Asian Journal of Medicine and Health

18(9): 94-106, 2020; Article no.AJMAH.60751
ISSN: 2456-8414

Evaluation of the Effects of *Pediococcus acidilactici* Isolated from Wara, a Nigerian Milk Product, in the Prevention of Diarrhea and the Modulation of Intestinal Microflora in Wistar Rats

Temidayo Emmanuel Olajugbagbe[1*], Samson Oludare Ayodeji Odukoya[2] and Bridget Okiemute Omafuvbe[1]

[1]Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun state, Nigeria.
[2]Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife, Osun state, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors TEO, SOAO and BOO designed the study. Author TEO performed the statistical analysis and wrote the first draft of the manuscript. Authors SOAO and BOO managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJMAH/2020/v18i930240
Editor(s):
(1) Dr. Ashish Anand, G.V. Montgomery Veteran Affairs Medical Center, USA.
Reviewers:
(1) Nihad Khalawe Tektook, Middle Technical University, Iraq.
(2) Marcos Gabriel Guidoli Cátedra de, Universidad Nacional del Nordeste, Argentina.
Complete Peer review History: http://www.sdiarticle4.com/review-history/60751

Original Research Article

Received 22 June 2020
Accepted 28 August 2020
Published 08 September 2020

ABSTRACT

Animals have been used in the evaluation of probiotic potentials of lactic acid bacteria for the development of functional food as only *in-vitro* tests may not be enough to ascertain the probiotic ability of an organism and its safety in a living host. The action of a probiotic *Pediococcus acidilactici* strain isolated from *Wara*, a Nigerian milk product, in the treatment of Diarrhea in wistar rats infected orally with Diarrhoeagenic Enterotoxigenic *Escherichia coli* as well as the survival and microflora modulation of the probiotic strain in the gastrointestinal tract were evaluated. Five groups of seven rats each were infected and treated as the case may be with the pathogen and probiotic respectively. Each group received specific treatments for 30 days, during which the animals were closely monitored and the faecal samples were analyzed for the trend of *E. coli* and LAB counts. Also, specific organs of the Gastrointestinal tract (GIT) were examined for any histomorphological

**Corresponding author: E-mail: olajugbagbetemidayo@yahoo.com;*

disparity after 30 days of daily treatment. Clinical signs were observed in the pathogen challenged animals while the probiotic treated groups displayed less pronounced Diarrheal symptoms and a negligible *E. coli* count. The strain adhered to the mucosal wall and did not initiate any adverse effect on the organs of the GIT after treatment. It was concluded that *Pediococcus acidilactici* isolated from *Wara*, a Nigerian milk product, was able to improve gut health through pathogen exclusion and had no adverse effect on the general health of the host.

Keywords: Probiotic; Pediococcus acidilactici; Wara; diarrhea; Escherichia coli.

ABBREVIATIONS

BV : Blood Vessels
CC : Chief Cells
CT : Connective Tissues
DPX : Distrene Plasticizer and Xylene
GC : Goblet Cells
GP : Gastric Pits
IEC : Intestinal Epithelial Cells
IG : Intestinal Glands
IVS : Intravillous Space
LP : Lamina Propria
ME : Muscularis Externae
MM : Muscularis Mucosa
PC : Parietal Cells
SE : Surface Epithelium
SM : Sub-Mucosa
TES : Tissue Embedding Station
TFM : Temporary Fold of the Mucosa
V : Villi

1. INTRODUCTION

Probiotics are generally defined as live microorganisms that confer beneficial effects on the health of a host when administered in adequate amount [1]. Several *in-vitro* assays have been successful in the investigation of probiotic potentials of organisms but the use of animal models and human cells has gained much attention recently in the preparations and development of functional foods as the organisms might not replicate such attributes in a living host [2].

Previously, the use of antibiotics in reducing the frequencies of disorders such as Diarrhea was commonly employed but has since become a point of concern as antibiotics contribute greatly to the increase in bacterial antibiotic resistance [3]. Furthermore, the consumer's desire for safe food products with minimal or no chemical processing has shifted attention to the use of natural strategies one of which is the use of probiotics to improve gut development and health [4]. Many strains of lactic acid bacteria have been investigated for probiotic attributes in the development of functional foods based on their ability to survive harsh conditions and are considered to possess the generally regarded as safe 'GRAS' status. However, further basic and safety characteristics are still required before they can be considered as probiotics.

Investigation of probiotic effects of LAB *In-vivo* may be approached by either examining the quantitative and qualitative characteristics of bacterial microflora in animals using cultivation and/or molecular biology techniques, monitoring the effect of the probiotic on health indices or evaluating treatment efficiency indirectly by using it to cure an artificially induced disease [5].

Diarrheal diseases are one of the leading causes of deaths in children below five years accounting for about 16% deaths annually estimated at 150,000 and 2.5 billion cases in Nigeria and globally respectively [6]. Enterotoxigenic *E. coli* (ETEC) is associated with infant and traveler's diarrhea and it initiates infection in a host by colonizing the gastrointestinal tract (GIT) and producing heat labile and/or heat stable toxins [7]. Probiotic bacterial strains, however, provide protection from such pathogens by the reduction of biofilm development and inhibition of adhesion to the intestinal walls [8]. Due to their long history in the development of functional foods, strains of *Lactobacillus* and *Bifidobacterium* are mostly used as probiotics but recently, strains of other bacterial species are reported to possess probiotic abilities [9]. For instance, the probiotic potentials of different strains of *Pediococcus acidilactici* has been reported and documented [10,11,12]. It is known for its ability to survive serious stress conditions such as acid and bile conditions of the GIT thus colonizing it and modulating the intestinal microbiota [13].

The probiotic strain used in this study was isolated from *Wara*, a Nigerian cheese curd locally made from fresh cow milk. The strain was identified by 16S RNA gene sequence as *Pediococcus acidilactici*. It is Gram-positive

cocci, Catalase negative and exhibits excellent ability to survive high acidic conditions as well as other stress conditions of gastro-intestinal tract. The aim of the study was to investigate the therapeutic tendency of the probiotic strain against a Diarrheagenic ETEC strain in Wistar rats and to evaluate its effect on the host's microflora and organs of the GIT after 30 days of daily feeding.

2. MATERIALS AND METHODS

Pediococcus acidilactici strain was stored and maintained on deMan Rogosa and Sharpe Agar (MRSA) slant in low temperature (4^{o}C). The strain was re-suspended in deMan Rogosa and Sharpe Broth (MRSB) and sub-cultured on MRSA plates. The pure colonies were subjected to Gram staining and Catalase test to confirm the organism's viability before the animal model experiments. Rats aged 6–8 weeks old (120-180 g) were housed under natural conditions of light and dark cycle at room temperature and humidity with free access to feed and water. The animals were obtained from the animal house, Multi-Diciplinary Laboratories, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria.

