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Efficacy Evaluation of Heat Attenuated Lactobacillus Rhamnosus Stored at Different Shelf-Life Conditions in Experimental Model of Castor Oil-Induced Diarrhea

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KEYWORDS

Heat attenuated probiotic, *Lactobacillus rhamnosus*, Castor oil, Diarrhea, Gut dysbiosis, Membrane integrity.

ABSTRACT:

Introduction: Acute watery diarrhea is a major problem especially in the paediatric population. Heat attenuated probiotics is a widely known therapeutic option with numerous health benefits. But evidences suggest that altered storage condition can alter the cell membrane integrity of heat attenuated probiotics, no studies have evaluated the efficacy of heat attenuated probiotics in diarrhea condition and simultaneously evaluated the impact of different storage conditions on anti-diarrheal efficacy of heat attenuated probiotics.

Objectives: The current research evaluated the anti-diarrheal efficacy of heat attenuated *Lactobacillus rhamnosus* stored in different storage conditions.

Methods: Heat attenuated probiotic was stored at 25°C ± 60% RH and 40°C ± 75% RH for 12 months. At baseline, months 6 and 12, representative samples from the storage conditions were collected and evaluated for anti-diarrheal efficacy. Male Albino Wistar rats (n=64) were randomised into groups to receive either saline, loperamide, or representative samples of heat attenuated probiotics at different storage conditions. Following diarrhea induction by castor oil, rats were evaluated for onset of diarrhea, number and weight of diarrhea faeces, and GI transit length using charcoal meal test.

Results: Heat attenuated probiotics and loperamide therapy significantly reduced diarrhea onset duration, total number and mass of diarrhea faeces, and intestinal transit length compared to disease group with loperamide group showing better efficacy than the heat attenuated probiotics group. Efficacy was reduced with therapy of heat attenuated probiotics stored at 25°C for 6 and 12 months by 31.20% and 38.26%, and that stored at 40°C for 6 and 12 months by 35.61% and 43.55%, respectively, compared to heat attenuated probiotics therapy stored at baseline condition. Similar results were observed for all evaluated parameters.

Conclusions: The results of this study shows that heat attenuated probiotics can be considered as effective measure for the management of acute watery diarrhea. The study also highlights the importance of optimal storage condition for maintaining the cell membrane integrity of heat attenuated probiotics that have direct influence on its overall efficacy.

1. Introduction

Diarrhea is defined as the passing of three or more loose/liquid stools per day leading to increased stool frequency, high fluid level in stools, and rapid dehydration and malabsorption of nutrients (1,2). Diarrhea is considered a major health concerns negatively affecting the quality of life (3,4). Among the various patient population, the pediatric age group are the most sensitive population for developing diarrhea and hence have the highest prevalence rate (3). With ~1.6 million deaths every year, diarrhea is a major cause of global mortality and morbidity (5). Numerous factors are linked with the development of diarrhea condition (6,7).

Trillions of micro-organisms reside in the human gastrointestinal tract (GIT) that plays important role in maintaining the normal health of the individual (1,8). Gut dysbiosis leads to the reduction of friendly microbes and increase of pathogens (1,8). Numerous evidences have

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highlighted gut dysbiosis as a major cause to various GIT-related disease conditions, including diarrhea (1). Dysbiosis leads to intestinal barrier disruption and damage of intestinal integrity, that leads to increased absorption of pathogenic toxins, reduced formation of beneficial compounds (like short-chain fatty acids (SCFAs)), and increased level of intestinal inflammation (9,10). Altogether, gut dysbiosis causes alteration of the intestinal permeability, leading to more water release in the intestinal lumen and reduced water reabsorption leading to diarrhea (9,10).

The current treatment options for diarrhea primarily includes pharmacological approaches, including oral rehydration salts (ORS) to regain lost fluids and electrolytes (11–13). Agents such as loperamide and racecadotril are also used to normalize the frequency of bowel movements. Antibiotic therapy is opted in case of bacterial infections as cause of diarrhea. Probiotics are one of the treatment options that are gaining traction for use in diarrhea condition as these therapies are capable to reduce gut dysbiosis and promote gut eubiosis (1,8,14).

