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Effects of Oral Administration of Camel Milk and Urine on Gut Microbiota: Biochemical and Microbiological Profiling in Rats

Samah O. Noor, Manal S. Alenini

Biology Department, Faculty of Science, King Abdulaziz University, Jeddah, KSA
Email: magdammali@hotmail.com

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Abstract

Human intestinal tract contained a diverse number of microbial communities which performed a significant role in human health. The presence of gut microbiota was affected mainly by diet. Camel milk is the source of nutrition and provides all the essential nutrients for growth. It has great significance in the treatment of liver, spleen, and anemic infections. Camel urine has also many medical advantages. In this study we examined the effect of camel milk and urine and a mixture of both (milk + urine) on the growth of Gut microbiota using an *in vivo* animal model. Fresh fecal samples were collected before and after administration of the tested materials. After that, the microbial analysis was conducted via culturing, denaturing gradient gel electrophoresis and metabolic analysis via high-performance liquid chromatography (HPLC). The result indicated that the numbers of bacterial groups were increased after the first dose. Coliform group have significant increase when given a mix of milk and urine compared to control group with $P < 0.05$. Bifidobacterium group have significant increase in their number in the Milk and Mix groups compared to control group with $P < 0.05$. The concentration of Short-chain fatty acids in fecal samples was increased in Milk and Mix groups compared to control group. In conclusion, drinking camal milk, urine or a mix of both increased the growth of Gut microbiota.

Keywords

Camal Milk, Urine, Gut Microbioa, HPLC, DGGE

1. Introduction

The human gastrointestinal tract (GIT) extends from the oral cavity to the rec-

tum and is colonized by a large number of microorganisms. There are more than 10^{14} organisms in the human colon alone with at least 400 species, approximately 10 times more than the sheer number of somatic cells [1] [2]. Microbiota in the colon and rectum is much more diverse and larger than the population of upper parts of GIT. The main objectives of the microbiota in the intestine are metabolic (Trophic function), barrier effect (protective) and enhancement of the immune system [3]. The colon is the main site for fermentation due to slower transit rate and rich nutritional environment. The main products of gut bacterial fermentation in the colon are the short-chain fatty acids (SCFAs). SCFAs stimulate the cell proliferation and differentiation of intestinal enterocytes [3] [4] [5]. The techniques for the study of microbial communities in the intestine are diverse, generally two methods are applied: Culture-based and Culture-Independent techniques including Polymerase Chain Reaction (PCR), Denaturing Gradient Gel Electrophoresis (DGGE), Fluorescence *in situ* hybridization, Cloning and Sequencing [6] [7] [8].

For many decades, the natural products were applied in the fields of medicine, pharmacy, biology and have very essential role in health care and prevention of diseases [9]. Animals have been a source of some interesting compounds that can be used as drugs. One of these is Camels that have a medical importance through their milk and urine. Medical uses of camel milk and urine which were known in previous generations were proven by modern scientific research. The lactoferrin found in camel milk (ten times greater than in cow milk), has anti-microbial and anit-viral activities such as lysozyme and immunoglobulins [10] [11]. Also it is effective against pathogens including *Staphylococcus aureus* and *E. coli* [12]. Camel's urine has medicinal properties through its use as a disinfectant agent for wiping wounds and sores. It also helps to block hair damage and baldness, and can be applied as a remedy for dandruff [13]. Moreover, camel's urine may be helpful to treat blood clots and dropsy (deficiency of albumin and potassium). The Camel's urine also provides a panacea for abdominal pains, liver infection such as hepatitis and digestive diseases [14]. Prof. Faten Khorshid and his coworkers proved effectiveness of camel urine and the active substance in the treatment of many types of cancer, such as leukemia, lung cancer and colon cancer [15] [16] [17] [18] [19]. In this study, the effect of camel milk and a mix of both milk and urine on the growth of Gut microbiota and their metabolic activity *in vivo* animal model were determined. The effect of both treatments on the colonic rat tissue was also determined.

