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ORIGINAL

EVALUATION OF BA ZHENG SAN'S THERAPEUTIC EFFECTS ON RECOVERY AND PHYSICAL RESILIENCE:INSIGHTS FROM A MOUSE URINARY TRACT INFECTION MODEL

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ABSTRACT

Objective: To evaluate the therapeutic potential of Ba Zheng San in promoting recovery and mitigating infection-related damage using a mouse urinary tract infection (UTI) model, with implications for enhancing physical resilience and overall health. **Methods:** A mouse UTI model was established to assess the effects of Ba Zheng San. Mice were treated via gavage with varying concentrations of Ba Zheng San or no treatment (control) for five days. Mortality rates, body weights, and urine bacterial counts were recorded daily, and bacterial counts were assessed on treatment days 1, 3, and 5 using standard plate count methods. On day 5, bladder and kidney specimens were collected for gross morphological assessment, bacterial analysis, and histopathological evaluation using hematoxylin-eosin (HE) staining. **Results:** After five days of treatment, Ba Zheng San groups exhibited lower mortality rates compared to the untreated model group. The low-dose group showed an increase in average body weight compared to the model group, while the medium- and high-dose groups showed slight reductions. Urine, bladder, and kidney bacterial counts were significantly lower in all Ba Zheng San treatment groups compared to the model group. Histological analysis revealed reduced bladder and kidney damage, with less severe bladder congestion in Ba Zheng

San-treated groups compared to controls. However, no significant intergroup differences in kidney morphology were observed. **Conclusions:** Ba Zheng San demonstrated significant therapeutic benefits in reducing UTI-related mortality, bacterial load, and tissue damage in a mouse model. These findings highlight its potential for promoting recovery and resilience, making it relevant for applications in enhancing physical health and performance. Further research is warranted to explore its utility in human health, particularly for maintaining physical readiness and recovery in physically active populations.

KEYWORDS: urinary tract infection, morphological, hematoxylin-eosin, kidney morphological,

1. INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial infections, affecting millions of individuals worldwide each year. UTIs can cause significant discomfort, impair daily activities, and in severe cases, lead to systemic complications such as sepsis (Davis & Meltzer, 2007). Addressing these infections effectively is critical for minimizing their impact on overall health and physical performance (Reitzer & Zimmern, 2019). While antibiotics remain the standard treatment, the growing prevalence of antibiotic-resistant pathogens has highlighted the need for alternative or adjunctive therapies. Traditional herbal formulations, such as Ba Zheng San, have shown potential for managing UTIs due to their anti-inflammatory and antimicrobial properties, making them a promising candidate for therapeutic applications (Rath & Padhy, 2015; Spaulding et al., 2017; Wiles et al., 2008). Ba Zheng San, a classic formula in traditional Chinese medicine, is known for its ability to clear heat, drain dampness, and promote urination. It has been widely used for treating conditions associated with damp-heat accumulation, including UTIs. Despite its long history of use, there is a need for scientific validation of its efficacy and mechanisms of action through rigorous preclinical studies. Investigating its therapeutic effects in controlled experimental settings can provide valuable insights into its role in managing infections and supporting recovery. In sports and physical activity contexts, UTIs can significantly impair performance and recovery, particularly in athletes or physically active individuals (Terlizzi et al., 2017). The symptoms of UTIs, such as pain, fatigue, and systemic inflammation, may limit an individual's ability to train or compete effectively. (Yang et al., 2021). Moreover, severe infections can lead to prolonged recovery periods and increased susceptibility to further health complications. Exploring the potential of Ba Zheng San in enhancing recovery from UTIs and mitigating associated complications could offer new avenues for integrating traditional medicine into sports health management (McLellan & Hunstad, 2016). (Jaillon et al., 2014; Roelofs et al., 2006). This study aims to evaluate the therapeutic effects of Ba Zheng San using a mouse UTI model, focusing on its ability to reduce bacterial load, mitigate tissue damage, and

promote recovery. (Chromek et al., 2006). By analyzing mortality rates, bacterial counts, and histopathological changes, this research seeks to provide a comprehensive understanding of how Ba Zheng San may support recovery from UTIs. (Hopkins et al., 1995; Yadav et al., 2010). The findings could have broader implications for its application in sports and physical rehabilitation (Carey et al., 2016), particularly in enhancing resilience and maintaining physical health in active populations (Engelsöy et al., 2019).