Probiotics are viable microorganisms that confers health benefits. In diarrhea condition, probiotics belonging to the *Lactobacilli*, *Bifidobacterium*, and *Saccharomyces* genera are widely studied (1,2,8). Numerous studies have demonstrated that probiotics are safe therapy and can be utilized in various health conditions (15,16). Considering special patient population like immunocompromised patients and patients with high risk to develop infections, probiotics therapy requires utmost caution considering the ability of probiotics to invade the systemic circulation and provoke abnormal immune activity (17,18). Postbiotics are probiotics-derived products that have healthy benefits (19). Currently, probiotic-derived metabolites (including SCFA and other nutrients), probiotic cellular fragments (including exopolysaccharides and probiotic lysates), and whole dead probiotic cells (also known as paraprobiotics and ghost probiotics (ghostbiotics)) are the postbiotics available (19,20). Postbiotics are better alternatives to probiotics in a number of ways, such as better and consistent production yield, predictable safety in special patient population, no risk of resistance or mutations, and ease of use for product development with other useful ingredients (19). Heat attenuated probiotics is one of the attractive postbiotic therapeutic approach which is gaining traction for development and use in clinical practice. The mechanism of action of heat attenuated probiotics are different compared to live probiotics. In case of live probiotics, the colonization of the probiotics in the GIT occurs and further leads to the release of various compounds that are responsible for the beneficial effects, while in case of heat attenuated probiotics, the major focus is given to maintain the integrity of the probiotic cell membrane which contains various components (like lipoteichoic acid) that are responsible for the beneficial effects (17,18,21). From this notion, it can be stated that the heat attenuated probiotics' cell membrane integrity is very crucial and is required to be maintained throughout shelf life period for desired therapeutic benefits. Among the numerous determinants, storage condition is an important determinant that have effect on the cell membrane integrity of heat attenuated probiotics. While numerous studies have shown that storage conditions have direct impact on the cell membrane integrity, studies evaluating the effect of different storage conditions on the anti-diarrheal efficacy of heat attenuated probiotics is lacking.

2. Objectives

The current study aimed to investigate the efficacy of heat attenuated probiotic *Lactobacillus rhamnosus* in experimentally induced diarrhea. We also investigated the effect of different shelf-life storage conditions and duration on the anti-diarrheal efficacy of heat attenuated probiotics. The results can help identify the heat attenuated probiotics as an attractive treatment option for the management of diarrhea condition and also provide insights into the importance of optimal storage conditions for maintaining the desired therapeutic efficacy throughout shelf life period.

3. Methods

3.1. Investigational probiotic procurement and storage

The investigational heat attenuated probiotic *L. rhamnosus* was kindly provided by Sundyota Numandis Probioceuticals Pvt. Ltd. (Ahmedabad, India). The brief manufacturing process involved the initial growth of *L. rhamnosus* seed culture in growth medium followed by batch fermentation. The biomass obtained by batch fermentation was then heated at optimal temperature for brief duration to form the heat attenuated probiotic biomass. The obtained biomass was then centrifuged and spray-dried to form the heat attenuated probiotic powder.

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The powder was stored in aluminium bags at -20°C. The initial strength of the heat attenuated probiotic was 300 billion cells per gram powder. The heat attenuated probiotic was analysed for its strength by flow cytometry method and then transferred in different aluminium bags. The bags were then stored at 25°C with 60% relative humidity (RH) and 40°C with 75% RH for 12 months. For evaluating the anti-diarrheal efficacy, representative sample of the heat attenuated probiotic from particular storage condition and duration were collected and stored at -20°C to prevent any cellular membrane damage. Following the entire storage duration and collection of sample from each storage condition and duration (baseline and months 6 and 12), the anti-diarrheal efficacy was evaluated as per the procedure described in the following sections.

3.2. Experimental animals

Healthy adult male Wistar rats (150-250 gm) were obtained from K.B. Raval College of Pharmacy (Gujarat, India). The animals were housed in propylene cages at 24°C ± 4°C with 12h light/dark cycle. The access to standard chow diet and water was free to all the rats and one week acclimatization period was considered for baseline stabilization. The entire study procedure was reviewed and approved by the ethics committee of the K.B. Raval College of Pharmacy (Gujarat, India) (Proposal No.: KBRCP/2025/11/03).