2.1 Preparation of Indicator Organism

Pathogenic indicator organism, Diarrhoeagenic Enterotoxigenic *Escherichia coli* was obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. The organism was freshly grown in nutrient broth for 18 h at 37^{o}C. The culture was centrifuged at 10,000 rpm for 10 minutes and the cells were suspended in Phosphate Buffered Saline (PBS) (pH 7.2) and standardized to 0.5 McFarland to obtain 2×10^{8} CFU/mL of bacterial cells using a spectrophotometer [14].

2.2 Preparation of Test Organisms

For experimental inoculation, 18 h old MRSB cultures of the probiotic LAB isolate was centrifuged at 8000 rpm for 10 mins and pellets were washed with PBS (pH 7.2). The cells were re-suspended in PBS (pH 7.2) and standardized to contain 1x10^{9} CFU/mL using a spectrophotometer.

2.3 Experimental Design

Thirty-five male albino rats were used for the study and the rats were randomly divided into 5 groups, each containing 7 rats. The experimental rats were fed intra esophageally via oral cannula with microorganisms. Group I (Pathogen–infected) animals were challenged once orally with a single dose (1 mL) of the previously standardized cell suspension of ETEC, Group II (Pathogen–LAB) animals were challenged once orally with a single dose (1 mL) of the previously standardized cell suspension of ETEC and after 24 h, were fed orally with 1.5 mL of the standardized LAB suspension daily for 30 days. Group III (Pathogen–LAB in Milk) animals were challenged once orally with a single dose (1 mL) of the standardized cell suspension of ETEC and after 24 h, the animals were fed orally with 1.5 mL of standardized LAB in milk in ratio 1:1 daily for 30 days. Group IV (LAB alone) animals were fed daily with 1.5 mL of the standardized cell suspension of LAB for 30 days and Group V (Control) animals were fed daily with a dose of (1 mL) PBS (pH 7.2) for 30 days.

2.4 Clinical Observation in Rats

Clinical observations such as behavioral pattern, salivation, stool consistency, lethargy, sleep, posture and response to handling were made on animals prior to exposure to the pathogen (ETEC) and test (probiotic LAB) organisms. Following administration, the rats were observed again for clinical signs each day for 30 days and observations were recorded.

2.5 Enumeration of Pathogens in Feaces

Pathogens in the faecal samples of animals in all the groups were enumerated. One gram of freshly passed faecal sample of rats was suspended in 9 mL normal saline in sterile stomacher bags and macerated. The mixture was further diluted serially up to 10^{-6} and 0.1 mL of diluted faecal sample was spread-plated on Eosin Methylene Blue (EMB) agar plates, incubated at 37^{o}C for 24 hours and colony forming units (CFU) were counted.

2.6 Enumeration of LAB in Feaces

1 g of freshly voided faecal samples of rats in all the groups were suspended in 9 mL normal saline and serially diluted to obtain 10^{-6} dilution after proper maceration in stomacher bags. 0.1 mL of appropriately diluted sample was spread-plated on MRS agar, incubated at 37ºC for 24 – 48 hours and colony forming units (CFU) were counted.

2.7 Gross Examination, Sacrifice and Organ Cropping

After appropriate treatments in respective groups, the rats were fasted overnight and sacrificed 24 hours after the last administration through cervical dislocation. The stomach, duodenum, transverse colon and rectum of the animals were aseptically and carefully excised and the tissues were fixed in 10% buffered formalin (Phosphate buffer and formalin in ratio 9:1) at pH 7.2 to prevent putrefaction and autolysis before histological assessment [15].

2.8 Histological Assessment

The fixed tissues were processed for paraffin wax embedding, stained with Gram stain to detect the pathogenic (ETEC) and probiotic (LAB) organisms and stained with Haematoxylin and Eosin to detect any histomorphologic disparity in the mucosa lining of the gastrointestinal organs. After staining, the tissues were examined under the light microscope [15].

2.9 Gram Stain

A section of the tissue was smeared on a grease-free slide and left to dry for adequate fixing. The fixed smear was flooded with crystal violet for 2 minutes and rinsed briefly under gently running tap water. Thereafter, slide was flooded with Gram's iodine for 2 minutes and differentiated in acetone for 3-5 minutes and the slide was rinsed under gently running tap water. The smear was counter-stained with 1% safranin for 10 seconds. The slide was washed in water and blotted dry. Dehydration was done using aniline-xylene (2:1) for 2 minutes. The slide was viewed under light microscope with 100 X objective under oil-immersion. Gram positive organism (LAB) stained blue-black while the Gram negative organism (*E. coli*) stained shades of red.

2.10 Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using Graph pad prism v.6.0 to determine significant difference between means. Statistical significance was set at 0.05 ($P = 0.05$). Values were recorded as mean ± standard error of mean.

3. RESULTS

After inoculation with a single dose of standardized (2×10^8 CFU/mL) diarrhoeagenic ETEC, the infected animals in group 1 were observed to release semi-solid stool consistently as well as presence of a mucoid substance in their feaces (Plate 1) for a period of 2-5 days post infection while animals in other groups released more compact feaces during this period. Also, during this period, a drop in appetite was observed in the ETEC challenged animals leading to weight loss and body weakness.

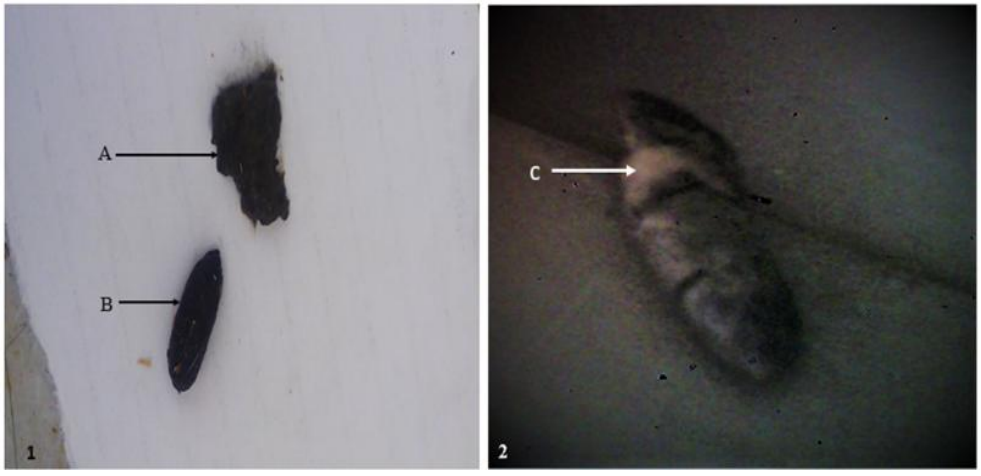

Plate 1. Changes observed in the appearance of experimental animal's feaces. A-Semi solid stool sample from a representative animal in group 1 after infection with ETEC, B-Compact faecal sample from a non-infected representative animal in group 5, C-Presence of mucoid substance in the faecal sample of an infected animal