2. Methods

2.1 Experimental Reagents

The traditional Chinese medicine (TCM) Ba Zheng San consists of nine ingredients: *Armand clematis stem, Plantain seed, Common knotgrass herb, Rhubarb root and rhizome, Cape jasmine fruit, Lilac pink herb, Talc, Common rush, and Liquorice root*. Ingredients were mixed in equal parts (according to mass) then distilled water was added to the mixture as a 10:1 mass ratio of distilled water:TCM. Next, the mixture was stirred until completely dissolved then the liquid mixture was stored at -80 °C overnight. Next, the liquid was lyophilized then the resulting solid TCM was ground to generate a powder. Thereafter, the powder was sealed to keep it dry and stored at room temperature. For use in experiments, 8 g of Ba Zheng San powder (determined using an electronic balance) was added to 20 mL of phosphate buffered saline (PBS) then the mixture was shaken until thoroughly mixed. Next, the concentration of the mixture was adjusted to 400 mg/mL then the mixture was filtered through a 0.2-micron filter to remove microbial contamination and stored at 4 °C. For use in experiments, the stock solution was diluted to generate solutions of different concentrations that were stored at 4 °C. The levofloxacin solution was prepared by adding 250 mg of levofloxacin powder to 5 mL PBS then the mixture was shaken until the solid was completely dissolved. Thereafter, the levofloxacin concentration was adjusted to 50 mg/mL by addition of PBS then the tube of solution was sealed and stored frozen at -80 °C. Preparation of LB medium was conducted in-house.

3. Experimental Methods

To generate the bacterial stock culture, 5 mL of autoclaved LB liquid medium was added to a 15-mL glass test tube then 50 μL of thawed bacterial solution was added to the LB in the tube. Next, the tube was placed in an incubator and incubated overnight at 37 °C with continuous shaking at 180 r/min. Six-week-old C57BL/6 female mice (purchased commercially) were placed in a sterile clean environment for one week to adapt to the new environment while they received unrestricted access to food and water. All animal experiments were conducted in strict accordance with relevant

regulations regarding humane care and use of laboratory animals.

3.1 Establishment of a mouse UTI model

After consulting relevant literature regarding a suitable number of bacteria and method of inoculation for establishing animal UTI models, we used the following method to construct a mouse UTI model. On the day before bacteria were inoculated into urinary tracts of mice, mice were subjected to water deprivation. Just prior to urethral bacterial inoculation, urine was drained from bladders of mice by gently pressing on the lower abdomen of each mouse. Next, mice were anesthetized then each mouse was placed in a biosafety cabinet, fixed supine on a board, then its urethra and surrounding areas were wiped with an alcohol-soaked cotton ball. Thereafter, a 26G intravenous catheter was slowly inserted into the urethra of each mouse at an angle of 30° to the plane to a depth of about 0.6 cm. Next, the catheter needle was removed then a 1-mL syringe containing the bacterial solution was inserted into the catheter and the bacterial solution was gently injected into the bladder of each mouse to deliver 1 × 108 colony-forming units (CFUs). Thereafter, inoculated mice were placed in cages and deprived of water for an additional 6 h then mice received unrestricted access to normal drinking Sterile water.

