Brief Report

DOI: 10.5582/bst.2015.01172

Effect of Shufeng Jiedu capsules as a broad-spectrum antibacterial

Yanyan Bao*, Yingjie Gao*, Xiaolan Cui**

Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China.

Summary **This study sought to investigate the broad-spectrum antibacterial action of an alternative medicine, Shufeng Jiedu capsules (SFJDC). Antibacterial testing was performed to determine whether SFJDC had broad-spectrum antibacterial action *in vitro*, and testing was performed to verify whether SFJDC prevented death due to a *Streptococcus* or *Staphylococcus aureus* infection in mice. Results of antibacterial testing suggested that SFJDC are a broad-spectrum antibacterial and that SFJDC are superior to Lianhua Qingwen capsules as a broad-spectrum antibacterial. Results of testing revealed that SFJDC lowered the mortality rate, it reduced mortality, it increased average survival time, and it increased the lifespan of mice dying due to a *Staphylococcus aureus* or *Streptococcus* infection. Thus, SFJDC could become a complement to broad-spectrum antimicrobials in clinical settings.**

Keywords: Shufeng Jiedu capsules, broad-spectrum antibacterial, *Staphylococcus aureus*, *Streptococcus*

1. Introduction

Antibiotics are a class of drugs used to inhibit bacterial growth or kill bacteria. They can inhibit or kill bacteria by blocking the synthesis of bacterial cell walls, increasing bacterial membrane permeability, inhibiting protein synthesis, or blocking replication and transcription of bacterial DNA. Antibiotics come in various types, such as aminoglycosides, quinolones, macrolides, and β-lactams. Wide-spectrum antibiotics can strongly inhibit most Gram-negative bacteria as well as Gram-positive bacteria, and they can inhibit *Rickettsia*, *Spirochetes*, and some *Protozoa* spp. However, the long-term overuse of broad-spectrum antibiotics results in many dangers: drug resistance, side effects, and an imbalance in intestinal flora (*1-7*). With extensive use of broad-spectrum antibiotics in clinical settings, resistance to antibiotics has increased, *e.g.* extended spectrum β-lactamases are able to hydrolyze penicillins, carbapenems, and amoxicillin. Thus, there is an urgent need for an alternative in clinical settings.

Shufeng Jiedu capsules (SFJDC) are a traditional Chinese medicine that is mainly used to treat upper respiratory tract infections such as the flu, swelling and pain in the throat, mumps, and strep throat (*8*). In China, SFJDC have been listed as a drug to combat avian influenza after years of clinical observations. This product is approved as a traditional Chinese medicine to "reduce heat and remove toxins" (*qingwen jiedu*) and "drain the lungs and eliminate heat" (*xuanfei xiere*). SFJDC have previously been shown to be active in the experimental model used in the present study. Lianhua Qingwen capsules (LHQWC) are another traditional Chinese medicine used to treat upper respiratory tract infections, and this medicine has antimicrobial activity according to previous studies (*8,9*), so it served as a positive control in the current study. A recent study by the current authors indicated that SFJDC have some effect on a bacterial infection with few adverse reactions and good tolerability, but their broader antibacterial activity has not been known. Thus, the current study sought to explore the broad-spectrum antimicrobial action of SFJDC.

2. Materials and Methods

2.1. *Test substance*

All investigations were performed with a single batch (No.150602) of SFJDC kindly provided by the

Released online in J-STAGE as advance publication February 15, 2016.

*These authors contributed equally to this works.
***Address correspondence to:*
Dr. Xiaolan Cui, Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China.
E-mail: cuixiaolan2812@126.com

manufacturer Jiren Pharmaceutical (Anhui, China). In accordance with a SFJDC dose of 6.24 g/day for adults (per 60 kg body weight), equivalent doses on the basis of allometric means in mice would be about 1.1 g/kg/day. Based on this calculation, doses in animal experiments correspond to approximately half, one, or two times the average equivalent dose for a human adult. Hence, mice received doses of 0.55, 1.1, or 2.2 g/kg/day. All treatments were administered by gavage. SFJDC were suspended in distilled water and administered to mice in a volume of 0.2 mL/10 g.

2.2. *Reference drug*

LHQWC (batch No. A1409089) produced by Shijiazhuang Yiling Pharmaceutical (Hebei, China) served as a reference drug. In accordance with the dose in humans (4.2 g/60 kg/day), an equivalent oral dose on the basis of the allometric mean was selected for mice (0.77 g/kg/day).