2. Material and Methods

2.1. Experimental Animals

Fifteen albino wistar male rats of the same age with a body weight of 200 - 250 g were collected from the Animal House Unite of King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. All the studies were conducted in accordance with the ethics and regulation of local governing au-

thorities. Animals were housed in respective plastic cages and divided into three groups: Milk, Mix and control groups. Each group has 5 animals. All experiments were run in the same controlled environment (21˚C ± 2˚C, 65% relative humidity and 12: 12 hour light/dark cycle).

2.2. Collection of Camel Products and Treatment Methods

Camel milk and urine were collected from camels that graze in south Jeddah, Saudi Arabia from adult female in the morning in sterilize bottles and kept at 4˚C until used. A daily dose of camel milk (1.8 ml/200 g) was given to the first rat group and a dose of 2.4 ml/200 g of mix (camel milk + urine, v/v) was given to the rat second group through oral gavages for one week. The third group is a control that drank water instead of milk or urine.

2.3. Collection of Fecal Sample

Fresh fecal samples were collected at different time points in sterile plastic containers. Containers were kept under anaerobic conditions in anaerobic Jar. Each week, three fecal samples/week were collected from each rat for two weeks, labeled, and then stored at −20˚C until used.

2.4. Plate Counting

The bacterial groups that were tested, the selective media and culture conditions that were used were shown in Table 1. Fecal samples were dissolved in water (10% w/v) and serial dilutions for each fecal sample were prepared. The plats which contained the selective media were inoculated with 100 µl of each dilution. Then, all plates were incubated at 37˚C or 45˚C under proper conditions.

2.5. Determination of Amount of Short Chain Fatty Acid

Short chain fatty acids (SCFAs) like Acetic, Propionic, Isobutyric and Lactic were investigated in fecal samples of the three tested animal groups. Water extract from fecal samples were prepared [20] and 10 µl of the extract was injected directly into HPLC System (Shimadzu LC-20AS Liquid Chromatography) using a C18 column (Shimadzu, 250 × 4.6 mm) at 60˚C. Diluent of Sulfuric acid and 2% methanol was used as a mobile phase at a flow rate of 1 ml/min. The SCFAs were detected using a UV detector set at wavelength of 210 nm.

Table 1. Selective Media and incubation condition that were used to cultivation gut bacteria.

The selective media	Bacteria	Incubation Temperature	Incubation Period	Incubation Condition
MRS agar	Lactobacilli	37˚C	7 days	Anaerobic
Beerns agar	Bifidobacterium	37˚C	7 days	Anaerobic
Azide blood agar	Streptococci	45˚C	3 days	Aerobic
MacConkey agar	Coliform	37˚C	1 day	Aerobic

2.6. DNA Extraction and PCR

Bacterial DNA was extracted from 200 mg of frozen fecal samples (−20˚C) using QIAamp DNA stool Mini kit, according to the manufacturer's instructions. The DNA concentration was measured using Nanodrop spectrophotometer and quality was evaluated using gel electrophoresis. The DNA that isolated from collected fecal samples was used as a template in PCR amplification. The V3 region of 16S rRNA was targeted to amplify the total DNA bacterial community using universal primer. The forward primer (341GC-F) (5'**CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG G** CCT ACG GGA GGC AGC AG 3') GC-clamp is in boldface) and the reverse primer (534R)(5'-ATT ACC GCG GCT GCT GG-3') were used [21]. The PCR products were purified with Ethanol/EDTA/Sodium Acetate precipitation method following Applide Biosystems protocol (2002). The DNA concentration and purity were measured at 260 and 280 nm using Nanodrop spectrophotometer.

2.7. DGGE

In this study, the DNA that was extracted from fecal samples from all experimental groups of rats at different time points were analyzed using DGGE which was performed using Bio-Rad Protein 11 system, essentially as described by [22]. DGGE with 6% polyacrylamide gel containing 30% - 50% linear denaturant gradient (100% denaturant corresponds to 7 M urea and 40% deionised formamide) was used. Sample (190 ng) of each purified PCR product was loaded onto the gel. DGGE was performed at 60˚C and 200 V for 1 hr in 1 × TAE buffer. Gels were stained with ethidium bromide (20 µl in 200 ml of 1 × TAE buffer for 20 min) and then visualised under UV light and photographed using gel documentation. All the samples were analyzed on the similar DGGE run to avoid the probable influence of variations in electrophoretic conditions between different runs. DGGE images were analysed by TotalLab Quant software.