3.1 Count of Pathogens in Feaces of Animals in Each Treatment Group

E. coli count in the feaces of animals in group 1, which were fed with a single dose of standardized (2 × 10^8 CFU/mL) cell suspension of ETEC, significantly increased (63.36 × 10^4 ± 3.7) (P < 0.0001) after 3 days of administration till the 15th day after which a steady decline was observed (Fig. 1). However, for animals in group 2, fed with a single dose of standardized ETEC cell suspension and then 1.5 mL of standardized (1 × 10^9 CFU/mL) cell suspension of LAB in PBS after 24 h, there was a significant increase (37.66 × 10^4 ± 1.45) (P < 0.0001) in the *E. coli* count within the first 3 days of administration after which a decline was observed. Similarly for animals in group 3, fed with 1.5 mL of standardized cell suspension of LAB in milk (1:1) 24 h after they were fed with a single dose of ETEC, there was a significant increase (41.32× 10^4 ±3.7) (P < 0.0001) in *E. coli* count within 3 days of administration after which there was a decline in the pathogen count. Furthermore, there was no significant difference in the faecal *E. coli* count of animals in group 4 fed with 1.5mL of standardized cell suspension of LAB when compared with the control group.

3.2 Count of LAB in Feaces of Animals in Each Treatment Group

Lactic acid bacteria count was observed to be low in group 1 animals, which were challenged once with 1 mL of standardized (2 × 10^8 CFU/mL) cell suspension of ETEC. While there was an increase in LAB count in the feaces of animals in group 2, which were treated with 1.5 mL of standardized cell suspension of LAB 24 h after they were challenged with a single dose of standardized cell suspension of ETEC after 12 days. However, the highest significant increase (125×10^4 ± 5.2) (P < 0.0001) in LAB count was observed in group 3 animals, which were treated with standardized cell suspension of LAB in milk for 30 days 24 hours after they were challenged with a single dose of standardized cell suspension of ETEC. Similarly, significant increase (73×10^4 ± 1.7) (P < 0.0001) in LAB count was observed in group 4 animals which were treated with 1.5 mL of standardized LAB cell suspension daily for 30 days. While LAB count in group 5 animals, which were administered with 1 mL of PBS daily was low compared to the groups treated with standardized suspension of probiotic LAB (Fig. 2).

The representative micrographs of smears of rat's stomach, duodenum, transverse colon and rectum stained with Gram stain are as presented in Figs. 3-6. Diarrhoeagenic ETEC, which are Gram negative organisms, were more abundant in the stained smears of pathogen treated group (Group 1) than Gram positive organisms after 30 days. Furthermore, the probiotic LAB strain chosen for the present study adhered well to the mucosal wall as there was higher occurrence of Gram positive organisms in the stained tissues of animals treated with probiotic LAB in groups 2, 3 and 4 while the occurrence of Gram negative organisms was very low and negligible after 30 days. Also, representative photomicrographs obtained after 30 days from the animals fed with 1 mL PBS (Group 5) showed fewer occurrence of both Gram positive and Gram-negative organisms in all the stained smears.

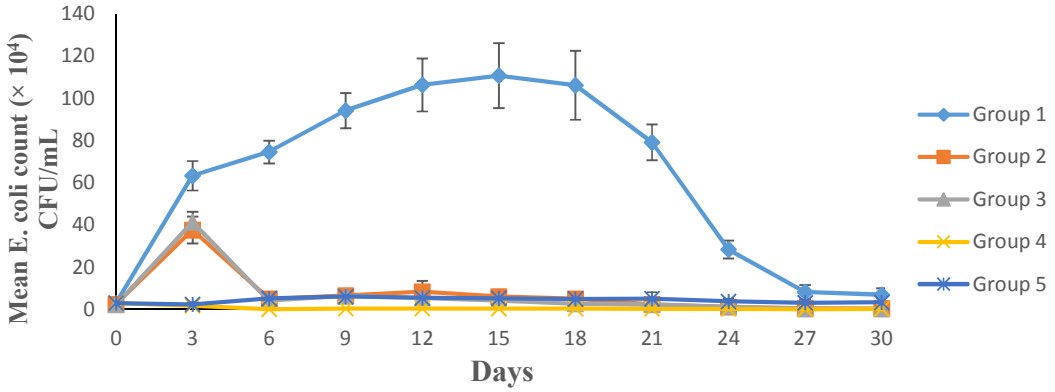

Fig. 1. *E. coli* count in feaces of animals in different treatment groups for 30 days

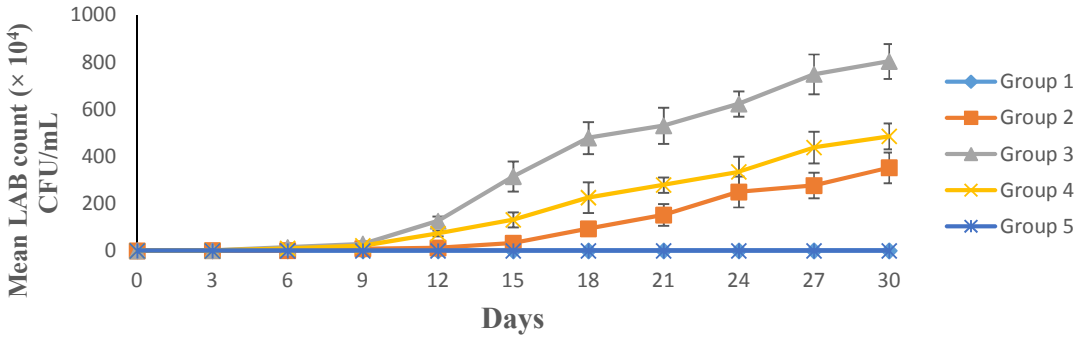

Fig. 2. LAB count in feaces of animals in different treatment groups for 30 days morphology of stained organ smears