Amoxicillin capsules (batch No. 50505005) produced by Zhuhai United Laboratories (Guangdong, China) also served as a reference drug. This product is a broad-spectrum antibiotic. In accordance with the dose in humans (2.0 g/60 kg/day), an equivalent oral dose on the basis of the allometric mean was selected for mice (0.363 g/kg/day).

2.3. *Bacteria*

Staphylococcus aureus (26003, 361), a *Staphylococcus* sp. (26101), *Staphylococcus epidermidis* (Standard strain, 108), *Streptococcus* spp. (10, 12), *Escherichia coli* (44113, 117, 178), *Pseudomonas aeruginosa* (209, 210, 211), *Streptococcus pneumonia* (31001, 163), a *Proteus* sp. (29108), *Neisseria gonorrhoeae* (29106), and *Candida albicans* (standard strain) were acquired from Chinese Pharmaceutical and Biological Products and were stored at -80°C until use.

2.4. *Animals*

Specific-pathogen-free CD-1 (ICR, male and female, 18-20 g) mice were all purchased from Charles River (Beijing, China). All animal work was performed under the guidelines of the China Academy of Chinese Medical Sciences and was approved by the China Academy of Chinese Medical Sciences Committee.

2.5. *Broad-spectrum antibacterial testing in vitro*

S. aureus (26003, 361), a *Staphylococcus* sp. (26101), *Staphylococcus epidermidis* (Standard strain, 108), *Streptococcus* spp. (10, 12), *E. coli* (44113, 117, 178), *P. aeruginosa* (209, 210, 211), *S. pneumonia* (31001, 163), a *Proteus* sp. (29108), *N. gonorrhoeae* (29106), and *C. albicans* (Standard strain) were each cultured for 24 h with nutrient broth at 37°C, and then the turbidity was adjusted to three hundred million bacteria per milliliter with nutrient broth according to Maxwell turbidimetry. All of the bacterial solutions were further diluted a thousand times with nutrient broth and 10 μL was added to 96-well plates. Fifty, 25, 12.5, 6.25, 3.125, 1.5625, and 0.87125 mg/mL of SFJDC and LHQWC were each added to the bacterial solution and cultured for 24 h at 37°C. Afterwards, the bacteria in solution were compared with normal bacteria.

2.6. *Mouse model of death due to an S. aureus infection*

A total of 120 mice were randomly distributed according to body weight into a model control group, a LHQWC group, an amoxicillin group (2.75 mL/kg), and three SFJDC groups (0.55, 1.10, and 2.20 g/kg). Drugs were orally administered daily for 3 days at a volume of 0.2 mL/10 g. Animals in the model control group were administered 0.2 mL/10g of distilled water under the same conditions. Beginning on day 3, the mice were treated with intraperitoneally injected with 0.2 mL *S. aureus*. The day after infection, the number of deaths in each group was recorded for 7 consecutive days.

2.7. *Mouse model of death due to a Streptococcus infection*

A total of 120 mice were randomly distributed according to body weight into a model control group, a LHQWC group, an amoxicillin group (2.75 mL/kg), and three SFJDC groups (0.55, 1.10, and 2.20 g/kg). Drugs were orally administered daily for 3 days at a volume of 0.2 mL/10g. Animals in the model control group were administered 0.2 mL/10g of distilled water under the same conditions. Beginning on day 3, the mice were intraperitoneally injected with 0.2 mL of a *Streptococcus* sp. The day after infection, the number of deaths in each group was recorded for 7 consecutive days.

2.8. *Statistical analysis*

Data were analyzed using SPSS software. A χ^2 test was used to statistically analyze the reduction in mortality, and a *t*-test was used to statistically analyze the average survival time. A $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1. *Effect of SFJDC as a broad-spectrum antibacterial in vitro*

A clear broth indicated that a drug effectively inhibited bacteria, and the minimum inhibitory concentration of SFJDC and LHQWC for a bacterium indicated the antibacterial action of the drug. Results indicated

that SFJDC were a broad-spectrum antibacterial that inhibited all 18 species of bacteria. SFJDC were more effective at killing *S. aureus* and *Staphylococcus*, and SFJDC were superior to LHQWC as a broad-spectrum antibacterial (Tables 1-3).

3.2. *Prevention of death from an S. aureus infection with SFJDC in mice*

As shown in Table 4, the *S. aureus* control group had a mortality rate of 95% and an average survival time of 1.4 day. Compared to the control group, the 3 groups receiving SFJDC had a lower mortality rate of 70%, 75%, and 80%, respectively. The 3 groups receiving SFJDC had a reduction in mortality of 26.32%, 21.05%, and 15.79%, respectively. The group receiving 0.55g/kg of SFJDC had a significantly increased average survival time ($p < 0.01$). The 3 groups receiving SFJDC had a significant increase in lifespan of 107.14%, 82.14%, and 71.43%, respectively.