3.3. Experimental procedure

After the acclimatization period, rats were fasted for overnight with free access to water. Following fasting, rats were randomized in eight groups with 8 animals per group. The groups were as follows, normal control (NC), disease control (DC), standard control (SC), heat attenuated probiotic at baseline condition (P1), heat attenuated probiotic stored in 25°C from month 6 and 12 (P2 and P3), and heat attenuated probiotic from 40°C from month 6 and 12 (P4 and P5). Based on the randomization schedule, NC and DC group rats were administered saline water, while SC group rats were treated with loperamide (5 mg/kg/rat). The heat attenuated probiotic dose was selected to be 20 mg/rat which corresponds to approximately six billion cells of baseline heat attenuated probiotic strength. As we aimed to identify the effect of different storage conditions and duration on the anti-diarrheal efficacy, 20 mg/rat dose was administered to rats in the P1-P5 groups from the respective storage conditions and duration as described above. After 2 hours of therapy supplementation, single oral gavage dose of castor oil (1 ml/rat) was administered to all rats (except the NC group rats who were gavaged saline water). Ricinoleic acid is an ingredient present in castor oil that is responsible for irritating the intestines leading to widespread intestinal inflammation, excessive fluid loss, and reduced water retention. Due to this activity, castor oil is widely utilized agent for inducing diarrhea in animal models.

3.4. Evaluation parameters

Following castor oil oral gavage, rats were immediately placed in separate propylene cages. The cages had adsorbent paper at the cage floor to collect the diarrhea faeces. The onset time of diarrhea (in minutes), number of diarrheal faeces, and weight of diarrheal faeces (in gm) were evaluated. The evaluation was performed for four hours following castor oil administration. The rats were then allowed to naturally recover from the condition for 7 days. After 7 days, the entire study was repeated and after 30 minutes of castor oil therapy, the rats were orally gavaged single dose of charcoal meal (1 ml of 10% w/v in 0.5% CMC). Following ~30 minutes of charcoal meal therapy, the rats were sacrificed, intestines collected, and evaluated for charcoal transit length, indicator of GI transit length.

3.5. Statistical analysis

Data was expressed in mean ± SD in pre-designed datasheet. GraphPad Prism software was utilized for statistical analysis using one way ANOVA with post-hoc Tukey test. A p-value of <0.05 was set as threshold for statistical significance.

4. Results

4.1. Onset of diarrhea

Diarrhea leads to faster onset of diarrhea and anti-diarrheal therapies prolongs the duration of diarrhea onset. As per the hypothesis, the DC group rats showed rapid induction of diarrhea while loperamide and heat attenuated probiotics therapy significantly prevented the diarrhea onset duration (**Figure 1**). Loperamide therapy was significantly better than the heat attenuated probiotics therapy. Among the heat attenuated probiotics group, difference in storage temperature and duration exhibited variable efficacy to prolong the onset of diarrhea. Altogether it was observed that the higher

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storage temperature and longer duration of storage resulted in reduction in the overall efficacy of heat attenuated probiotics. Compared to heat attenuated probiotic stored at baseline storage condition, gradual reduction in efficacy was observed for both the heat attenuated probiotics stored at 25°C and 40°C stored for 6 and 12 months, respectively. Heat attenuated probiotics therapy stored at 25°C for 6 and 12 months showed 31.20% and 38.26% faster diarrhea onset, respectively, while heat attenuated probiotics therapy stored at 40°C for 6 and 12 months showed 35.61% and 43.55% faster onset of diarrhea, respectively, compared to therapy with baseline condition stored heat attenuated probiotics (**Figure 1**). This indicates the importance of optimal storage temperature and duration for optimal efficacy of heat attenuated probiotics throughout shelf-life period.