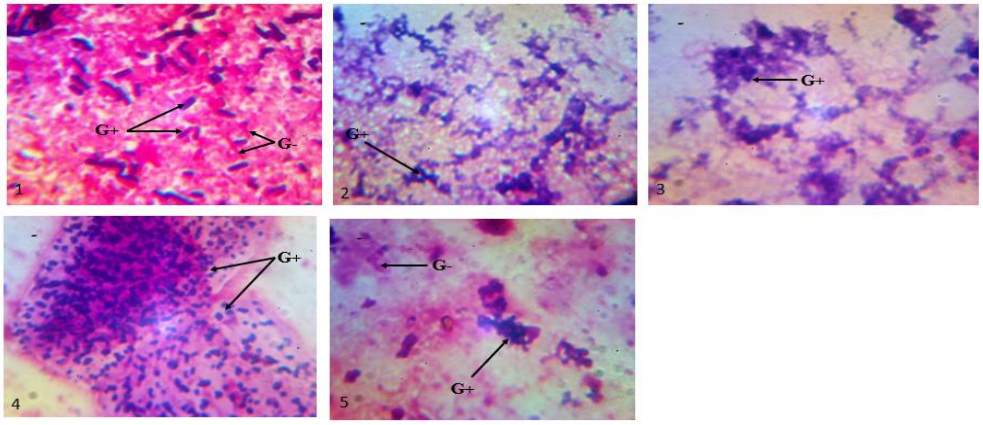

Fig. 3. Representative micrographs of smears of rat's stomach mucosa subjected to gram stain

Observe: 1, 5: Presence of Gram positive (G+) and Gram negative (G-) bacteria while in 2, 3, and 4, Gram positive (G+) bacteria mostly seen

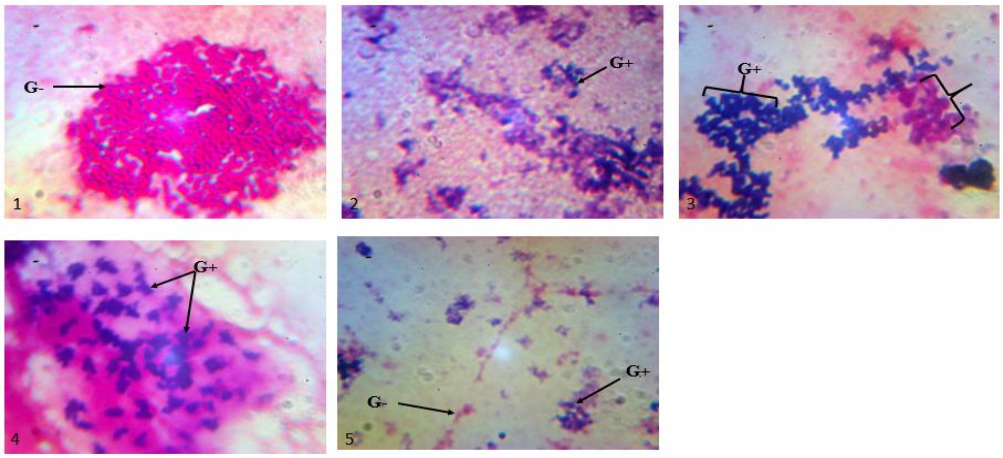

Fig. 4. Representative micrographs of smears of rats' duodenum mucosa subjected to gram stain

Observe: 1 Presence of numerous Gram negative (G-) bacteria closely arranged, 2 and 4, Gram positive (G+) bacteria mostly seen. Gram positive and Gram-negative bacteria were present in 3 and 5

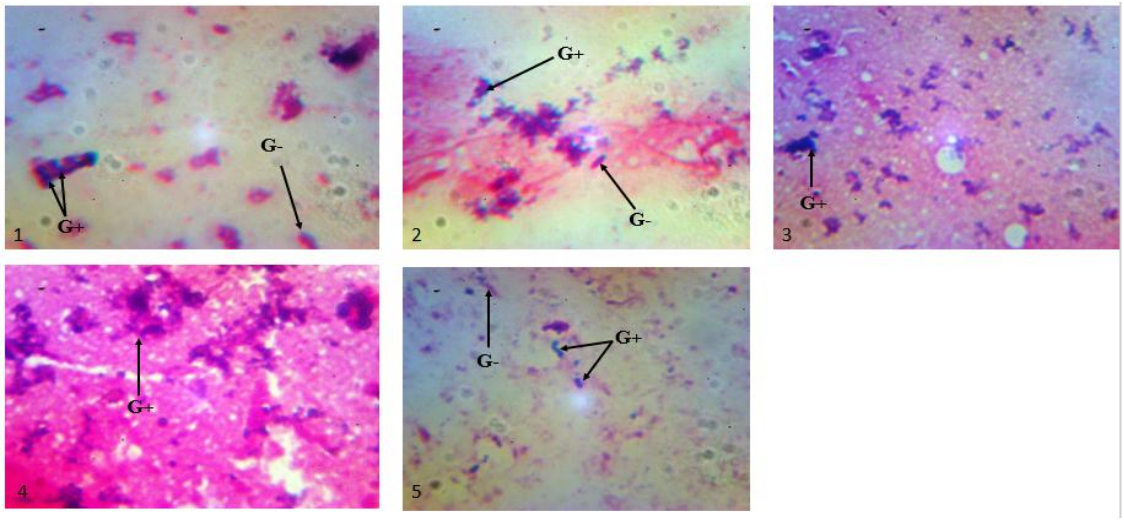

Fig. 5. Representative micrographs of smears of rats' transverse colon mucosa subjected to gram stain

Observe: 1, 2 and 5, Presence of Gram positive (G+) and Gram negative (G-) bacteria while in 3 and 4, Gram positive (G+) bacteria mostly seen