3.3. *Preventing death due to a Streptococcus infection with SFJDC in mice*

As shown in Table 5, the *Streptococcus* control group had a mortality rate of 70% and an average survival

Table 1. The anti-bacterial effects of SFJDC *in vitro*

Name of bacterium	Bacterium No.	SFJDC (mg/mL)							Control
		50	25	12.5	6.25	3.125	1.5625	0.87125	
Staphylococcus aureus	26003	-	-	-	-	-	-	+	+
Staphylococcus aureus	361	-	-	-	-	-	+	+	+
Staphylococcus sp.	26101	-	-	-	-	-	-	+	+
Staphylococcus epidermidis	Standard strain	-	-	-	-	-	+	+	+
Staphylococcus epidermidis	108	-	-	-	-	-	+	+	+
Streptococcus sp.	10	-	-	-	+	+	+	+	+
Streptococcus sp.	12	-	-	+	+	+	+	+	+
Escherichia coli	44113	-	+	+	+	+	+	+	+
Escherichia coli	117	-	+	+	+	+	+	+	+
Escherichia coli	178	-	+	+	+	+	+	+	+
Pseudomonas aeruginosa	209	-	-	+	+	+	+	+	+
Pseudomonas aeruginosa	210	-	-	-	+	+	+	+	+
Pseudomonas aeruginosa	211	-	+	+	+	+	+	+	+
Streptococcus pneumonia	31001	-	-	-	-	-	+	+	+
Streptococcus pneumonia	163	-	-	+	+	+	+	+	+
Proteus sp.	29108	-	+	+	+	+	+	+	+
Neisseria gonorrhoeae	29106	-	-	-	-	+	+	+	+
Candida albicans	Standard strain	-	-	-	+	+	+	+	+

"-": inhibitory effect; "+": no inhibitory effect.

Table 2. The anti-bacterial effects of LHQWC *in vitro*

Name of bacterium	Bacterium No.	LHQWC (mg/mL)							Control
		50	25	12.5	6.25	3.125	1.5625	0.87125	
Staphylococcus aureus	26003	-	-	+	+	+	+	+	+
Staphylococcus aureus	361	-	-	+	+	+	+	+	+
Staphylococcus sp.	26101	-	-	+	+	+	+	+	+
Staphylococcus epidermidis	Standard strain	-	-	+	+	+	+	+	+
Staphylococcus epidermidis	108	-	-	+	+	+	+	+	+
Streptococcus sp.	10	-	-	+	+	+	+	+	+
Streptococcus sp.	12	-	+	+	+	+	+	+	+
Escherichia coli	44113	-	+	+	+	+	+	+	+
Escherichia coli	117	-	+	+	+	+	+	+	+
Escherichia coli	178	-	+	+	+	+	+	+	+
Pseudomonas aeruginosa	209	-	+	+	+	+	+	+	+
Pseudomonas aeruginosa	210	-	+	+	+	+	+	+	+
Pseudomonas aeruginosa	211	-	+	+	+	+	+	+	+
Streptococcus pneumonia	31001	-	-	+	+	+	+	+	+
Streptococcus pneumonia	163	-	+	+	+	+	+	+	+
Proteus sp.	29108	-	+	+	+	+	+	+	+
Neisseria gonorrhoeae	29106	-	-	-	+	+	+	+	+
Candida albicans	Standard strain	-	-	+	+	+	+	+	+

"-": inhibitory effect; "+": no inhibitory effect.

time of 3.2 days. Compared to the control group, the 3 groups receiving SFJDC had a lower mortality rate of 20%, 30%, and 40%, respectively. The groups receiving 0.55 or 1.1 g/kg of SFJDC had a significant reduction in mortality ($p < 0.01$) of 71.43%, 57.14%, and 42.86%, respectively. The groups receiving 0.55 or 1.1 g/kg of SFJDC had a significantly increased average survival time ($p < 0.01$). The 3 groups receiving SFJDC had a significant increase in lifespan of 84.38%, 68.75%, and 51.56%, respectively.

