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Effects of Jiawei Qiwei Baizhu powder-containing serum on the proliferation and osteogenic differentiation of rat bone marrow mesenchymal stem cells by enhancing proliferation and alkaline phosphatase activity

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Abstract: **Background**: Jiawei Qiwei Baizhu Powder (JQBP) has been widely applied in clinical practice for the treatment of pediatric short stature associated with spleen–kidney deficiency and dampness accumulation, showing favorable therapeutic outcomes. Bone marrow mesenchymal stem cells (BMSCs) play a pivotal role in skeletal growth and development through their proliferative and osteogenic differentiation capacities. **Objectives**: This study aimed to explore the potential cellular mechanisms underlying the therapeutic effects of JQBP on pediatric short stature by investigating the effects of JQBP-containing serum on the proliferation and early osteogenic differentiation of rat BMSCs *in-vitro*. **Methods**: JQBP-containing serum and normal control serum were prepared using a serum pharmacology approach. BMSCs were isolated from Sprague–Dawley rats using the whole bone marrow adherence method and identified by flow cytometry. Cell proliferation under different serum concentrations was dynamically assessed using the CCK-8 assay. Based on proliferation outcomes, the optimal serum concentration was selected for osteogenic induction experiments. After 7 days of osteogenic induction, early osteogenic differentiation was evaluated by alkaline phosphatase (ALP) staining and quantitative grayscale analysis. **Results**: CCK-8 assays demonstrated that JQBP-containing serum significantly promoted BMSC proliferation, with the 1% concentration showing the most stable and sustained proliferative effect. Under osteogenic induction conditions, ALP staining revealed markedly enhanced ALP activity in the 1% JQBP-containing serum group compared with the induction-only group and the 1% normal serum group ($P < 0.05$). Quantitative grayscale analysis further confirmed significantly lower grayscale values in the 1% JQBP-containing serum group, indicating the strongest ALP activity among all groups. **Conclusion**: JQBP-containing serum effectively promotes BMSC proliferation and enhances early osteogenic differentiation *in-vitro*. These findings suggest that JQBP may facilitate bone growth by directly activating osteogenic precursor cells, providing experimental evidence for integrating traditional Chinese medicine into modern growth-related therapeutics.

Keywords: Alkaline phosphatase; Bone marrow mesenchymal stem cells; Cell proliferation; Drug-containing serum; Jiawei Qiwei Baizhu powder; Osteogenic differentiation; Short stature; Traditional Chinese medicine

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INTRODUCTION

Short stature in children is a common clinical issue in pediatric endocrinology, defined as an individual's height being more than 2 standard deviations below the population mean for the same race, gender, and age under similar living conditions (Chiarelli *et al*., 2020; Hokken-Koelega *et al*., 2023). It not only affects the quality of life and psychological well-being during childhood but may also indicate underlying systemic diseases. Linear bone growth primarily depends on endochondral ossification at the growth plates of long bones, which is precisely regulated by a complex network involving genetics, nutrition, endocrine factors, and local cytokines (Trompet *et al*., 2024; Allen *et al*., 2021; Faienza *et al*., 2021). The growth hormone and insulin-like growth factor-1 (GH-IGF-1) axis serves as the primary endocrine regulator of childhood growth. Recombinant human growth hormone (rhGH), as the current mainstream treatment, is effective but has limitations, including high cost, the need for daily injections, suboptimal response in some patients, and potential side effects (Cammisa *et al*., 2025; Baron *et al*., 2015; Maghnie *et al*., 2022; Galetaki *et al*., 2024). Therefore, exploring multi-target, mild-acting, and highly compliant adjuvant or alternative therapies, especially discovering safe and effective formulas from Traditional Chinese Medicine (TCM), holds significant clinical and scientific value.

In TCM, based on clinical manifestations, short stature can be categorized under "Wu Chi" (five delays), "Gan Syndrome" (infantile malnutrition), or "Xu Lao" (consumptive disease) (Liao *et al*., 2024; Li *et al*., 2022; Lee *et al*., 2020). The core pathogenesis is often attributed

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to deficiency of both the spleen and kidney. The kidney is considered the "congenital foundation," governing bone development and producing marrow. The Yellow Emperor's Inner Classic clearly states that the abundance or decline of kidney essence directly governs children's growth, development and bone strength. The spleen is the "acquired foundation," the source of qi and blood generation and governs the muscles and limbs. Children often have "spleen insufficiency." If the spleen's transportive and transformative functions are weak, the transformation of food and water into essential nutrients is impaired, leading to inadequate generation of qi and blood. Consequently, the limbs and skeleton lack nourishment, resulting in growth retardation (Liao *et al*., 2024). Therefore, "tonifying the kidney and replenishing essence to strengthen bones and fortifying the spleen and replenishing qi to promote growth" is the fundamental therapeutic principle in TCM for treating children with growth retardation (Li *et al*., 2022; Lee *et al.*, 2020).