3.2 Calculation of drug dose

The dose of Ba Zheng San was calculated according to the body surface area of an adult mouse of mass 20 g. The mass of daily gavage treatment administered to each 20-g mouse was about 80 mg, which contained Ba Zheng San high-, medium-, and low-dose concentrations of 800, 400, or 200 mg/kg-d, respectively, that corresponded to drug equivalents of 1×, 1/2×, and 1/4× the adult dose. Based on the adult human levofloxacin dosage of 400 mg/d, the daily dose of levofloxacin that was administered to mice was about 50 mg/kg.

3.3 Therapeutic effect of Ba Zheng San on UTI model mice

Seventy-two mice were assigned to 6 groups (12 mice/group): Normal group, Model group, Ba Zheng San high-dose group, Ba Zheng San middle-dose group, Ba Zheng San low-dose group, and Levofloxacin group. Treatments containing PBS alone (Normal and Model groups) or Ba Zheng San were administered on days 1, 3, and 5 by gavage beginning 6 h after urethral inoculation with the bacterial suspension. Mice in the Levofloxacin group received a daily subcutaneous injection of 20 μL of Ba Zheng San (50mg/kg) in addition to the daily 50-mg/kg dose of levofloxacin for 5 days. All mice were weighed daily at a time point half-way between consecutive gavages then mouse body weights were recorded. Mice were examined daily to detect deaths then mortality rates for the six groups of mice were calculated

at treatment completion.

3.3.1 Effect of Ba Zheng San on bacterial counts in urine and organs of mice with UTIs

Urine samples were collected on days 1, 3, and 5 after bacterial inoculation. After urine collection, a 20-μL urine sample was added to 180 μL of PBS then the bacterial suspension was mixed and serial dilutions of the bacterial suspension were plated on agar plates. After growth of plated bacteria was allowed to proceed for 1d, CFU counts were recorded and used to calculate numbers of bacteria in the original urine samples. After 5 days of gavage administration, mice were sacrificed and bladders and kidneys of mice were completely removed, the weight of each organ was recorded, then organs were photographed and examined for gross pathological changes (visible to the naked eye). Next, grinding beads and 1 mL of PBS were added to each organ specimen then specimens were pulverized using a grinder. After dilution, numbers of bacteria in bladder and kidney specimens were counted by plating organ tissue suspensions on agar plates then organs were fixed in 4% paraformaldehyde solution, paraffin embedded, dewaxed in water, stained, dehydrated, and sealed in transparent resin. Finally, stained organs were observed under a microscope then differences in organ pathological changes among the different groups were compared.

3.4 Data processing and analysis

GraphPad Prism8 software was used for data analysis, processing, and mapping. The unpaired two-tailed Student's t-test was used to analyze the data between groups, with different levels of statistical significance of intergroup differences indicated by * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$). Adobe Illustrator CC 2018 software was used to crop and splice images together.

4. Results

4.1 Changes in body weights of mice in each group

As shown in Figure 1, during the 5-day gavage administration cycle, Normal group average body weight continually increased. Meanwhile, body weight curves of mice in the Ba Zheng San low-dose group and Levofloxacin group overlapped slightly as average body weights of both groups initially decreased for 2 days then increased (but were always lower than that of the Normal group). By contrast, body weights of mice in high- and medium-dose Ba Zheng San groups and the Model group continually decreased, with the Ba Zheng San high-dose group losing the most significant amount of weight.

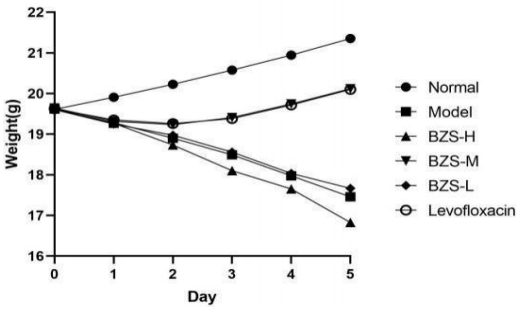

Figure 1: Trends in mean body weight of mice in each group.