Based on Gram staining, bacteria were divided into two categories, Gram-positive bacteria and Gram-negative bacteria. Most pyogenic bacteria are Gram-positive bacteria that can produce exotoxins that make people sick, while most intestinal bacteria are Gram-negative bacteria that produce endotoxins that make people sick. A broad-spectrum antibiotic can strongly inhibit most Gram-negative bacteria as well as Gram-positive bacteria, and it can inhibit *Rickettsia*, *Spirochetes*, and some *Protozoa* spp. The current study examined the broad-spectrum antimicrobial activity of SFJDC by antibacterial testing *in vitro* and *in vivo*. *In vitro* testing was done with six genera of Gram-positive bacteria, *i.e. S. aureus* (26003, 361), a *Staphylococcus* sp. (26101), *S. epidermidis* (Standard strain, 108), *Streptococcus* spp. (10, 12), *S. pneumonia* (31001, 163) and *C. albicans* (Standard strain), and four genera of Gram-negative bacteria, *i.e. E. coli* (44113, 117, 178), *P. aeruginosa* (209, 210, 211), a *Proteus* sp. (29108), and *N. gonorrhoeae* (29106). Results indicated that SFJDC are a broad-spectrum antibacterial and that SFJDC inhibited all 18 species of bacteria, but a larger dose of SFJDC was needed to kill Gram-negative bacteria than Gram-positive bacteria. This suggests that SFJDC are more effective at killing Gram-positive bacteria. SFJDC were most effective at killing *S. aureus* and *Staphylococcus*, and SFJDC was superior to LHQWC as a broad-spectrum antibacterial.

S. aureus is the number one cause of hospitalization and surgery for children, and the leading cause of bacteraemia in people > 65 years of age (*10,11*). A recent review (*12*) has noted alarming rates of *S. aureus* infections /100,000 population/y. Over the past few decades, bacteremia rates have been 20-38%, and this rate jumps to > 100 for people over the age of 70. *S. aureus* is the pathogen that is most frequently involved in combined infections (*13-16*). The current study investigated the ability of SFJDC to prevent death from an *S. aureus* infection in mice. Compared

Table 3. The minimum inhibitory concentration (MIC) of SFJDC for different bacteria

Name of bacterium	Bacterium No.	MIC (mg/mL)	
		SFJDC	LHQWC
Staphylococcus aureus	26003	1.5625	25.0000
Staphylococcus aureus	361	3.1250	25.0000
Staphylococcus sp.	26101	1.5625	25.0000
Staphylococcus epidermidis	Standard strain	3.1250	25.0000
Staphylococcus epidermidis	108	3.1250	25.0000
Streptococcus sp.	10	12.5000	25.0000
Streptococcus sp.	12	25.0000	50.0000
Escherichia coli	44113	50.0000	50.0000
Escherichia coli	117	50.0000	50.0000
Escherichia coli	178	50.0000	50.0000
Pseudomonas aeruginosa	209	25.0000	50.0000
Pseudomonas aeruginosa	210	12.5000	50.0000
Pseudomonas aeruginosa	211	50.0000	50.0000
Streptococcus pneumonia	31001	3.1250	25.0000
Streptococcus pneumonia	163	25.0000	50.0000
Proteus sp.	29108	50.0000	50.0000
Neisseria gonorrhoeae	29106	6.2500	12.5000
Candida albicans	Standard strain	12.5000	25.0000

Table 4. Prevention of death due to a *Staphylococcus aureus* infection with SFJDC in mice

Groups	Dose (g/kg)	Number of animals	Mortality rate (%)	Reduction in mortality (%)	Average survival time (d)	Increase in lifespan (%)
Control	-	20	95	-	1.40 ± 1.35	-
LHQWC	0.77	20	80	15.79	2.30 ± 2.43	64.29
Amoxicillin	0.363	20	10	89.47**	6.40 ± 1.85**	357.14
SFJDC	0.55	20	70	26.32	2.90 ± 2.77**	107.14
SFJDC	1.1	20	75	21.05	2.55 ± 2.65	82.14
SFJDC	2.2	20	80	15.79	2.40 ± 2.46	71.43

**$p < 0.01$ compared to control.

Table 5. Preventing death due to a *Streptococcus* infection with SFJDC in mice

Groups	Dose (g/kg)	Number of animals	Mortality rate (%)	Reduction in mortality (%)	Average survival time (d)	Increase in lifespan (%)
Control	-	20	70	-	3.20 ± 2 .63	-
LHQWC	0.77	20	35	50	5.10 ± 2.69**	59.38
Amoxicillin	0.363	20	0	100**	7.00 ± 0.00**	118.75
SFJDC	0.55	20	20	71.43**	5.90 ± 2.27**	84.38
SFJDC	1.1	20	30	57.14**	5.40 ± 2.52**	68.75
SFJDC	2.2	20	40	42.86	4.85 ± 2.72	51.56