2.8. Histological Studies

To investigate the histological changes in the rat colons, sigmoid colon sections, stained using Hematoxylin and Eosin and examined using light microscope [23].

2.9. Statistical Analysis

Statistical analysis was applied using the statistical Package software for Social Science (SPSS for windows, version 16). The significance of the difference between groups was determined using the One-Way ANOVA. The difference was regarded significant when $P < 0.05$ and non-significant when $P > 0.05$.

3. Results

3.1. Plate Count Data

After administracion of camel milk and mix (camel milk and urine) to rats for a week, seven rat fecal samples were collected during 2 weeks. Each fecal sample

was cultivated on the selective media to investigate the different groups of bacteria in the rat feces. The data revealed that using camel milk or a mix of both milk and urine increased all types of tested bacteria compared to control (zero time). Results of the enumeration of cultivable bacteria are presented in Figure 1 and Figure 2.

The results also indicated that the Coliform group has the highest growth in the Mix fed group with 1.6×10^9 cfu/ml, after the first week. This increase was significant compared to control group with $P < 0.05$. The growth of the same group of bacteria was also increased in Milk fed group (2.2×10^5 cfu/ml) compared to control. The enumeration of Streptococci bacteria showed the highest growth in the Mix fed group with 1.3×10^5 cfu/ml, while 1.7×10^4 cfu/ml in the Milk feed group. However this increase was not significant in comparison to control group ($P > 0.05$). The results also showed that the Lactobacilli group was increased in numbers to 2.4×10^6 cfu/ml and 2.3×10^6 cfu/ml in Milk and Mix groups, respectively. Bifidobacterium group showed similar increase in their growth with 1.42×10^6 and 1.46×10^6 cfu/ml in the Milk feed group and Mix fed group, respectively. This increase was significant compared to the control group with $P < 0.05$. In the milk fed group, the growth of Coliform and Streptococci

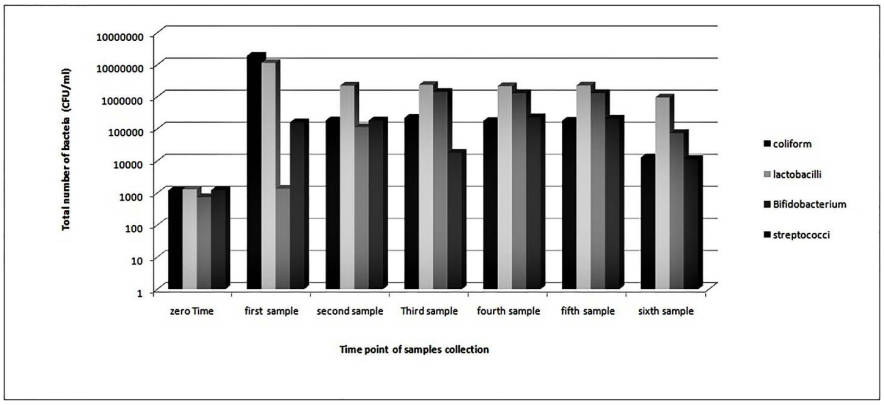

Figure 1. Enumeration of total number of bacteria (CFU/ml) from fecal samples of rats after oral administration of camel milk for two weeks.

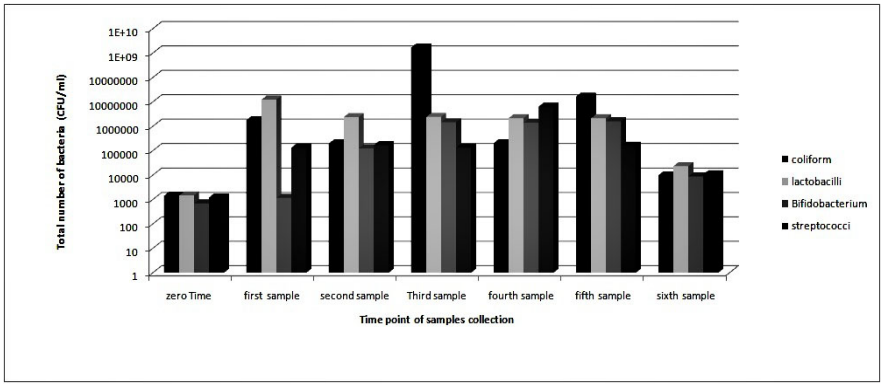

Figure 2. Enumeration of total number of bacteria (CFU/ml) from rat fecal samples after oral administration of a mixture of milk + urine for two weeks.