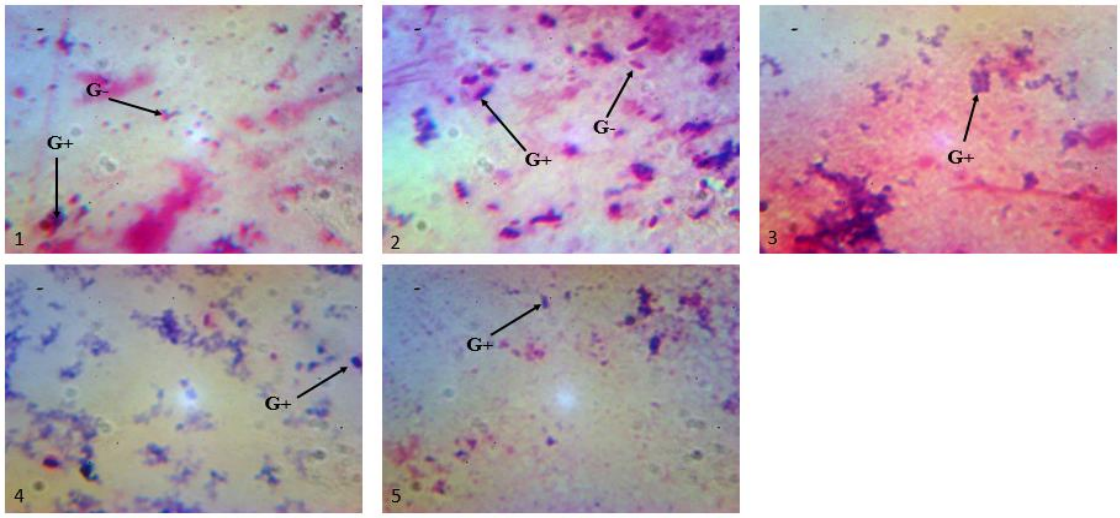

Fig. 6. Representative micrograph of smears of rats' rectum mucosa subjected to gram stain

Observe: 1, 2, Presence of Gram positive (G+) and Gram negative (G-) bacteria while in 3, 4 and 5, Gram positive (G+) bacteria mostly seen

3.3 Haematoxylin and Eosin Stain of Organ Sections

The representative micrographs of sections of rat's stomach stained with Haematoxylin and Eosin stains show that the mucosa, submucosa and muscularis externae layers in the stomach of representative animals were intact. The epithelium (innermost part of the mucosa) formed different invaginations known as the gastric pits while the lamina propria (middle part of the mucosa) with strongly eosinophilic parietal cells and the muscularis mucosa (outermost part of the mucosa) with prominent layer of smooth muscle were intact across the groups (Fig. 7). In the duodenum, the mucosa, submucosa and the muscularis externae were intact. In the mucosa are prominent intestinal epithelial cells (IEC), plicae circularis with prominent intervillous space (IVS) and laminar

propria (Fig. 8). In the transverse colon, temporary folds of the mucosa with numerous goblet cells (GC) were observed for representative animals in each of the groups. Furthermore, the muscularis mucosae, submucosa, muscularis externae were intact across the groups with distinct inner circular and prominent outer longitudinal layers (Fig. 9). In the rectum, the temporary fold of rectal mucosa with projecting surface epithelium as well as goblet cells (GC) were prominent across groups. Also, other layers of the GIT such as the muscularis mucosae, submucosa, muscularis externae were intact across all groups (Fig. 10).

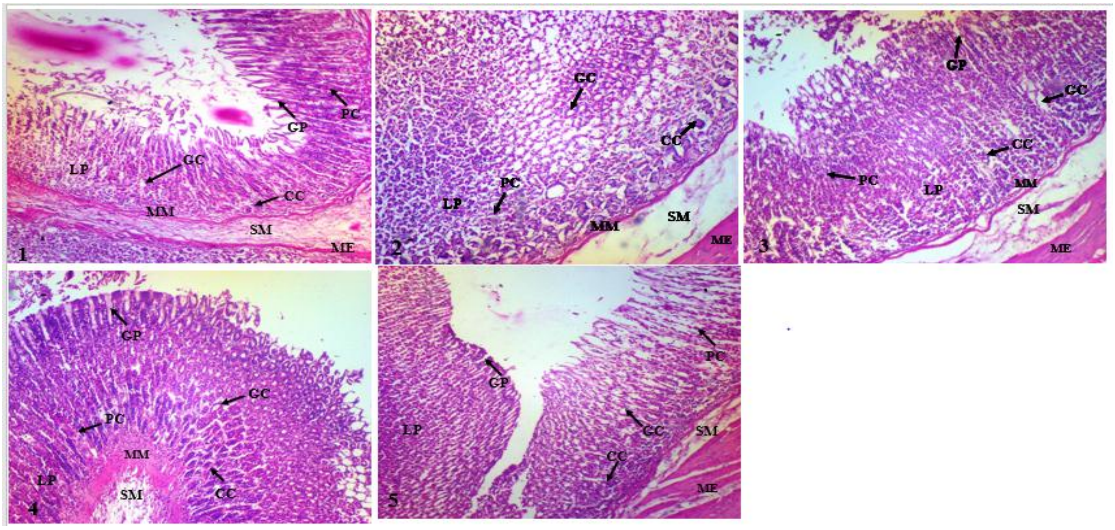

Fig. 7. Micrographs of sections of rats' stomach mucosa subjected to H & E stains

Intact arrangements in the mucosa with distinct gastric pits (GP), lamina propria (LP) with strongly eosinophilic parietal cells (PC) having round centrally located nucleus and strongly basophilic chief cells (CC) in their mucosae and goblet cells (GC). Muscularis mucosa (MM), submucosa (SM), muscularis externae (ME) layers are intact across the groups. Magnification: 100x

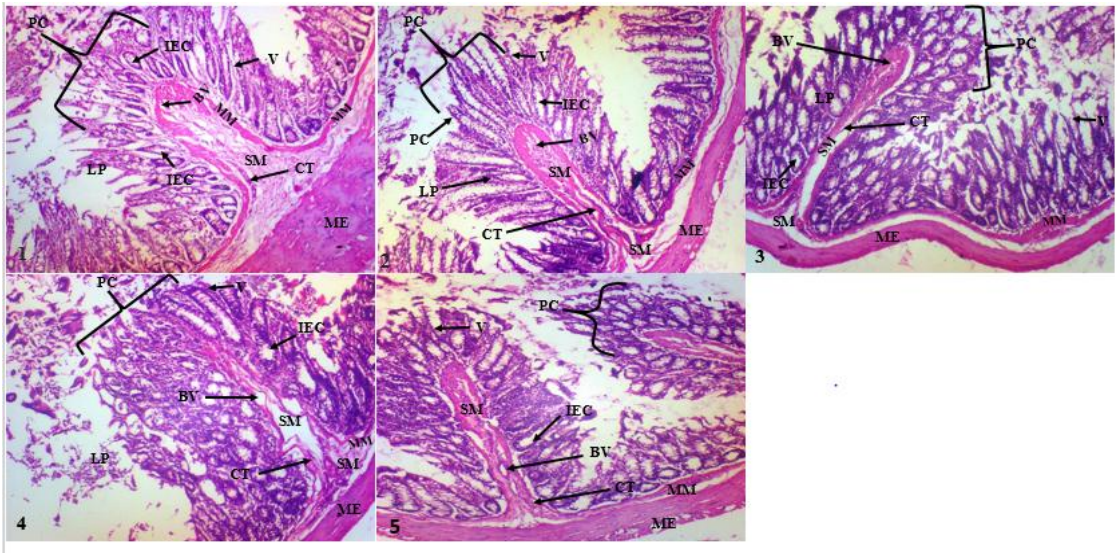

Fig. 8. Micrographs of sections of rats' duodenum subjected to H & E stains

Permanent spiral folds or elevations of the mucosa plicae circulares (PC), with prominent villi (V) and intestinal epithelial cells (IEC). Submucosa (SM) with loose connective tissues (CT) and blood vessels (BV) and muscularis mucosal as well as the muscularis externae (ME) are intact in these groups. Magnification:100x