**$p < 0.01$ compared to control.

to the control group, the 3 groups receiving SFJDC had a lower mortality rate and reduced mortality. The group receiving 0.55 g/kg of SFJDC had a significantly increased average survival time ($p < 0.01$) and a significantly increased lifespan. Findings suggest that SFJDC prevented death due to an *S. aureus* infection in mice. This study also investigated the ability of SFJDC to prevent death due to a *Streptococcus* infection in mice. Compared to the control group, the 3 groups receiving SFJDC had a lower mortality rate. The groups receiving 0.55 or 1.1 g/kg of SFJDC had a significantly reduced mortality ($p < 0.01$). The groups receiving 0.55 or 1.1 g/kg of SFJDC had a significantly increased average survival time ($p < 0.01$), and all 3 groups receiving SFJDC had a significantly increased lifespan. These findings suggest that SFJDC could become an alternative to broad-spectrum antimicrobials in clinical settings.

Results of the current study indicated that the antibacterial action of SFJDC is inversely correlated with the dose. This finding may be related to the absorption of a traditional Chinese medicine. Previous studies found that absorbance of traditional Chinese medicines was inversely correlated with the dose in a certain range, but the reason for this phenomenon has yet to be determined.

References

1. Coopman SA, Johnson RA, Platt R, Stern RS. Cutaneous disease and drug reactions in HIV infection. N Engl J Med. 1993; 328:1670-1674.
2. Titanji R, Trofa A. Hypokalemia associated with ticarcillin-clavulanic acid. Maryland Med J. 1993; 42:1013-1014.
3. Sattler FR, Weitekamp MR, Ballard JO. Potential for bleeding with the new beta-lactam antibiotics. Ann Intern Med. 1986; 105:924-931.
4. Linton AL, Clark WF, Driedger AA, Turnbull DI, Lindsay RM. Acute interstitial nephritis due to drugs: Review of the literature with a report of nine cases. Ann Intern Med. 1980, 93:735-741.
5. Heim-Duthoy KL, Caperton EM, Pollock R, Matzke GR, Enthoven D, Peterson PK. Apparent biliary pseudolithiasis during ceftriaxone therapy. Antimicrob Agents Chemother. 1990; 34:1146-1149.
6. Appel GB. Aminoglycoside nephrotoxicity. Am J Med. 1990; 88:16S-20S.
7. Hopkins S. Clinical toleration and safety of azithromycin. Am J Med. 1991; 91:40S-45S.
8. Tao Z, Yang Y, Shi W, Xue M, Yang W, Song Z, Yao C, Yin J, Shi D, Zhang Y, Cai Y, Tong C, Yuan Y. Complementary and alternative medicine is expected to make greater contribution in controlling the prevalence of influenza. Biosci Trends. 2013; 7:253-256.
9. Lin LX, Li SX, Zheng FY. [Use of *in vitro* bionic digestion and biomembrane extraction for metal speciation analysis, bioavailability assessment, and risk assessment of Lianhua Qingwen capsules]. Zhongguo Zhong Yao Za Zhi. 2014 Jun; 39:2330-2335.
10. Proctor RA. Is there a future for a *Staphylococcus aureus* vaccine? Vaccine. 2012; 30:2921-2927.
11. Proctor RA. Challenges for a universal *Staphylococcus aureus* vaccine. Clin Infect Dis. 2012; 54:1179-86.
12. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015; 28:603-661.
13. Fisher A, Webber BJ, Pawlak MT, Johnston L, Tchandja JB, Yun H. Epidemiology, microbiology, and antibiotic susceptibility patterns of skin and soft tissue infections, Joint Base San Antonio-Lackland, Texas, 2012- 2014. MSMR. 2015; 22:2-6.
14. Kapadia BH, Berg RA, Daley JA, Fritz J, Bhave A, Mont MA. Periprosthetic joint infection. Lancet. 2015; pii:S0140-6736(14)61798-0.
15. Lin WT, Wu CD, Cheng SC, Chiu CC, Tseng CC, Chan HT, Chen PY, Chao CM. High prevalence of methicillin-resistant *Staphylococcus aureus* among patients with septic arthritis caused by *Staphylococcus aureus*. PLoS One. 2015; 10:e0127150.
16. Parvizi J, Alijanipour P, Barberi EF, Hickok NJ, Phillips KS, Shapiro IM, Schwarz EM, Stevens MH, Wang Y, Shirtliff ME. Novel developments in the prevention, diagnosis, and treatment of periprosthetic joint infections. J Am Acad Orthop Surg. 2015; 23(Suppl):S32-43.

(Received December 14, 2015; Revised December 27, 2015; Re-revised January 28, 2016; Accepted January 29, 2016)