were decreased after stopping the milk doses while the decrease was not clear in case of *Lactobacilli* and *Bifidobacterium*. In the mix fed group, the growth of all bacterial groups decreased after the last dose.

3.2. Metabolic Analysis

In the HPLC chromatograms that were obtained from fecal water analysis, different concentrations of SCFAs were obtained. The result of the average concentrations of the SCFAs is presented in Figure 3. The concentration of lactate increased in the milk feed group by 85%, while its concentration decreased in the mix feed group by 45% after the first dose. After administration of milk and urine, the concentration of acetate in the mix feed group was decreased by 59%, while its concentration in the milk feed group was increased by 63%. The concentration of Isobutyrate in the milk and mix groups was increased by 57% and46%, respectively. However concentration of propionate acid was decreased after starting dosing in the mix group by about 39%. In the milk group its concentration was increased by about 77%.

3.3. DGGE Analysis

Figure 4 showed the DGGE profile where each line represent a sample from

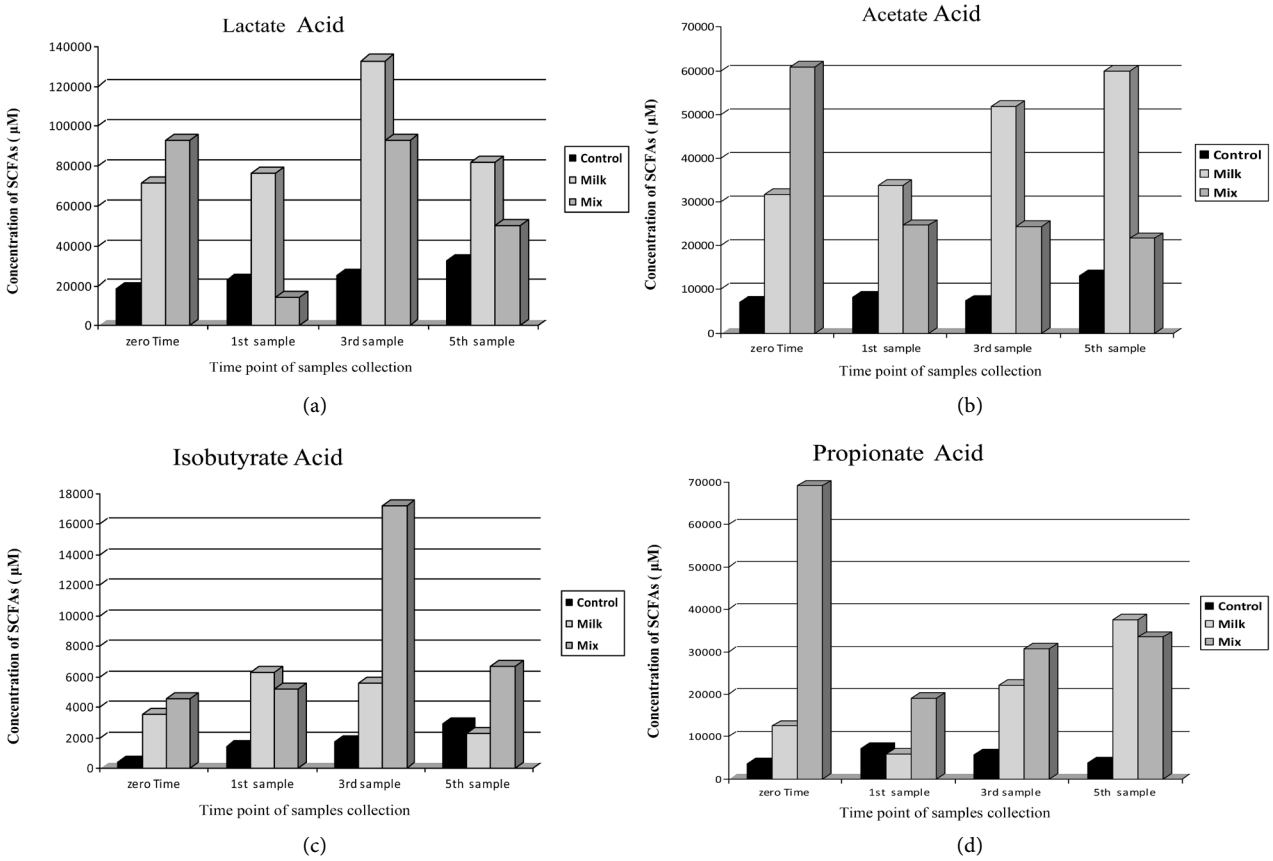

Figure 3. The average of concentration (µM) of SCFAs that analysis by HPLC for the Control, milk and mix groups before and after dosing. (a) Lactate acid; (b) Acetate acid; (c) Isobutyrate acid; (d) Propionate acid.