4.2 Changes in mortality of different groups of mice during treatment

During mouse UTI model development, the number of inoculated bacteria per mouse (1×10^7 CFU, 1×10^8 CFU, and 1×10^9 CFU) was found to be associated with mortality rate. No deaths were associated with inoculation of 1×10^7 CFU, while post-inoculation urine bacterial counts gradually decreased during the 5-day treatment period, thus indicating that a UTI model could not be successfully generated through urethral inoculation of this number of bacteria. By contrast, inoculation of mice with 1×10^9 CFU was associated with a significantly greater mortality rate (exceeding 90%) by 5 days post-inoculation. Ultimately, 1×10^8 CFU was selected to establish the UTI model. Figure 2 presents death data of mice in each group. Figure 2(A) shows survival curves for each group that indicate all mice in the Normal group survived for more than 5 days. Meanwhile, survival curves of Ba Zheng San high-dose, Ba Zheng San medium-dose, and Levofloxacin groups coincided such that survival rates of all three groups on day 5 were lower than the survival rate of the Normal group and were greater than the survival rate of the Model group. 2(B) shows mortality rates for each group of mice. As compared to the Model group mortality rate (33%), mortality rates of all Ba Zheng San treatment groups were significantly reduced, with the lowest mortality rate observed for the Ba Zheng San medium-dose group (8.33%). Taken together, these results suggest that Ba Zheng San treatment reduced UTI-associated mortality rates.

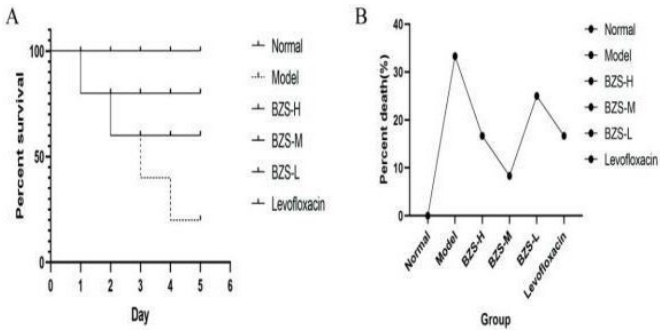

Figure 2: Survival and mortality of mice with UTIs. (A) Survival curves of mice in each group. (B) Mortality rates for each group of mice.

4.3 Changes of bacterial counts in urine, bladders, and kidneys of mice with UTIs after Ba Zheng San treatment

4.3.1 Changes of bacterial counts in mouse urine samples during Ba Zheng San treatment

On days 1, 3, and 5 of the treatment cycle, urine samples were collected for plate count determinations to assess bacterial counts in urine samples collected from mice in each group. As shown in Figure 3, although urine bacterial counts obtained for all groups continually increased during the 5-d treatment cycle, the urine bacterial count of the Model group was significantly greater than urine bacterial counts obtained for the other groups. Notably, although urine bacterial counts of all three Ba Zheng San groups trended upward during the 5-day treatment course, they were significantly lower than Model group counts on days 3 and 5 of treatment.

Moreover, comparisons of urine bacterial counts of Ba Zheng San high-dose, medium-dose, and low-dose groups on day 5 of treatment revealed that Ba Zheng San low-dose group counts were greater than counts obtained for the other Ba Zheng San groups, which were similar between high-dose and low-dose groups; Meanwhile, Levofloxacin group counts were significantly lower than counts obtained for all other groups.

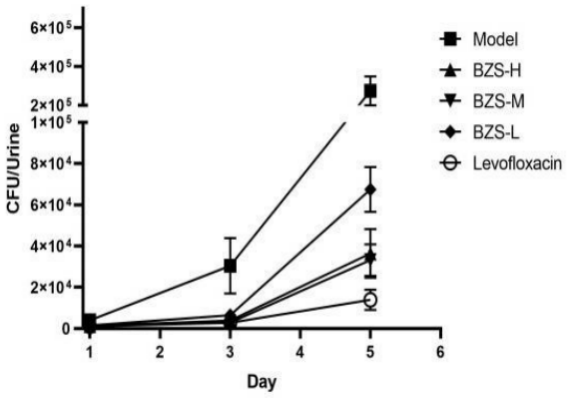

Figure 3: Effect of Ba Zheng San treatment on numbers of urine bacteria in mice with UTIs.