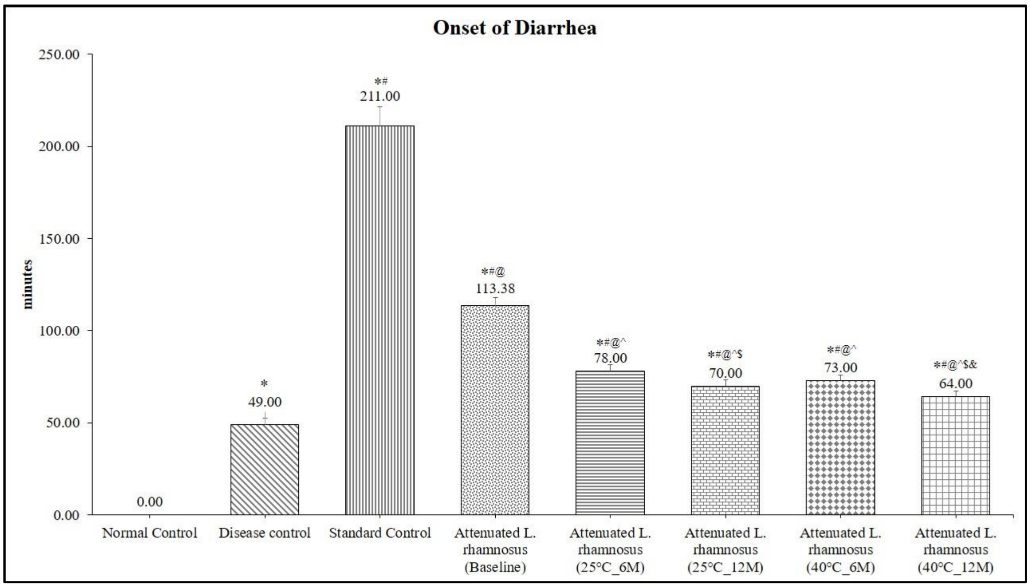

Figure 1: Efficacy of heat attenuated probiotics in onset of diarrhea. *$p < 0.05$ v/s Normal group; $^{\#}p < 0.05$ v/s Disease group; $^{@}p < 0.05$ v/s Standard control group; $^{\wedge}p < 0.05$ v/s Heat attenuated *L. rhamnosus* (Baseline) group; $^{\$}p < 0.05$ v/s Heat attenuated *L. rhamnosus* (25°C_6M) group; $^{\&}p < 0.05$ v/s Heat attenuated *L. rhamnosus* (40°C_6M) group.

4.2. Number and weight of diarrhea faeces

The number and weight of diarrhea faeces was significantly increased in the DC group rats and was suppressed by the loperamide and heat attenuated probiotics therapy (**Figure 2**). Increasing storage temperature and duration caused reduction in the overall efficacy of heat attenuated probiotics. Therapy with heat attenuated probiotics stored at 25°C for 6 and 12 months showed 23.51% and 27.43% higher number of diarrhea faeces, and therapy with heat attenuated probiotics stored at 40°C for 6 and 12 months showed 29.31% and 31.35% higher number of diarrhea faeces, respectively, compared to therapy with heat attenuated probiotics from baseline storage condition (**Figure 2**). The %defecation inhibition compared to DC group rats was found to be 88.01%, 31.98%, 16.04%, 13.38%, 12.05%, and 10.71% in the standard group, heat attenuated probiotics group from baseline storage condition, 25°C for 6 and 12 months, and 40°C for 6 and 12 months, respectively.

Similar trend was observed for the diarrhea faeces weight parameter. Therapy with heat attenuated probiotics stored at 25°C for 6 and 12 months showed 42.88% higher weight of diarrhea faeces, and heat attenuated probiotics therapy stored at 40°C for 6 and 12 months showed 45.98% and 51.89% higher weight of diarrhea faeces, respectively, compared treatment with heat attenuated probiotics from baseline storage condition (**Figure 3**).

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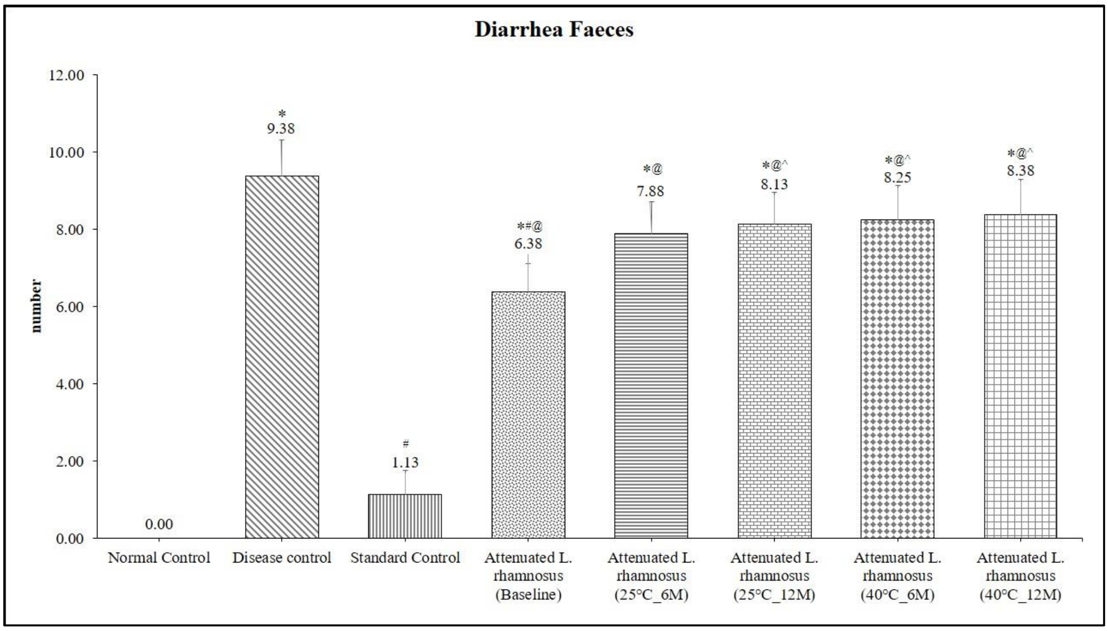

Figure 2: Efficacy of heat attenuated probiotics in reducing diarrhea faeces number. *$p < 0.05$ v/s Normal group; #$p < 0.05$ v/s Disease group; @$p < 0.05$ v/s Standard control group; ^$p < 0.05$ v/s Heat attenuated *L. rhamnosus* (Baseline) group.