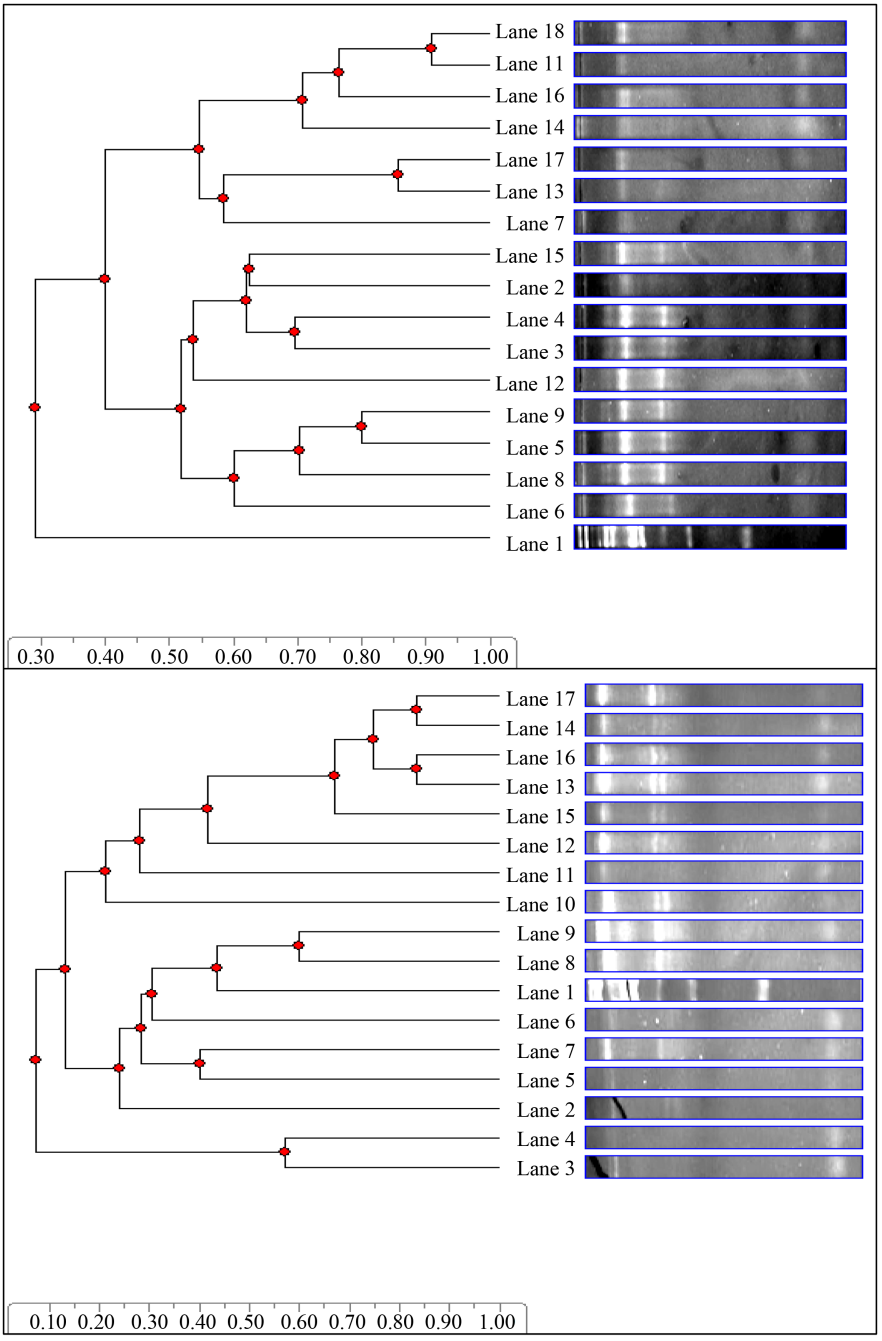

Figure 4. Dendrogram showing similarity between bacteria in milk group (a) and Mix group (b).

individual rat. The bands in each line represent different bacterial group. The intensity of the band may provide an assessment of the fraction of the target bacterial group in the sample. The microbial diversity was evaluated by the count of bands existence in the DGGE patterns.

The milk feed group showed a stable number of bands through the dosing period. The mean of number of bands was 8 ± 1 bands. When comparing the intensity of bands in this group, most bands had an increase in their intensity after

administrated of milk. The bands with R_f = 0.009, 0.026 and 0.849 were increased by about 2 - 3 folds and these increases were significant (P = 0.001). One band with R_f = 0.196 has imperceptibly increase. The increase in the intensity of those bands indicates an increase in the numbers of these bacterial groups.

Interestingly, in the group that was feed on mixture of milk and urine, the bacterial diversity remained the same after the first three doses while after the fourth dose there was a substantial increase in microbial diversity (number of bands 9 ± 1) with P= 0.003. Additionally, although there were no increase at the first three time points, the intensity of one of the bands (R_f = 0.101) has ten folds increase after the third feeding point. This increase was translated into a rise in number of this particular group of bacteria. On the other hand there was a significant decrease in the intensity of another band (R_f = 0.331) which indicates the loss of numbers of this bacterial group (Table 2).