4.3.2 Changes of bacterial counts in bladders and kidneys of mice with UTIs after Ba Zheng San treatment

After mice with UTIs received 5-day Ba Zheng San treatments, bladder and kidney specimens were collected, pulverized, and plated then bacterial counts were calculated and bacterial loads in organs were plotted. As shown in Figure 4, Ba Zheng San treatment reduced numbers of bacteria in bladder and kidney tissues, with even low-dose Ba Zheng San treatment significantly reducing bladder bacterial counts ($p < 0.05$), as shown in Figure 4(A).

Meanwhile, low-dose group Ba Zheng San treatment significantly reduced bladder bacterial counts ($p < 0.05$), with more significant bacterial count reductions observed for high-dose and middle-dose Ba Zheng San treatments ($p < 0.01$). As shown in Figure 4(B), low-dose Ba Zheng San treatment significantly reduced kidney bacterial load ($p < 0.01$), while high-dose and medium-dose Ba Zheng San treatments reduced kidney bacterial loads more significantly than low-dose Ba Zheng San treatment ($p < 0.001$).

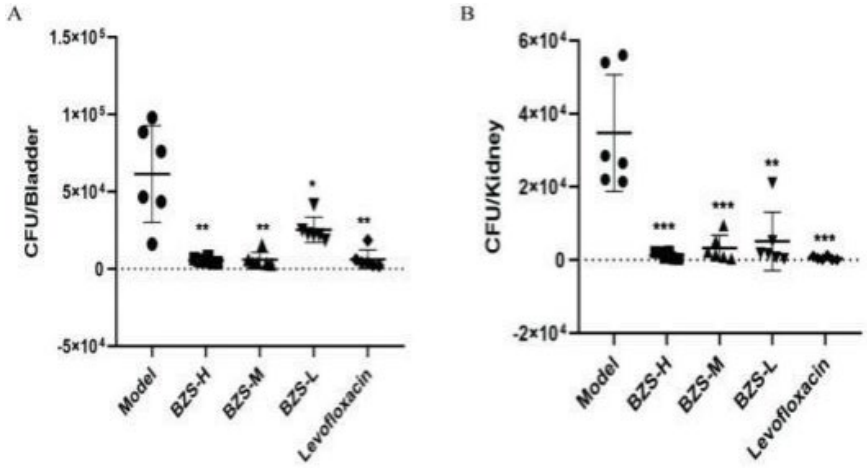

Figure 4: Effect of Ba Zheng San on bacterial counts obtained from bladder and kidney specimens of mice with UTIs. (A) Effect of Ba Zheng San treatment on bladder bacterial count. (B) Effect of Ba Zheng San treatment on kidney bacterial count.

4.4 Changes in bladder and kidney pathology of mice in different groups after treatment

4.4.1 Bladder and kidney morphological differences as assessed from photographs of organs of UTI-afflicted mice of different treatment groups

After bladders and kidneys of mice in each group were harvested, the organs were photographed (Figure 5) then examined to detect intergroup differences in bladder and kidney morphologies after the 5-day treatment cycle. In Figure 5(A), it can be seen that Normal group bladder color appears transparent, while the Model group bladder appears dark red with signs of congestion.

Meanwhile, less obvious bladder congestion and smaller bladder size were observed in the Ba Zheng San high-dose group than in the Model group, while Ba Zheng San middle-dose and low-dose group bladders exhibited less severe hyperemia as compared to that of the Model group. By contrast, no significant intergroup differences in gross kidney morphology and color are apparent, as shown in Figure 5(B).