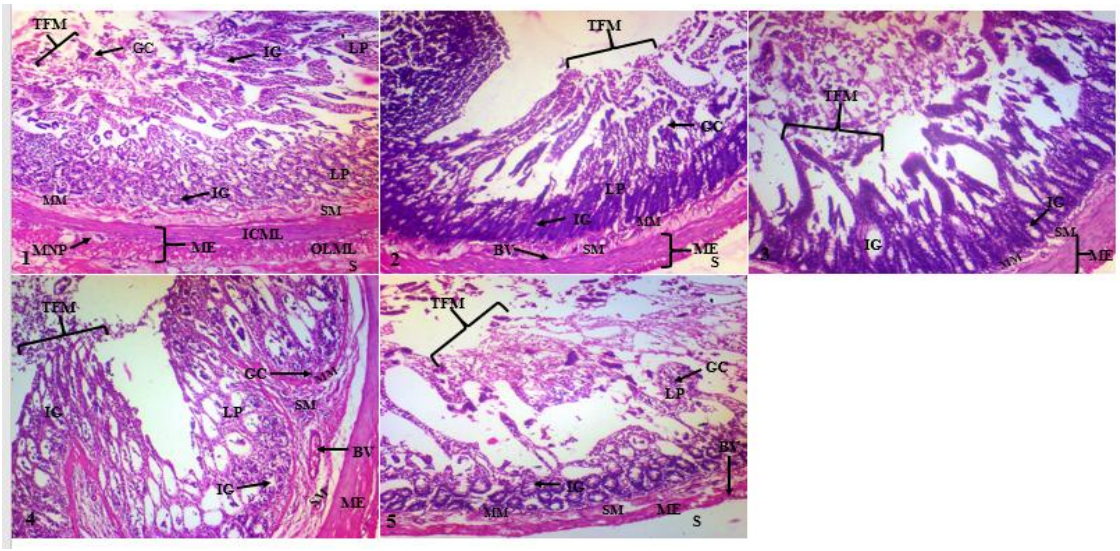

Fig. 9. Micrographs of sections of rats' transverse colon subjected to H & E stains

Temporary fold of the mucosa (TFM) across the groups. Goblet cells (GC) are distinct in all the groups interspersed among the columnar absorptive cells of the intestinal epithelium. Prominent blood vessels (BV) were also found around the Submucosa in groups 2 and 4. Muscularis mucosae (MM), submucosa (SM), muscularis externae (ME) with its distinct inner circular (ICML) and outer longitudinal muscle layers (OLML) are prominent across the groups. Magnification: 100x

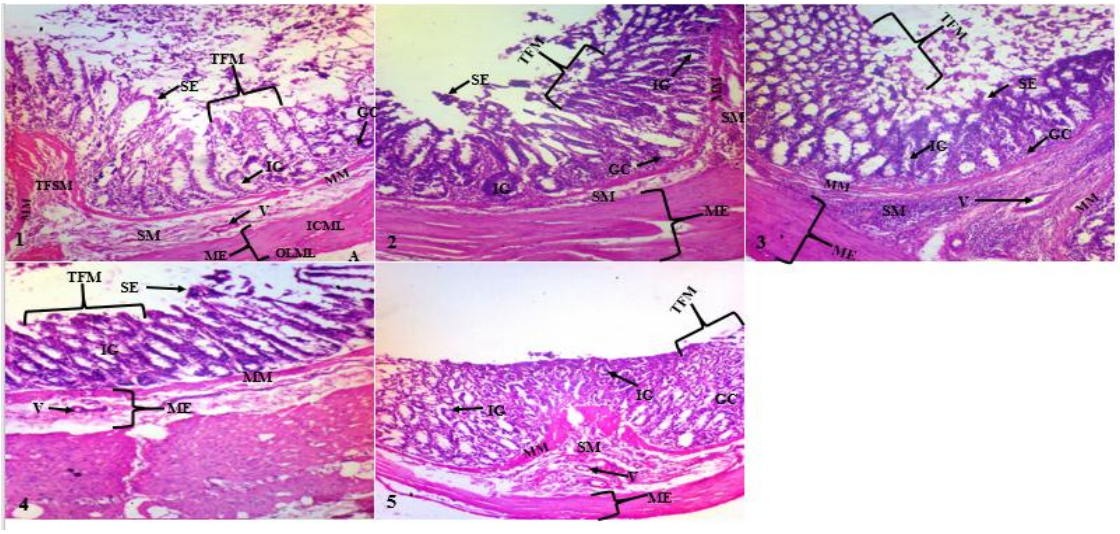

Fig. 10. Micrographs of sections of rats' rectum subjected to H & E stains

Temporary fold of rectal mucosa (TFM) with their projecting surface epithelium (SE) with prominent intestinal glands (IG) in the mucosa across the groups. The mucularis mucosae (MM), submucosa (SM) with prominent venules (V), thick muscularis externae (ME) are intact across the groups. Magnification: 100x

4. DISCUSSION

Diarrhea is characterized with discharge of watery stools more than 3 times/day sometimes with the presence of blood or mucus in the stool, as well as loss of appetite, weight loss and general body weakness [16]. Enterotoxigenic *Escherichia coli* (ETEC) has been reported to be the leading cause of bacterial diarrhea amongst the various *Escherichia coli* pathotypes. ETEC strains initiate diarrhea by colonizing the small intestine followed by the production of enterotoxins. They are one of the most common causes of diarrhea in children and traveler's

diarrhea in developing countries [17]. In the present study, observations such as appetite and weight loss, watery stool and appearance of mucus indicated the initiation of diarrhea in the experimental animals. Also, the observation of clinical signs of diarrhea within 2-5 days of infection with ETEC is consistent with the report that clinical signs of diarrhea are usually identified within 72 hours of exposure to the pathogen [16].

