory factors in diarrhea-induced mice

The results in the table above show a significant increase in the values of inflammatory factors in the group of mice with diarrhea and untreated, as the inflammatory factors (TNF-α and IL-6) were recorded at (230.8, 126.15) respectively, compared to the healthy control group, which was (179.5, 96.17). Treatment with the probiotic and dietary fiber with carrot powder and brown rice had a significant effect at ($P>0.05$) in reducing the concentrations of inflammatory factors, as the T3 treatments recorded (205.3, 115.8), the T4 treatments (195.7, 110.63), the T5 treatments (200.5, 111.17), and the T7 treatments (119.5, 217.5), while the T7 treatments recorded (176.2, 105.5) and outperformed the rest of the treatments.

The results were consistent with those of [1] regarding the elevated cytokines associated with castor oil diarrhea.They demonstrated that excessive production of reactive oxygen species (ROS) is a major contributor to oxidative stress, which leads to protein oxidation. This oxidation activates inflammatory mediators and signaling pathways associated with inflammation. Furthermore, excessive ROS production can cause tissue damage and stimulate an inflammatory response.As for the group of animals with diarrhea treated with the probiotic, the results showed a significant decrease in the level of inflammatory factors. The therapeutic bacteria affect inflammatory factors by regulating the inflammatory balance. The therapeutic bacteria contribute to regulating the balance between inflammation and its control, thus preventing an excessive immune response that can lead to chronic inflammation [18].

By promoting gut health, therapeutic bacteria play a vital role in gastrointestinal health. They support the growth of beneficial bacteria in the gut and reduce the growth of harmful bacteria, thus decreasing the risk of gastrointestinal infections and contributing to a reduction in overall systemic inflammation [3]. By regulating the immune response, therapeutic bacteria modulate immune system activity and reduce its overreaction, thereby decreasing the severity of inflammation. These bacteria also influence the regulation of cytokine secretion, which are regulatory proteins that play a pivotal role in activating and inhibiting inflammatory processes [31]. By reducing the typical proportion of harmful bacteria, the presence of therapeutic bacteria prevents or slows their growth, thus reducing the chances of infections and diseases. Furthermore, by improving overall immune function, the presence of therapeutic bacteria can enhance the immune system, making the body more capable of fighting infections and inflammation [6].The results were consistent with those of [5], showing a significant decrease in TNF-α and IL-6 levels in diabetic rats treated with L. plantarum cells for 8 weeks. They also aligned with [29], who reported a significant decrease in Tnf-a and IL-6 levels in individuals with brain injuries in the intensive care unit. Furthermore, the results were consistent with those of [37], who observed a significant decrease in TNF-α and IL-6 levels in adults with inflammation due to fat accumulation and obesity after 12 weeks of feeding them yogurt containing the therapeutic bacteria L. plantarum. The results were similar to [39] with a significant decrease in the level of TNF-α and IL-6 for people with type 2 diabetes who took dietary supplements containing L. rhamnosus and Lb. acidophillus bacteria.The results also showed a significant decrease ($P>0.05$) in the concentration of inflammatory factors in the group of animals infected and treated with a diet of carrot powder and brown rice. Acute and chronic inflammation in mice decreased significantly, and the results were similar to the effect of the well-known anti-inflammatory drug diclofenac. This is attributed to the fact that extracts of various types of carrots (such as carota, gummifer, and maximus) contain terpenes and flavonoids, or to their antioxidant activity. Carrot components contain a number of terpenes such as alpha-pinene, beta-caryophyllene, beta-bisabolene, sabinene, limonene, and alpha-longipine, in addition to a group of polyphenols such as luteolin, kaempferol, apigenin, caffeic acid, quercetin, and vitamin C, all of which are known for their ability to neutralize free radicals and reduce oxidative stress (Mornin and others). The results (2003) were consistent with those of [38], showing a significant decrease in TNF-α and IL-6 levels in mice induced with formaldehyde, suggesting that carrot components possess anti-inflammatory effects. Interestingly, the disease was reduced in mice fed a high dose of carrot seed extract. In another study by [28], carrot seed extract compounds (2,4,5-trimethoxybenzaldehyde, oleic acid, trans-asarone, and geraniol) were found to have anti-inflammatory properties due to the inhibition of cyclooxygenase enzymes, providing significant anti-inflammatory benefits even when compared to anti-inflammatory drugs such as aspirin, ibuprofen, naproxen, and Celebrex.Studies have also shown that apricots, as a rich source of bioactive plant compounds, have protective and therapeutic effects against liver fibrosis, oxidative stress, and the inflammatory response in animal models of non-alcoholic steatohepatitis (NASH) induced by a high-fat diet (HFD). The results of [30] demonstrated that including apricots in the diet limits the progression of hepatic steatosis and reduces damage caused by free radicals. This effect is mainly attributed to its high content of carotenoids, especially beta-carotene, which act as potent antioxidants.Significant improvements in oxidative stress markers, such as decreased malondialdehyde (MDA) levels and increased concentrations of total glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx), were observed in mouse models of carbon tetrachloride-induced liver damage. These findings suggest that consuming beta-carotene-rich apricots contributes to reducing oxidative stress, improving fatty liver status, and protecting hepatocytes from damage. Furthermore [17] demonstrated a significant decrease in Tnf-α and IL-6 levels, indicating pectin's role in regulating the inflammatory response in the colon, specifically by inhibiting the production of inflammatory cytokines. The results also showed that mice fed orange pectin had lower levels of these cytokines compared to those fed acidic pectin. This suggests that pectin not only acts as a prebiotic regulator of the gut microbiota, but also directly affects host cells by modulating the immune and inflammatory response. Beta-carotene also has antioxidant properties, which help reduce damage caused by free radicals that contribute to the inflammatory process. By regulating the immune system, beta-carotene acts as an immune regulator, helping to stimulate and activate the immune system to fight infection and inflammation [45].