4.3. Gastrointestinal transit length

Castor oil therapy significantly increased the GI transit length in the DC group rats, while treatment with loperamide and heat attenuated probiotics showed anti-diarrheal efficacy and thereby reduced the GI transit length with the greatest effect observed with loperamide therapy (**Figure 4**). Similar to previous observations, higher storage temperature and longer storage duration reduced the heat attenuated probiotics anti-diarrheal efficacy. Treatment with heat attenuated probiotics stored at 25°C for 6 and 12 months showed 12.92% and 17.02% greater GI transit distance, and therapy with heat attenuated probiotics stored at 40°C for 6 and 12 months showed 14.40% and 20.78% greater GI transit distance, respectively, compared to therapy with heat attenuated probiotics from baseline storage condition (**Figure 4**).

5. Discussion

Probiotics are among the most widely used therapies in various health conditions, due to their promising efficacy to reduce gut dysbiosis and promote healthy gut microbiome (1,2,8). Heat attenuated probiotics are probiotics that are not live but have the cell membrane integrity almost similar to the live probiotics. Numerous evidences suggests that heat attenuated probiotics are promising alternatives to conventional live probiotics in patient population who are more vulnerable to develop infectious conditions (17,18,21). Despite promising evidences, the use of heat attenuated probiotics in the management of diarrhea is limited, possibly due to lack of sufficient experimental evidence for efficacy. The current study investigated the efficacy of heat attenuated probiotic *Lactobacillus rhamnosus* in the management of castor oil-induced diarrhea in rats. The results of the current study shows that the heat attenuated probiotics are effective in the management of diarrhea condition. While numerous evidences have shown health benefits by probiotics particularly by restoring a gut microbiome and reducing gut dysbiosis through various mechanism of actions (22–25), the efficacy of heat attenuated probiotics are induced due to a completely different mechanism compared to live probiotics therapy.

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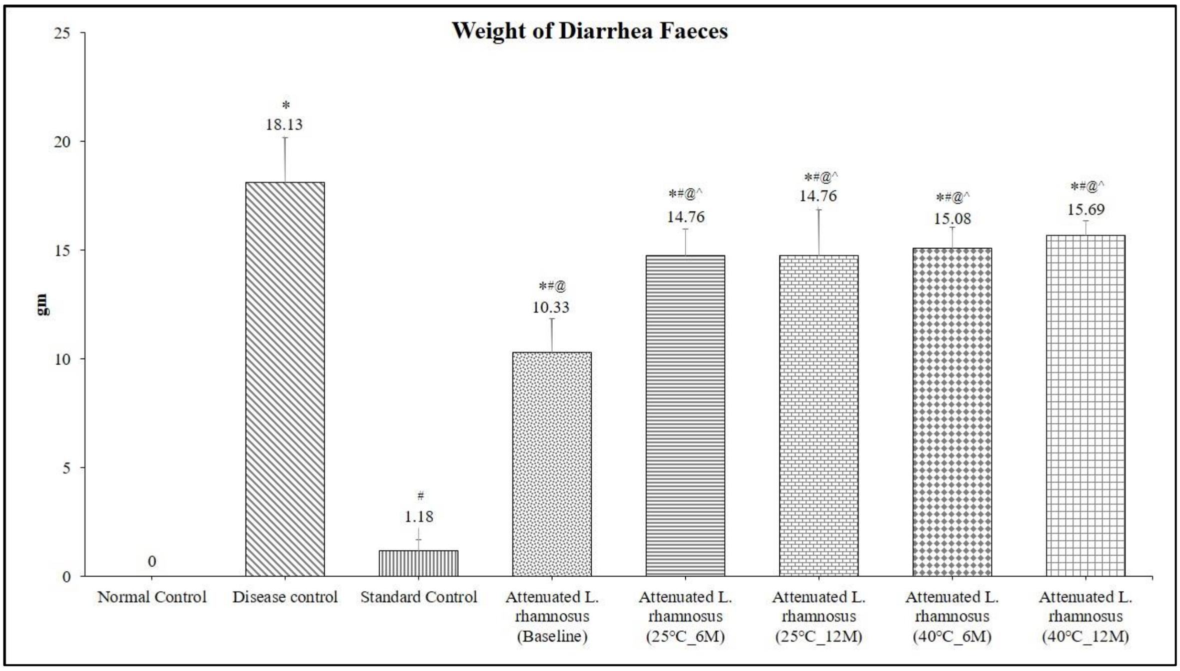