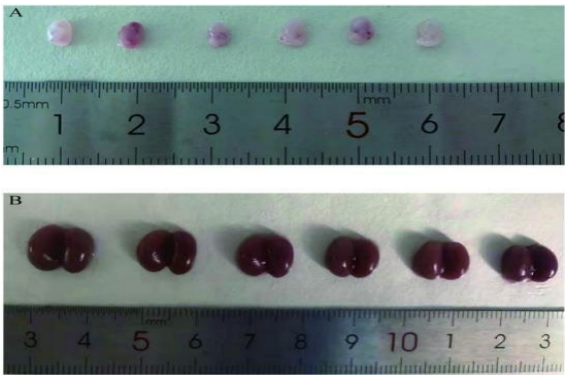

Figure 5: Photograph showing mouse bladder and kidney morphological changes induced by Ba Zheng San treatment. (A) Bladder morphological changes induced by Ba Zheng San treatment. (B) Kidney morphological changes induced by Ba Zheng San treatment. Note: Pictures from left to right correspond to specimens of the Normal, Model, Ba Zheng San high-dose, Ba Zheng San middle-dose, Ba Zheng San low-dose, and Levofloxacin groups.

4.4.2 Hematoxylin-eosin (HE) staining-based detection of bladder and kidney pathological changes for each group of UTI-afflicted mice

Tissue sections of Normal group bladders revealed no mucosal swelling, no epithelial degeneration, no cellular enlargement, no basal layer inflammation, and no edema, thus indicating that these specimens exhibited no pathologic features. By contrast, tissue sections of Model group bladders exhibited mucosal swelling, obvious transitional epithelial degeneration and swelling, more diffuse inflammatory cell infiltration around basal layer blood vessels, cellular enlargement with partial cytoplasmic transparency, and the presence of mucosal epithelium and inflammatory debris within the bladder cavity. In bladders of the Ba Zheng San high-dose group, individual areas of swelling within the mucosal layer, slight localized enlargement of the transitional epithelium, and a small amount of inflammatory exudation in the muscular layer tissue were observed. In bladders of the medium-dose Ba Zheng San group, the mucosal layer exhibited mild segmental swelling with a small amount of blood exudation detected in some basal layers. Meanwhile, cells in mucosal layers of bladders of the low-dose Ba Zheng San group were reduced in number and appeared slightly swollen and slightly segmented. Within bladder interstitial muscle tissue layers, loose circular muscles and numerous accumulated inflammatory cells (mainly eosinophils) were observed. By contrast, no mucosal swelling was observed in bladders of the Levofloxacin group, while reduced cell sizes, sporadic areas of stromal inflammation, and areas of slightly localized transitional epithelium enlargement were observed in the absence of inflammatory muscle layer exudates.Examination of Normal group renal tissue pathology revealed no glomerular enlargement, no mesangial cell proliferation, no glomerular congestion and swelling, and no obvious lesions within the renal interstitium. By contrast, Model group glomeruli were enlarged to varying degrees,

glomeruli were partially divided into valves, bursae were exudated, mesangial cells were enlarged, renal tubulointerstitial area blood was exudated, and renal tubules were swollen. In high-dose Ba Zheng San group kidneys, glomeruli were not significantly enlarged, renal tubules appeared relatively normal, the interstitium appeared only slightly congested, and the mesangium was not significantly thickened. In Ba Zheng San medium-dose group kidneys, renal cysts were enlarged, glomeruli were shrunken, inflammatory cells were present within renal tubules, and the interstitium was slightly thickened. In kidneys of the Ba Zheng San low-dose group, renal cysts were enlarged, glomeruli were divided into lobes, some glomeruli were congested, a small amount of renal interstitium congestion was observed, epithelial cells were swollen, and inflammatory cells were distributed around blood vessels. In the Levofloxacin group, no obvious proliferation of mesangial cells, no protein in tubules, no congestion in the interstitium, and no swelling in renal tubules were observed.