Consuming brown rice in mice also showed a reduction in inflammatory factors, significantly lowering IL-6 and TNF-α levels. This reflects its anti-inflammatory effect and its protective role in limiting tumor development. These results are consistent with those of [35], who observed a significant decrease in TNF-α and IL-6 levels. Ren et al. noted that a high-fat diet caused a significant increase in IL-6 and TNF-α levels in the model group compared to the control group, leading to an increased inflammatory response and the development of tumor changes. However, sprouted brown rice significantly reduced IL-6 and TNF-α levels, reflecting its anti-inflammatory effect. This is attributed to brown rice being rich in γ-oryzanol, which is responsible for several pharmacological properties, such as cholesterol-lowering and anti-inflammatory effects. Brown rice also improved glucose tolerance and insulin resistance. The studied medicinal plants contain important antioxidants and possess anti-inflammatory properties. Research indicates that they can influence several inflammatory pathways in the body, including inhibiting inflammatory enzymes such as cyclooxygenase and lipoxygenase, which play a key role in the inflammatory process. By inhibiting the formation of inflammatory cytokines, they reduce the severity of inflammation and decrease harmful oxidative reactions. The results showed that the use of a probiotic supplement and dietary fiber supplementation with carrot powder and brown rice significantly increased the levels of inflammatory markers in the treated groups, leading to a significant improvement in the values of experimentally diagnosed diarrheal animals.

The efficacy of oral administration of a probiotic and

dietary fiber in restoring the normal gut flora balance in diarrhea-induced rats

Table (3) shows the effectiveness of oral administration of a probiotic and feeding on carrot powder and brown rice on the intestinal bacterial balance of laboratory mice experimentally induced with diarrhea using castor oil.