3.4. Histological Studies

Microscopic examination of the colon tissue showed that there are no changes in the structure of the colon tissues (colonic mucosa, submucosa and muscular), but there was an increase in the intensity of the mucus layer in the treaded groups compared to control group, as indicated in Figure 5.

4. Discussion

Microbiota in gastrointestinal tract plays a key role in human health [24]. Therefore, we must preserve the microbial community balanced and healthy. Camel's milk has many healing factors and all the essential nutrients for growth [25]. The current work, investigated the impact of these camel products on gut microbiota and their metabolic activity in the digestive system in rats.

The enumeration results of fecal bacteria which investigated before and after camel products administrations indicated that the numbers of tested bacterial groups were increased after the first doses. This might be due to the presence of Lactic acid bacteria (LAB) in camel milk, while some researchers reported that lactic acid bacteria (LAB) exist in numerous food products, also are part of natural microbial community in gastrointestinal tract [26]. The results of [19]

Table 2. The rate of flow (R_f) value and intensity of bands detected from DGGE gels.

Rat group	R_f value	Intensity of bands	
		Before dosing (zero time)	After dosing (after 2 weeks
Camel Milk	0.009	4970.17	8392.78
	0.026	4204.19	12153.93
	0.849	22322.36	53157.14
	0.196	81779.19	89460.79
Mix (camel milk + urine)	0.101	14008.48	142938.88
	0.331	32068.27	11281.34