It has been reported that there is an increase in the population of Enterobacteriaceae during disease in a susceptible host although the mechanism behind this is not fully known [18]. The proliferation of *E. coli* within the first 15 days of infection in group 1 animals, which were challenged with infective dose of ETEC, as isolated from their feaces could be due to the ability of the pathogen to colonize the mucosal site, suppress the host immune system and initiate disease within this period. It has also been reported that the level of oxygen in the gastrointestinal epithelium supports the proliferation of *E. coli*, based on the ability of the organism to regulate genetic networks for oxygen sensing [19]. The increased LAB count in animals treated with probiotic organisms in this study indicated the ability of the probiotic strain to survive gastric and intestinal conditions in the animal gut. This is very essential for the organism to elicit beneficial effects in the host. This observation is consistent with other results that recorded a significant increase in the LAB count of the faecal samples of experimental animals treated with probiotics [14]. Furthermore, the higher count of LAB in the feaces of animals in group 3 (animals treated with LAB in milk) could be due to the ability of the probiotic organism to utilize the additional nutrients present in the milk such as calcium, vitamins, carbohydrates and proteins for proliferation. Thus indicating the use of milk and other suitable dairy products as a veritable carrier for probiotic administration in a living host [20].

The decrease in the *E. coli* count in the feaces of animals treated with probiotics 3 days after they have been challenged with the pathogen (groups 2 and 3) signals the inhibitory effect of the probiotic strain on the pathogen. Probiotics possess the ability for modification of the physiological homeostasis of the intestinal microbiota. They block the invasion of intestinal cells by pathogenic bacteria and modulate the intestinal immune response to up-regulate immunoglobulins, down-regulate inflammatory cytokines and protect mucosal barrier [21]. Furthermore, probiotics are expected to adhere well enough to the mucosal layer in the GIT since they need to compete with other pathogens for nutrient uptake [22]. The abundance of Gram-positive organisms in the Gram stained organs of animals treated with probiotics indicates the adhesive ability and the resistance to harsh luminal gastrointestinal tract conditions exhibited by the probiotic strain. Also, the lower occurrence of Gram-negative organisms in animals treated with the probiotic LAB is due to the activity of the organism to maintain gut microbiota and reduce the occurrence of pathogenic organisms in the gut. Probiotics are able to achieve this phenomenon of competitive exclusion by competing vigorously with pathogens for binding sites through the production of antimicrobial metabolites such as organic acids, bacteriocins and successful competition for available nutrients [23].

The high occurrence of Gram-negative organisms in the duodenum of group 1 animals challenged with the pathogen obtained after Gram stain could be due to the stress conditions the animals were exposed to during the period of the experiment. This is supported by a previous report which illustrated that stress levels can initiate the increase of *E. coli* concentration in the proximal sections (duodenum and jejunum) of the digestive tract [24]. Furthermore, the proliferation of *E. coli* could encourage the growth of other pathogens such as *Bacteroidetes* sp. which are Gram negative bacteria largely associated with the GIT [25].

Hematoxylin and Eosin stains have been very essential for histologic examination and the recognition of various tissue types and the morphologic changes that form the basis for contemporary cellular pathology [26]. Histologically, the morphological integrity of the organs (stomach, duodenum, transverse colon and rectum) stained with hematoxylin and eosin stains was preserved across the groups in the present study. The result obtained in group 1 animals which were challenged with ETEC indicates the possibility of the animals to have recovered naturally from the induced diarrhea before 30 days. It has been reported that individuals infected with ETEC can recover within 7-12 days of infection depending on the host's immune system and the virulence of the pathogen involved [27]. An important factor considered in the administration of probiotics is that the probiotic strain must be tolerated by the

host's immune system and be able to stimulate the immune system against pathogens and not by itself provoke local or general pathogenic, allergic, mutagenic or carcinogenic reactions [22]. The intact mucosal arrangement without any histomorphologic disparity observed in animals treated with the standardized cell suspension of the probiotic strain in PBS (groups 2 and 4) and in milk (group 3) indicates the safety of the probiotic both in a normal suspension and in a carrier system over a long period of consistent intake. This is consistent with the evaluation of the effects of a probiotic LAB in the prevention of *Helicobacter pylori* in the stomach tissue of mice [28]. Furthermore, the intact mucosal arrangement observed in the control animals (Group 5) suggests that healthy animals were selected for the study and there was no environmental contamination or possible error during the experiment.

5. CONCLUSION

In conclusion, the results obtained from this study indicates that *Pediococcus acidilactici* isolated from *Wara* was able to act as a therapeutic agent against a Diarrhoeagenic Enterotoxigenic *E. coli* and improve gut health through the adherence to mucosal wall and exclusion of pathogenic bacterial strains from the organs of the GIT without any adverse effect on the wellbeing of the host.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee, Ethical clearance for all procedures involving animals was obtained from the Health Research Ethic Committee (HREC), Institute of Public Health, Obafemi Awolowo University, Ile-Ife, Nigeria with the number IPH/OAU/12/1026.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the members of staff of the OAU multidisciplinary laboratory for the assistance rendered during the animal model experiment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Joint Food and Agriculture Organization and World Health Organization (FAO/WHO). Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food. London, Ontario, Canada. 2002;1-11.
2. Tezel BU. Preliminary *in-vitro* evaluation of the probiotic potential of the bacteriocinogenic strain *Enterococcus lactis* PMD74 isolated from ezine cheese. J. Food Qual. 2019;1-12. Available:https://doi.org/10.1155/2019/4693513
3. Cho JH, Zhao PY, Kim IH. Probiotics as a dietary additive for pigs. J. Anim. Vet. Adv. 2011;10(16):2127-2134. DOI: 10.3923/javaa.2011.2127.2134
4. Yirga H. The use of probiotics in animal nutrition. J Prob Health. 2015;3:132. DOI: 10.4172/2329-8901.1000132
5. Varankovich NV, Nickerson MT, Korber DR. Probiotic based strategies for therapeutic and prophylactic use against multiple gastrointestinal diseases. Frontier Microbiol. 2015;6:685. DOI: 10.3389/fmicb.2015.00685
6. Peter AK, Umar U. Combating diarrhea in Nigeria: The way forward. J. Microbiol Exp. 2018;6(4):191-197. DOI: 10.15406/jmen.2018.06.00213
7. Roussel C, Cordonnier C, Livrelli V, Van de Wiele T. Blanquet-Diot S. Enterotoxigenic and enterohemorrhagic *Escherichia coli*: survival and modulation of virulence in the human gastrointestinal tract. *Escherichia coli*-Recent Advances on Physiology, Pathogenesis and Biotechnology Applications. 2017;1:3-24. Available:http://dx.doi.org/10.5772/intechopen.68309
8. Ferreira AF, Pereira-Manfro WF, Rosa ACP. Diarrhoeagenic *Escherichia coli* and probiotic activity against food-borne pathogens: A brief review. Gastroenterol. Hepatol. 2017;7(5):00248. DOI: 10.15406/ghoa.2017.07.00248
9. Mohammadi M, Tajabadi EM, Pakpour B. Restoration of morphine-induced amnesia by *Pediococcus acidilactici*, an indigenous

probiotics of Iran in male wistar rats. Arch. Med. Lab. Sci. 2018;4(1):1-8.