Figure 3: Efficacy of heat attenuated probiotics in weight of diarrhea faeces. *p < 0.05 v/s Normal group; #p < 0.05 v/s Disease group; @p < 0.05 v/s Standard control group; ^p < 0.05 v/s Heat attenuated *L. rhamnosus* (Baseline) group.

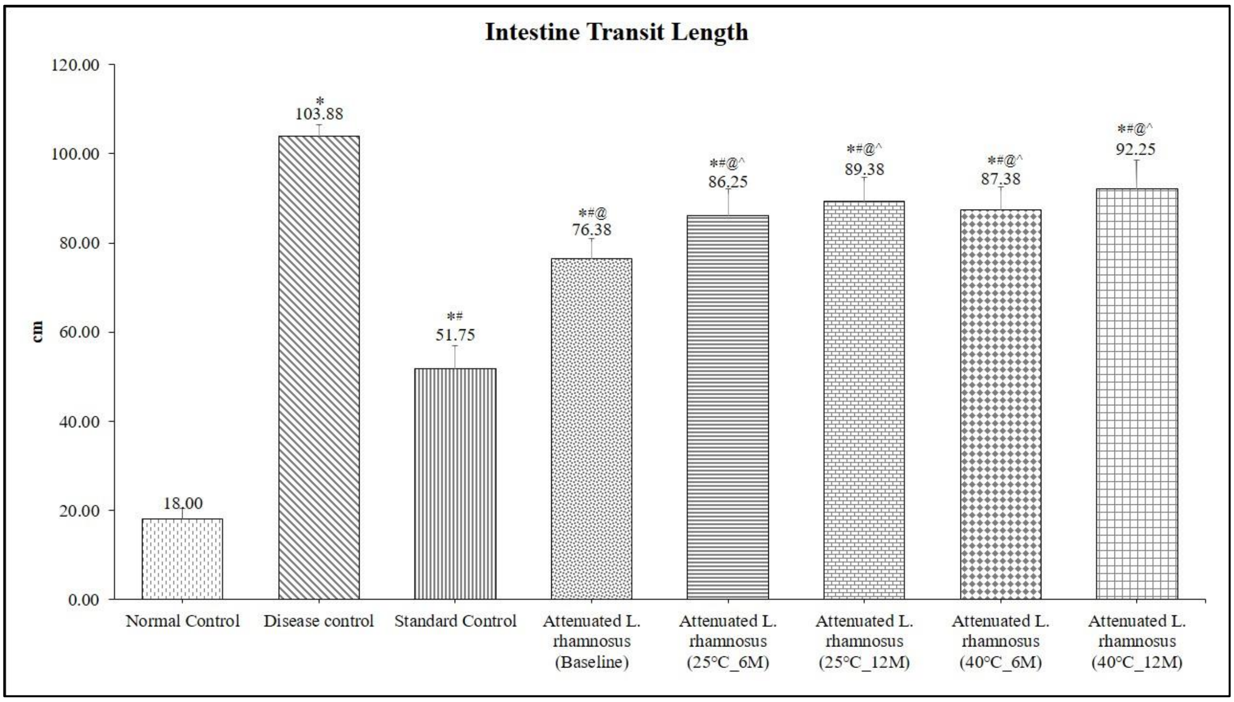

Figure 4: Efficacy of heat attenuated probiotics on GI transit length. *p < 0.05 v/s Normal group; #p < 0.05 v/s Disease group; @p < 0.05 v/s Standard control group; ^p < 0.05 v/s Heat attenuated *L. rhamnosus* (Baseline) group.

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The heat attenuated probiotics are manufactured with utmost care to ensure that the cell membrane integrity is maintained similar to the live probiotics. This process ensures that the cell membrane components remain intact, which are responsible for their beneficial effects (17,18,21). As heat attenuated probiotics are an attractive treatment option, it is very important that the cell membrane components remains intact during the entire storage duration to maintain the desired efficacy. Storage temperature have a huge influence of the stability of products, including the products with heat attenuated probiotics throughout shelf-life period. Improper storage conditions, including storage at high temperatures or in humid environments, significantly affects the probiotics' characteristics (26–28). The observations of current study shows that high storage temperature and longer storage duration progressively reduces the anti-diarrheal efficacy of heat attenuated probiotics. Such observations helps us to envisage the importance of optimal storage conditions for ensuring the cell membrane integrity of heat attenuated probiotics throughout their shelf life duration.