The results showed that the effect of inducing diarrhea with castor oil in laboratory animals in group (T2) caused a significant increase ($p<0.05$) in the number of intestinal bacteria in both the small and large intestines, which were at 6.7 and log CFU/g 7.9, respectively, compared to their numbers in the control group (T1), which were at 4.2 and log CFU/g 4.9, respectively. Meanwhile, the number of lactic acid bacteria in the small intestine decreased, with log CFU/g numbers of 5.4 and 4.9, respectively, compared to their numbers in the control group, which were at 8.5 and 7.7, respectively. Oral administration of the probiotic and feeding with dietary fiber in carrot powder and brown rice caused a modification in the numbers of bacterial species, reducing the types of coliform bacteria and increasing the numbers of lactic acid bacteria, but they did not reach the normal number of them in the control group (T1), as the numbers of coliform bacteria in the small intestine were between 4.7 to 5.4 log CFU/g and the types of lactic acid bacteria were between 6.2 to 7.4 log CFU/g, and the numbers of coliform bacteria in the large intestine were between 5.2 to 6.5 log CFU/g and the numbers of lactic acid bacteria were between 6.2 to 8.3 log CFU/g respectively.

The results are consistent with those of [51], who demonstrated a clear disruption of the intestinal bacterial balance due to castor oil. They also align with [50], who showed an increase in enteric bacteria and a decrease in lactic acid bacteria in the intestines of rats in group T2 due to increased intestinal

peristalsis, leading to increased bacterial loss and a decrease in their numbers. Furthermore, these results are consistent with those of [44], who demonstrated in a human experiment that castor oil causes intestinal permeability, resulting in a decrease in the number of gut flora.

The improved health of the laboratory mice is associated with a significant decrease in the number of colonies of enteric bacteria compared to the number of colonies of lactic acid bacteria. This can be explained by the fact that types of probiotic bacteria possess multiple characteristics that make them efficient in improving the health condition of the host due to their ability to produce bacteriocins, as well as to produce hydrogen peroxide, which kills other microbes, as well as to produce short-chain fatty acids and mucous substances, and their ability to activate the immune system and compete with pathogenic microbes for the site of attachment and food consumption in the intestines of the host, all of which contribute to improving the health condition of the host [33].

The results also indicated a positive balance in the number and quality of the natural flora in the intestines of animals given the probiotic compared to the pathogenic microbial species. This information was consistent with what [16] stated, who found that giving probiotics as nutritional supplements in the diet of newly weaned piglets reduced the total number of E. coli bacteria in the fecal sample and attributed this to their ability to produce the substances mentioned above.Studies in this field have shown that gut health is key to maintaining overall health, as the natural gut flora plays a major role in metabolism and nutrient absorption. Therefore, an optimal and positive balance of gut flora is a priority for achieving host health [26]. Furthermore, probiotic bacteria are diverse protein combinations whose presence in the gut positively impacts the health of body cells and tissues, ultimately leading to positive health effects in the host [27]. The above explains why the predominance of lactic acid bacteria in probiotics is associated with the improvement observed in laboratory animal groups fed probiotic diets.The results also agreed with what [54] reported regarding the reduction of coliform bacteria numbers in laboratory piglets following oral administration of lactic acid bacteria. The results also agreed with [53], who showed that lactic acid cell extract led to a decrease in coliform bacteria numbers [36] stated that lactic acid bacteria play an important role in stimulating the body's immunity through their effects in multiple ways, including maintaining a positive balance of intestinal flora. Several studies have indicated the important role of lactic acid bacteria, which are naturally present in the intestines of humans and animals, in maintaining microbial balance within the intestine [15]. The role of these bacteria is due to their secretion of lactic acid, as well as the production of hydrogen peroxide and bacteriocin, in addition to their ability to adhere to the intestine [34], to act as inhibitory factors in reducing the number of pathogenic bacteria, in addition to blocking the sites of attachment of germs and toxins to epithelial cells, and they also work to modify the immune response of the host [25].