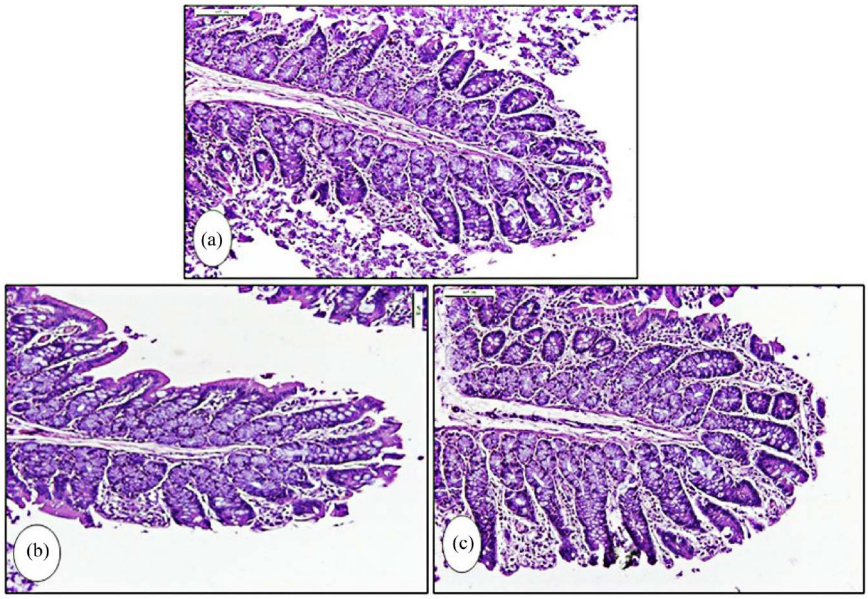

Figure 5. The effect of oral administration of Milk (b) and Mix (c) on colonic tissue of rats, showed normal structure of colonic inucosn. subinucosa and muscular and no differences are seen compared with control jeroup (a), except the mucous layer has increased in its thickness (H & E ×20).

stated that the camel milk represent a source of biological material in dairy products, due to the existence of lactic acid bacteria which are beneficial bacteria. Moreover, more free amino acids and peptides are found in camel milk than in bovine milk which is digested by microorganisms, and therefore, camel milk presents a higher metabolic activity when used in a culture preparation [27]. A study of [28] investigated the antimicrobial activity of Camel's urine; some normal bacteria were isolated from camel urine including *E. coli*, and lactic acid bacteria (LAB). In this study, DGGE method was selected for study gut microbiota, because the culture-dependent technique showed only culturable bacteria while molecular method monitor the entire DNA in the sample.

Most previous studies have monitored the shift in DGGE patterns by correlating the presence and absence of bands, or switch in the intensity of a single band on the same gel [29]. In this study, DGGE method was used as quantitative method and the intensity of the most bands that were detected in the gel for the study groups was increased, which indicates an increase in the numbers of the bacterial groups. On the other hand, there was a decrease in the intensity of other bands which indicates a drop in the number of some bacterial group.

The main products of gut bacterial fermentation in the colon are the short-chain fatty acids (SCFAs) which can be detected in fecal samples. However, the actual proportion of these products depends on many factors such as diet [30]. Variation in the gut microbiota composition is likely to affect metabolic function. The SCFAs investigated in this study were increased in Milk and Mix fed groups compared to control group. This might be due to the fundamental nutrients for growth and development that found in camel milk, including proteins

carbohydrates, fatty acids, minerals and growth factors [31].

In this study, sigmoid colon was selected to examine the histological changes in the colon using light microscope, where its main objective is to store feces until enters the rectum and expelled through the anus, and it is the site of wide range of complications. Ulcerative colitis and Crohn's (inflammatory bowel diseases) may occur here. Also, the diverticulitis is more common in the sigmoid than any other part of the bowel, as well as cancers that prefer the sigmoid colon. The mucus layer functions as a dynamic protective barrier and interacts with the commensal microbiota to keep a steady maintenance and balance. This homeostasis between mucus and the microbiota is damaged in a variety of intestinal disorders, including IBD, where as in these patients, mucosal inflammation was shown to be associated with a reduction in the diversity of the microbiota. More specifically, a loss of anaerobic bacteria such as Bacteroides, Eubacterium, and *Lactobacillus* species was reported [32] [33]. Our examined histological sections of sigmoid colon indicated no observable significant effect on tissues structure, except the mucus thickness which was increased in the treated rats by camel products compared to control group ($P < 0.05$).

5. Conclusion

The results obtained in this study suggested that the presence of free amino acids and Lactic acid bacteria in Camel milk may play an important role as a natural source to improve the microbial community in the gastrointestinal tract. These results may encourage the consumption of camel milk as a source of nutrition providing the essential nutrients for growth and development of our metabolic activity.

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