The current study have various strengths. Firstly, the current study is novel as no previous study have evaluated the efficacy of heat attenuated *L. rhamnosus* probiotic in the management of diarrhea condition in experimental animal model of castor oil-induced diarrhea. Many experimental studies have been previously conducted and shown that castor oil-induced diarrhea is an excellent experimental model for evaluating the anti-diarrheal effect of investigational therapies (29–32). In the current study, castor oil therapy successfully developed diarrhea in rats, which was significantly prevented by heat attenuated probiotics therapy. Secondly, the current study evaluated the effect of different storage conditions and duration on the anti-diarrheal efficacy of heat attenuated probiotics. Many speculations have been made regarding the influence of storage condition on the heat attenuated probiotics stability and efficacy, but to our knowledge, no study has evaluated this hypothesis. The current study is the first study that has successfully established a direct correlation between the storage condition and duration with the heat attenuated probiotics efficacy to mitigate diarrhea condition. The current study has certain limitations too. The current study used heat attenuated *Lactobacillus rhamnosus* as the investigational probiotic, while other probiotics species (including *Bifidobacterium* and *Saccharomyces*) are also widely used that might have different susceptibility to the storage conditions. As different probiotics have different cellular structure and components, the results of the current study cannot be directly extrapolated to other heat attenuated probiotics. But considering the fact that the current study is novel, it can pave the way for future well-designed experimental studies as well that can evaluate the effect of storage conditions on different heat attenuated probiotic species as well.

In conclusion, heat attenuated probiotics are promising therapeutic agents that have numerous health benefits. The current study highlights the potential of heat attenuated *Lactobacillus rhamnosus* probiotics in the management of diarrhea condition. The storage condition is very important aspect for maintaining the efficacy of heat attenuated probiotics throughout shelf life period. The results of current study shows that different storage condition and duration can lead to significant variability in the overall efficacy of heat attenuated probiotic therapies. These results help us to identify the importance of optimal storage conditions for maintaining the cell membrane integrity of heat attenuated probiotics and thereby maintaining the efficacy of therapy throughout shelf life period. Additionally, the current study can help future researchers to conduct more experimental and clinical studies to further validate the results of current study.

References

1. Kheni DB, Sureja VP, Deshpande SS, Dubey VP, Kansagra JJ. Lactobacillus rhamnosus GG in paediatric acute diarrhoea: A clinical perspective and systematic review of clinical studies published in the last decade. Chettinad Heal City Med J. 2024;4(13):135–45.
2. Mourey F, Sureja V, Kheni D, Shah P, Devang Parikh, Upadhyay U, et al. A multicenter, randomized, double-blind, placebo-controlled trial of Saccharomyces boulardii in infants and children with acute diarrhea. Pediatr Infect Dis J. 2020;39(11):e347–51.
3. Behera DK, Mishra S. The burden of diarrhea, etiologies, and risk factors in India from 1990 to 2019: Evidence from the global burden of disease study. BMC Public Health. 2022;22(1):92.
4. Das JK, Salam RA, Bhutta ZA. Global burden of childhood diarrhea and interventions. Curr Opin

