Schwann cells (SCs) are special gliocytes in the peripheral nervous system which play an important role in the genesis and development of peripheral nerves as well as in maintaining normal morphology and function. The proliferative response of SCs in diabetic peripheral neuropathy (DPN) was observed in animal experiments and clinical studies, which implied that SCs could promote axon repair and regeneration. However, there were differing reports as to the proliferation of rat SCs cultured in high glucose medium because these SCs were from tumors of the nervous system, such as Schwannoma cells (JS1 cells) and NF1T cells that were derived from neurofibromas. In this study, the effect of Jinmaitong (筋脉通, JMT) with medicated serum on the proliferation of primarily cultured SCs of rat sciatic nerves in high glucose medium was observed.

**METHODS**

**Material**

JMT, formulated by Peking Union Medical College Hospital and prepared by Beijing Jiulong Pharmaceutical Factory, is composed of milkvetch root, raw rehmannia root, red sage root, kudzuvine root, leech, dodder seed, grossy privet fruit and cassia barktre branchlet, etc. Each capsule of JMT contains 0.35 g crude herbs. The batch number was 061019. Neurotropin (Ntp) was purchased from the Organ Pharmaceutical Co., Ltd., Japan; one tablet was 4NU and the batch number was 06180. Other materials included Dulbecco’s minimum essential medium (DMEM control), 50 mmol/L glucose medium (50 mmol/L Glu), 75 mmol/L glucose medium (75 mmol/L Glu), as well as 50 mmol/L glucose medium, with different concentrations of JMT serum (undiluted, 1:2 diluted and 1:8 diluted) and Neurotropin (Ntp), respectively. The proliferation of SCs under different conditions was detected by MTT. SCs grew exuberantly in DMEM within 24-72 h, but slowed down at 96 h. The proliferation of SCs was inhibited in 50 mmol/L Glu and 75 mmol/L Glu after cultures of 48, 72 and 96 h, which showed that both were significantly different compared to the control group (P<0.01). The inhibition was more significant in 75 mmol/L Glu than in 50 mmol/L Glu (P<0.05). Spearman’s rho analysis revealed that the proliferation of SCs had a negative correlation with the concentration of glucose (r = -0.471, P<0.01). Excluding the time factor, partial correlation showed similar results (r = -0.679, P<0.01). After 48 h, the proliferation of SCs increased significantly in JMT1:2 and Ntp compared with 50 mmol/L Glu (control 0.437±0.019, 50 mmol/L Glu 0.367±0.035, JMT1:2 0.426±0.024, Ntp 0.422±0.013; P<0.01), and there were no statistically significant differences among the JMT groups, the Ntp group and the control group (P>0.05). Conclusions: The proliferation of SCs was inhibited in high glucose medium, and the inhibition was reduced by different concentrations of JMT serum, especially at JMT1:2.

**KEY WORDS** Schwann cells, high glucose, primary culture, Jinmaitong, proliferation, Chinese herbs’ serum pharmacology
S-100 protein, SABC kit, and DAB kit (Boster, China).

Main Instruments
Super-clean work bench at thousand-grade integrally and hundred-grade partly (Cell Center, Institute of Basic Medical Science, Peking Union Medical College, Chinese Academy of Medical Sciences), CO2 incubator (Forma 3111), centrifuge (ALC PK121R), thermostatic water bath (Xutemp XT5706), phase contrast microscope (Olympus CKX31SF), pure water system (Millipoe Elix/BiOs), enzyme micro-plate reader (BioRad Cada).

Primary Culture and Purification of SCs
SCs were isolated from the sciatic nerves of ten Wistar rats [4-day old, purchased from the Institute of Laboratory Animal of Chinese Medical Science Academy (CAMS), SCXK (Beijing) 2005-0013] which were killed by breaking their vertebrae, and were cultured using the tissue explant method. The medium was changed once every 2-3 days, and SCs were purified by the different speed adherence method after 5 days; G-418 (concentration 100 μg/mL) was added 7 days later. The medium contained DMEM, 20% FBS, 2 mmol/L glutamine, 1 mmol/L sodium pyruvate, 100 IU/mL penicillin, 100 μg/mL streptomycin and 12.5 mmol/L HEPES, which was filtered with 0.22 μm filter, and then stored at 4 °C for later use.

Identification of SCs
Method of SABC Cells from the third passage were seeded and incubated in a 6-well plate with a slide, 1 × 10⁵ per well, and then the prepared medium was added; finally, the cell slide was made. Three days later, the slide was fixed in acetone at 4 °C for 15 min, and soaked in 0.1% Triton-X 100 for 10 min; then 5% bovine serum albumin (BAS) was added and hatched after 20 min at room temperature; S-100 polyclonal antibody (1:100 dilution) was added at 37 °C and hatched after 60 min; a second antibody was added at 37 °C for 20 min, SABC was added at 37 °C and was hatched after 20 min, and 50 μL fresh DAB solution was added for 5-10 min. Results assessment: the cytoplasms staining brown indicated a positive result.

Preparation of JMT and Ntp Containing Serum
Wistar rats [male, 280-320 g, purchased from the CAMS, SCXK (Beijing) 2005-0013] were fed at the SPF laboratory animal installation under controlled temperatures 22 °C (20-25 °C), with a humidity of 45% (40%-70%) and noise <60 dB in a 12-h light/dark cycle. After being adaptively fed for 24 h, all the rats were divided into 3 groups. JMT group: rats were administered with JMT (15 times the dose of the adult, 1.3125 g/kg per day); Ntp group: with Ntp (15 times the dose of the adult, 4 NU/kg per day), and the control group: with distilled water. All treatments were given via gastrogavage twice daily for 3 days. Blood was collected from the carotid artery within 2 h after the last administration on the third day. The sera were centrifuged at 4 000 r/min for 10 min after being kept at room temperature for 2 h, and then were placed in 56 °C water for 30 min to inactivate the complements, filtered using 0.22 μm filter, and stored at -20 °C.

Grouping
The third passage SCs were digested with 0.05% pancreatin and 0.02% EDTA, seeded and incubated in a 96-well plate, 7 × 10³ per well, with 200 μ L of a different medium in each well. Grouping: Control group (Control): DMEM + 20% normal rat serum; 50 mmol/L glucose group (50 mmol/L Glu): DMEM+50 mmol/L glucose+20% normal rat serum; JMT1:1 group: DMEM+50 mmol/L glucose+20% JMT serum; JMT1:2 group: DMEM+50 mmol/L glucose+10% JMT serum +10% normal rat serum; JMT1:8 group: DMEM+50 mmol/L glucose+2.5% JMT serum +17.5% normal rat serum; Ntp group: DMEM+50 mmol/L glucose+20% Ntp serum; 75 mmol/L glucose group (75 mmol/L Glu): DMEM+75 mmol/L glucose+20% normal rat serum.

Proliferation of SCs
The MTT experiment was used to evaluate the proliferation of SCs after seeding for 24h, 48h, 72h and 96 h separately. The medium was removed, and then 200 μL 10% MTT was added into each well. After incubation at 37 °C for 2-4 h, the medium was removed, the cells were mixed with 200 μL DMSO in each well for 30 min, and then the OD values were measured at 570 nm wavelength with an enzyme micro-plate reader.

Statistical Analysis
Statistical analysis was performed with SPSS 13.0 software. Data were expressed as mean ± standard deviation, and statistical analysis was assayed with ANOVA. Correlation analysis was done using Spearman's rho correlation analysis and Partial correlation.

RESULTS
Morphological Observation of SCs under Inverted Phase Contrast Microscope
Under inverted phase contrast microscopy, SCs