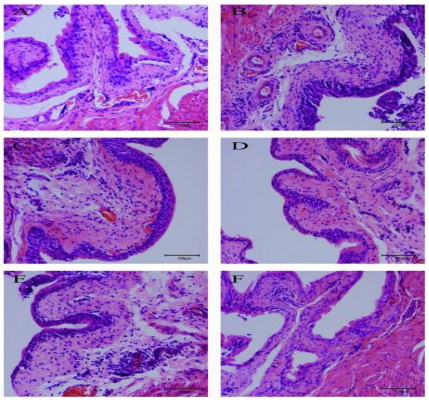

Figure 6: Photograph of slide showing HE-stained bladder tissue pathology after Ba Zheng San treatment of mice with UTIs. (A) Normal group. (B) Model group. (C) Ba Zheng San high-dose group. (D) Ba Zheng San medium-dose group. (E) Ba Zheng San low-dose group. (F) Levofloxacin group.

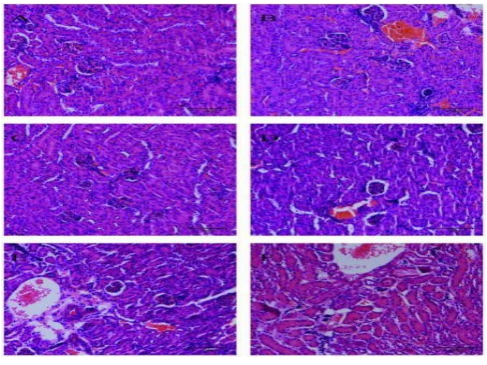

Figure 7: Photograph of slide showing changes in renal pathology induced by Ba Zheng San treatment of mice with UTIs. (A) Normal group. (B) Model group. (C) Ba Zheng San high-dose group. (D) Ba Zheng San medium-dose group. (E) Ba Zheng San low-dose group. (F) Levofloxacin group.

5. Discussion

In this study, we first established a mouse UTI model then assessed UTI-alleviating effects of different Ba Zheng San doses, as based on treatment-associated changes in body weight, mortality rate, survival curves, urine and organ bacterial loads, and bladder and kidney gross and microscopic pathological changes. During development of the mouse model, we screened effects associated with different numbers of inoculated bacteria (1×10^9 CFU, 1×10^8 CFU, 1×10^7 CFU). The results revealed that unacceptably high mortality resulted from inoculation of 1×10^9 CFU, while numbers of bacteria in urine and organs of mice inoculated with 1×10^7 CFU were too low for use as a UTI model. Therefore, we inoculated mice with an intermediate number of bacteria (1×10^8 CFU) to generate our mouse UTI model, which did not conflict with formal animal care and use regulations. With regard to inoculum volume, numerous published reports have indicated that an inoculum volume of 100 μL could be used to generate mouse UTI models (Demirel et al., 2020; Liu et al., 2015). By contrast, results of several studies suggest that urethral inoculation of 100 μL of bacterial suspension, an excessive inoculum volume associated with high injection pressure, caused reverse flow to occur along the ureter to the kidney that triggered the occurrence of vesicoureteral reflux (Song et al., 2007). Nonetheless, results of other studies demonstrated that this issue could be avoided through use of an inoculation volumes of 25-50 μL (Bottek et al., 2020), thus prompting us to inoculate mice with 50 μL of bacterial suspension to generate our mouse UTI model (Folmer et al., 2006). After the establishment of the mouse UTI model, mice with UTIs assigned to different groups received different treatments then survival and mortality rates were determined and compared among groups. Our results indicated that although Ba Zheng San treatment of mice reduced UTI-induced mortality, administration of increasing Ba Zheng San doses was associated with gradually decreasing body weights during the 5-day treatment cycle. Nevertheless, average mortality rate and average body weight of high-dose Ba Zheng San group mice were both lower than corresponding Model group values after 5 days of treatment. Moreover, during day 2 of the 5-day treatment course, watery stools were produced by mice in all Ba Zheng San groups but not by mice in Normal and Model groups. This result suggests that Ba Zheng San damages the spleen Yang of mice to a certain extent, resulting in loss of spleen movement and development of a watery stool. However, it is possible that the Ba Zheng San dose was too high, due to a potentially invalid body mass-based conversion from the human dose to the mouse dose, warranting further study (Armbruster et al., 2018; Erman et al., 2012). Notably, Ba Zheng San treatment of mice with UTIs was associated in a dose-dependent manner with significantly reduced urine, bladder, and kidney bacterial counts, with increasing Ba Zheng San treatment dose associated with greater reduction of bacterial counts. Taken together, in vivo results presented here and results of previous in vitro experiments indicate that Ba