Infect Dis. 2014;27(5):451–8.
5. Manetu WM, M'masi S, Recha CW. Diarrhea disease among children under 5 years of age: A global systematic review. Open J Epidemiol. 2021;11:207–21.
6. Camilleri M, Sellin JH, Barrett KE. Pathophysiology, evaluation, and management of chronic watery diarrhea. Gastroenterology. 2017;152(3):515-532.e2.
7. Field M. Intestinal ion transport and the pathophysiology of diarrhea. J Clin Invest. 2003;111(7):931–43.
8. Kheni DB, Sureja VP, Deshpande SS, Dubey VP, Kansagra JJ. A systematic mapping review of in-vitro and in-vivo evidences exploring the role of strain-specific probiotic Bifidobacterium longum W11. Int J Pharm Sci Drug Res. 2024;16(1):127–34.
9. Li Y, Xia S, Jiang X, Feng C, Gong S, Ma J, et al. Gut microbiota and diarrhea: An updated review. Front Cell Infect Microbiol. 2021;11:625210.
10. Sarker SA, Ahmed T, Brüssow H. Persistent diarrhea: A persistent infection with enteropathogens or a gut commensal dysbiosis? Environ Microbiol. 2017;19(10):3789–801.
11. Meisenheimer ES, Epstein C, Thiel D. Acute diarrhea in adults. Am Fam Physician. 2022;106(1):72–80.
12. Alam NH, Ashraf H. Treatment of infectious diarrhea in children. Pediatr Drugs. 2003;5(3):151–65.
13. Brandt KG, Antunes M de C, Silva AP da. Acute diarrhea: Evidence-based management. J Pediatr (Rio J). 2015;91(6 Suppl 1):S36–43.
14. Ahmadi E, Alizadeh-Navaei R, Rezai MS. Efficacy of probiotic use in acute rotavirus diarrhea in children: A systematic review and meta-analysis. Casp J Intern Med. 2015;6(4):187–95.
15. Lahtinen SJ. Probiotic viability - does it matter? Microb Ecol Health Dis. 2012;23.
16. Terpou A, Papadaki A, Lappa IK, Kachrimanidou V, Bosnea LA, Kopsahelis N. Probiotics in food systems: Significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. Nutrients. 2019;11(7):1591.
17. Wegh CA, Geerlings SY, Knol J, Roeselers G, Belzer C. Postbiotics and their potential applications in early life nutrition and beyond. Int J Mol Sci. 2019;20(19):4673.
18. Vinderola G, Sanders ME, Salminen S. The concept of postbiotics. Foods. 2022;11(8):1077.
19. Żółkiewicz J, Marzec A, Ruszczyński M, Feleszko W. Postbiotics—A step beyond pre- and probiotics. Nutrients. 2020;12(8):2189.
20. Kerksick CM, Moon JM, Jäger R. It's dead! Can postbiotics really help performance and recovery? A systematic review. Nutrients. 2024;16(5):720.
21. Kheni D, Sureja V, Deshpande S. Potential role of Lactobacilli spp. postbiotics in atopic dermatitis. Int J Res Publ Rev. 2024;5(4):10001–4.
22. Plaza-Diaz J, Ruiz-Ojeda FJ, Gil-Campos M, Gil A. Mechanisms of action of probiotics. Adv Nutr. 2019;10(Suppl_1):S49–66.
23. Ng S, Hart A, Kamm M, Stagg A, Knight S. Mechanisms of action of probiotics: Recent advances. Inflamm Bowel Dis. 2009;15(2):300–10.
24. Walker WA. Mechanisms of action of probiotics. Clin Infect Dis. 2008;46(Suppl 2):S87–91.
25. Sherman PM, Ossa JC, Johnson-Henry K. Unraveling mechanisms of action of probiotics. Nutr Clin Pract. 2009;24(1):10–4.
26. Jannah SR, Rahayu ES, Yanti R, Suroto DA, Wikandari R. Study of viability, storage stability, and shelf life of probiotic instant coffee Lactiplantibacillus plantarum subsp. plantarum Dad-13 in vacuum and nonvacuum packaging at different storage temperatures. Int J Food Sci. 2022;2022:1663772.
27. Mortazavian A, Ehsani M, Mousavi S, Rezaei K, Sohrabvandi S, Reinheimer J. Effect of refrigerated storage temperature on the viability of probiotic micro-organisms in yogurt. Int J Dairy Technol. 2007;60(2):123–7.
28. Cabello-Olmo M, Oneca M, Torre P, Díaz JV, Encio IJ, Barajas M, et al. Influence of storage temperature and packaging on bacteria and yeast viability in a plant-based fermented food. Foods. 2020;9(3):302.
29. Zhu J, Yu L, Fan Y, Zhang H, Li F, Li X, et al. Camelina sativa oil treatment alleviates castor oil-induced diarrhea in ICR mice by regulating intestinal flora composition. Evidence-Based Complement Altern Med. 2022;2022:5394514.
30. Abdelouafi B. In vivo anti-diarrheal activity of jujube honey on castor oil-induced diarrhea in mice. J Tradit Chinese Med. 2021;41(6):900–8.
31. Vanjari H, Mohan M, Saudagar P, Guthale G, Dalvi M. Protective effect of SKB_Gutbiotic against castor oil and E.coli induced diarrhea in laboratory animals. Microb Pathog. 2020;143:104078.
32. Barai P, Hossain KM, Rahman SMM, Mazid MF Al, Gazi MS. Antidiarrheal efficacy of probiotic bacteria in castor oil induced diarrheal mice. Prev Nutr Food Sci. 2018;23(4):294–300.