The results for the groups containing dietary fiber were consistent with those of [20], who demonstrated that dietary fiber plays a crucial role in restoring microbial balance. Their study showed that carrots altered the composition of the gut microbiome (the microbes that play a vital role in digestion and overall health). Mice that consumed carrots exhibited a healthy balance of gut bacteria. Furthermore, these mice had a higher number of bacteria that produce short-chain fatty acids (SCFAs), small molecules formed when bacteria break down dietary fiber. SCFAs help regulate energy metabolism and blood sugar levels, and they also support gut health. The study concluded that consuming carrots shifts gut bacteria toward a healthier balance, which benefits mice with type 2 diabetes. Carrots may help enhance the body's ability to regulate blood sugar levels and positively influence the composition of gut bacteria. Over a 16-week period, the effects of carrots on mice with type 2 diabetes were studied. The mice were placed on a high-fat diet to mimic the unhealthy diet of humans. They were divided into two groups: one group consumed a diet supplemented with 10% dried carrot powder, while the other group consumed a carrot-free diet. The results showed that the mice that consumed the carrot powder exhibited improved blood sugar regulation. Researchers also observed changes in the gut microbiota composition, specifically an increase in bacteria that produce short-chain fatty acids (SCFAs), which help regulate energy metabolism and support gut health. These findings are consistent with those of [8], who demonstrated that consuming large

amounts of rice fiber causes changes in microbial communities, specifically a significant increase in Bifidobacterium numbers and a significant decrease in Clostridium numbers. They also align with [21], who demonstrated that brown rice has an effect on the gut microbiota composition in diabetic mice. In humans, this supports the existence of a link between the nature and activity of gut microbes and the anti-obesity and anti-diabetic effects of brown rice. The results showed that oral administration of a probiotic and dietary fiber supplementation with carrot powder and brown rice resulted in a microbial balance in the gut.

Table 3 Effect of oral administration of probiotic and dietary fiber on the microbial balance of the intestines of laboratory rats induced with diarrhea

Treatment ±SDMean	Total number of bacterial species in the intestines of mice (x 10^8)			
	Large intestine		small intestine	
	intestinal bacteria	Lactic acid bacteria	intestinal bacteria	Lactic acid bacteria
T1	4.9 ± 0.27d	8.5 ± 0.27a	4.2±2.3d	7.7±0.3 a
T2	7.9 ± 0.27a	5.4 ± 0.31c	6.7±0.5a	4.9±1.4e
T3	6.1 ± 0.70c	7.6±0.3a	4.9±0.3b	6.5±1.3b
T4	5.9 ± 0.16c	8.1 ± 0.19a	5.4±2.6c	6.9±0.3b
T5	6.5 ± 0.25b	6.2 ± 2.21b	4.9±1.1d	6.7±0.4b
T6	6.4 ± 0.25b	7.4 ± 0.16a	5.1±0.4cd	6.2±2.3c
T7	5.2 ± 0.18c	8.3 ± 0.20a	4.7±3.3d	7.4±1.7a

Different letters on the rates in a single column indicate significant differences at a probability level of 0.05.

T1: Healthy Control T2: Infected Control T3: Infected + Lb.a T4: Infected + Lb.a + 20% Carrots T5: Infected + Lb.a + 20% Brown Rice T6: Infected + 10% Carrots + 10% Brown Rice T7: Infected + Lb.a + 10% Carrots + 10% Brown Rice

Conclusion

Oral administration of the probiotic Lactobacillus acidophilus, carrot powder, and brown rice resulted in a decrease in the concentration of Interleukin 6- (IL-6) and TNF-α .The gut microbiota of animals was balanced with oral administration of the probiotic Lactobacillus acidophilus and carrot and brown rice powder, particularly the probiotic alone or synergistically with dietary fiber, which was the most effective. The dietary fiber group (carrot or brown rice) showed moderate improvement, but less than the probiotic. While treatment with carrot or brown rice powder was helpful in alleviating symptoms, its effect was less consistent than that of the probiotic Lactobacillus acidophilus. This suggests that fiber/plant components may support recovery, but the mechanism may be different from or slower than that of beneficial,bacteria.*Lactobacillus acidophilus* bacteria have shown an effective role in reducing diarrhea; therefore, they can be considered a useful treatment option for reducing the severity of diarrheal diseases.The results showed that combining a probiotic with dietary fiber from a single plant or a mixture of both plants has a greater effect compared to using each alone, demonstrating a synergistic effect between the probiotic and fiber.

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