Zheng San may reduce in vivo bacterial counts by inhibiting UPEC adhesion to bladder and kidney tissues, while also exerting a diuretic effect (due to its moisturizing ingredients) that may promote urine excretion that washes bacteria out of the urinary tract. Regardless, although liquid intake and the daily gavage volume administered to mice was consistent among the groups, it was not possible to determine whether urine volumes of Ba Zheng San-treated groups were greater than urine volumes of other groups. Therefore, specific mechanisms underlying Ba Zheng San-associated reduced kidney and bladder bacterial counts are unknown, warranting further study (Klarström Engström et al., 2019; Yeh et al., 2019).In addition, pathological changes in tissues of each group were observed after staining of bladder and kidney tissue sections. As consistent with results presented in Figure 5(A), results of HE staining of mouse bladder specimens indicated that bladder health of Ba Zheng San-treated groups was better than that of the Model group, thus indicating that Ba Zheng San treatment alleviated UTIs in mice in vivo. Furthermore, results presented in Figure 5(B) revealed no significant intergroup differences in gross renal tissue pathology (as detected by naked-eye examination), while HE staining results of tissue sections revealed that Ba Zheng San treatment of mice with UTIs was associated with significantly improved kidney health. In this work, in vivo changes during 5-day Ba Zheng San treatment of mice with UTIs were monitored based on health indicators, including average weight, mortality rate, survival, urine bacterial count, bladder bacterial count, kidney bacterial count, naked-eye bladder and kidney pathological observations, and microscopic bladder and kidney pathological observations. In general, results obtained for the indicators suggest that Ba Zheng San is an effective treatment for UTIs, although some contradictory results were obtained for different doses of the TCM, as were obtained in previously reported in vitro experiments. Furthermore, the mechanism by which Ba Zheng San inhibits UPEC adhesion in mice with UTIs is still unclear, warranting further study.

6. Conclusion

This study demonstrates the therapeutic potential of Ba Zheng San in managing urinary tract infections (UTIs) through its ability to reduce bacterial load, mitigate tissue damage, and promote recovery in a mouse model. The results revealed that Ba Zheng San treatment significantly decreased mortality rates, bacterial counts in urine, bladder, and kidney tissues, and alleviated UTI-induced bladder and kidney damage. These findings validate its traditional use and highlight its efficacy as a complementary therapy for UTIs. From a sports and physical health perspective, the ability of Ba Zheng San to reduce infection severity and tissue damage has important implications for maintaining physical performance and resilience, particularly in active individuals. UTIs can severely impact physical activity by causing pain, fatigue, and systemic inflammation, which can delay recovery and hinder athletic

performance. The findings of this study suggest that Ba Zheng San may serve as an effective adjunct to conventional therapies, supporting quicker recovery and enabling individuals to resume physical activities more efficiently. Further research is needed to explore the mechanisms of Ba Zheng San's therapeutic effects, its long-term safety, and its efficacy in human populations. Investigating its potential role in enhancing recovery during high-stress or high-performance situations, such as in athletes, could provide valuable insights for integrating traditional medicine into modern sports and rehabilitation frameworks. This study lays the foundation for future work on combining natural remedies with evidence-based medicine to support physical health and performance in diverse populations.

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