10. Wang S, Yao B, Gao H, Zang J, Tao S, Zhang S, et al. Combined supplementation of *Lactobacillus fermentum* and *Pediococcus acidilactici* promoted growth performance, alleviated inflammation and modulated intestinal microbiota in weaned pigs. BMC Vet. Res. 2019;15(239):1-11. Available:https://doi.org/10.1186/s12917-019-1991-9
11. Abbasiliasi S, Tan JS, Bashokouh F. *In vitro* assessment of *Pediococcus acidilactici* kp10 for its potential use in the food industry. BMC Microbiol. 2017; 17(121):1-11. DOI: 10.1186/s12866-017-1000-z
12. Gupta A, Sharma N. Characterization of potential probiotic lactic acid bacteria- *Pediococcus acidilactici* Ch-2 Isolated from Chuli- A traditional apricot product of Himalayan region for the production of novel bioactive compounds with special therapeutic properties. J Food Microbiol Saf Hyg. 2017;2:119. DOI: 10.4172/2476-2059.1000119
13. Brousseau JP, Talbot G, Beaudoin F, Lauzon K, Roy D, Lessard M. Effects of probiotic *Pediococcus acidilactici* strain MA18/5M and *Saccharomyces cerevisae* subsp. *boulardii* strain SB-CNCM 1-1079 on feacal and intestinal microbiota of nursing and weaning piglets. Journal of Animal Science. 2015;93(11):5313-26. DOI: 10.2527/jas.2015-9190
14. Shukla G, Sharma G, Goyal N. Probiotic characterization of lactobacilli and yeast strains isolated from whey beverage and therapeutic potential of lactobacillus yoghurt in Murine Giardiasis. Am. J. Biomed. Sci. 2010;2(3):248-261. DOI: 10.5099/aj100300248
15. Avwioro OG. Tissue processing. Histochemistry and Tissue Pathology. 1st Edition. 2002;218-219 ISBN 978 35627-9-7.
16. Oghenesuvwe EE, Tedwins JO, Obiora IS, Lotanna AD, Ujam N, Okezie M. et al. Preclinical screening techniques for anti-diarrhoeal drugs: A comprehensive review. Am. J. Physiol. Biochem. Pharmacol. 2018;7(2):61–74. DOI: 10.5455/ajpbp.20180329014330
17. Raj V, Rai A, Singh M. Detection of bioflavonoids from methanol bark extracts of acacia catechu willd through HPTLC and their role as antidiarrhoeal agents. J. Analytical Pharmacol. Res. 2017;5(4): 00146. DOI: 10.15406/japlr.2017.05.00146
18. Rizzatti G, Lopetuso LR, Gibiino G, Binda C. Gasbarrini A. Proteobacteria: A common factor in human diseases. Biomed Res. Int. 2017;1-7. Available:doi.org/10.1155/2017/9351507
19. Holy O, Chmelar D. Oxygen tolerance in anaerobic pathogenic bacteria. Folia Microbiol (Praha). 2012;57(5):443-446. DOI: 10.1007/s12223-012-0149-y
20. Gasmalla M, Khadir K, Musa A, Aboshora W, Zhao W. Evaluation of some physicochemical parameters of three commercial milk products. Pak. J. Food Sci. 2013;23(2):62-65. ISSN: 2226-5899.
21. Samanta A, Mandal A, Patra A, Mandal S, Roy S, Pradhan S. et al. Impact of probiotic supplementation on intestinal microflora of rat under environmental hypobaric pressure. Int. J. Curr. Microbiol. Appl. Sci. 2014;3(8):282-291.
22. Shyamala G, Meenambigai P, Prabhavathi P, Raja R, Yesudoss L. Probiotics and its effects on human health- A review. International J. Curr. Microbiol. Appl. Sci. 2016;5(4):384-392. Available:http://dx.doi.org/10.20546/ijcmas.2016.504.046
23. Villena J, Kitazawa H. Probiotic microorganisms; A closer look. Microorganisms. 2017;5(17):1-3. DOI: 10.3390/microorganisms5020017
24. Gombosova L, Lazurova I, Zakuciova M, Curova K, Kmetova M, Petrasova D. Siegfried L. genes of intestinal *Escherichia coli* and their relation to the inflammatory activity in patients with ulcerative colitis and Chron's disease. Folia Microbiol. 2011;56:367-372. DOI: 10.1007/s12223-011-0051-z
25. Gibiino G, Lopetuso LR, Scaldeferri F, Rizzatti G, Binda C, Gasbarrini A. Exploring bacteriodetes: Metabolic key points and immunological tricks of our gut commensals. Dig. Liver. Dis. 2018;50(7): 635-639. Available:https://doi.org/10.1016/j.dld.2018.03.016
26. Fischer AH, Jacobson KA, Rose J, Zeller R. Hematoxylin and eosin staining of tissue and cell sections. CSH Protoc; 2008. DOI: 10.1101/pdb.prot4986
27. Gomes AT, Elias P, Scaletsky CA, Guth EC, Rodrigues F, Piazza MF, Ferreira CS,

Martinez B. Diarrhoeagenic *Escherichia coli*. Br J. Microbiol. 2016;47(1):3–30. Available:http://dx.doi.org/10.1016/j.bjm.2016.10.015

28. Afsahi A, Mahmoudi H, Ebrahimi A, Aeini Z, Esmaeili D. Evaluation of the effect of *Lactobacillus plantarium* probiotics produced from broad bean seed in prevention of *Helicobacter pylori* in stomach tissue of C57BL/6 mice. J. Can. Sci. Ther. 2018;10(4):085-092. DOI: 10.4172/1948-5956.1000524

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/60751