Qiwei Baizhu Powder (QBP) originated from the Song Dynasty and is composed of seven herbs: Atractylodis Macrocephalae Rhizoma (Bai Zhu), Ginseng Radix et Rhizoma (Ren Shen), Pogostemonis Herba (Huo Xiang), Puerariae Lobatae Radix (Ge Gen), Glycyrrhizae Radix et Rhizoma (Gan Cao), Pogostemonis Herba (Huo Xiang), Aucklandiae Radix (Mu Xiang) and Poria (Fu Ling). Originally, this formula was recognized for its efficacy in fortifying the spleen, replenishing qi, harmonizing the stomach and promoting fluid production (Zhang *et al*., 2024). In clinical practice, it is often modified by incorporating herbs that tonify the kidney, strengthen bones and enhance essence and marrow, resulting in a modified formulation known as Jiawei Qiwei Baizhu Powder (JQBP). This endows JQBP with dual functions of fortifying the spleen and tonifying the kidney. JQBP has demonstrated promising clinical potential in treating children with growth retardation due to spleen-kidney deficiency. Modern pharmacological studies have further confirmed that TCM herbs with spleen-fortifying and kidney-tonifying properties can improve bone metabolism and increase bone mineral density (Zhao *et al*., 2021; Wen *et al*., 2025; Zhang *et al*., 2021). However, systematic, in-depth experimental research into the specific cellular and molecular mechanisms by which this formula promotes bone growth, particularly its direct effects on osteoprecursor cell function, remains insufficient.

Bone marrow mesenchymal stem cells (BMSCs) are multipotent stem cells residing in the bone marrow microenvironment, capable of self-renewal and multi-lineage differentiation. BMSCs serve as precursor cells for osteoblasts. The proliferative capacity of BMSCs and their commitment to osteogenic differentiation are crucial links in maintaining the dynamic balance between bone formation and bone resorption, thereby ensuring skeletal structural and functional integrity (Chu *et al*., 2020; Purwaningrum *et al*., 2021; Gholami Farashah *et al*., 2023; Li *et al*., 2025). In the metaphysis beneath the growth plates of children's long bones, BMSCs are an important source of osteoblasts that participate in bone formation during the final stage of endochondral ossification, which is essential for longitudinal bone growth and modeling (Matsushita *et al*., 2020; Kurenkova *et al*., 2020). The proliferative vigor of BMSCs and their ability to differentiate into osteoblasts directly influence the rate and quality of new bone formation. Studies have shown that some kidney-tonifying and spleen-fortifying TCM herbs and their active components can promote the proliferation and osteogenic differentiation of BMSCs, which may provide a cytological basis for their "bone-strengthening" effects (Shen *et al.*, 2022; Huang *et al.*, 2024; Chen *et al.*, 2021). Therefore, investigating the effects of JQBP on the biological behavior of BMSCs is a key step toward elucidating the mechanism by which this formula promotes bone growth.

Chinese herbal formulas have complex compositions. After oral administration, they undergo digestion and metabolism *in vivo*, and the material basis for their eventual pharmacological actions is the components that enter the systemic circulation. The serum pharmacology method, which involves administering the herbal formula to animals and collecting their serum as the drug carrier for *in-vitro* experiments, can more closely simulate the drug's *in vivo* action. This method is considered relatively scientific and reliable for studying the mechanism of action of Chinese herbal formulas (Bochu *et al*., 2005; Liang *et al*., 2023; Gao *et al*., 2024).

Based on this background, the scientific hypothesis was proposed that JQBP might, through its active components entering the bloodstream, directly act on BMSCs to promote their proliferation and induce their differentiation into osteoblasts, thereby simulating the biological effects of "fortifying the spleen and tonifying the kidney". To test this hypothesis, this study prepared JQBP-containing serum. It systematically assessed its effects on proliferation and alkaline phosphatase (ALP) activity – an early osteogenic differentiation marker – in rat BMSCs in *vitro*. The aim was to explore the potential cellular mechanisms underlying this formula's ability to promote bone growth at the stem cell level, providing solid experimental evidence for its clinical application.

MATERIALS AND METHODS

Experimental animals

Fifteen clean-grade healthy male Sprague-Dawley (SD) rats were used. Ten adult rats weighing 180-220 g were used for the preparation of drug-containing serum. Another five young rats, weighing 80-100 g, were used for BMSC isolation. The Experimental Animal Center of Guangzhou University of Chinese Medicine provided all rats. They were housed in a clean-grade animal room under controlled

conditions: room temperature 22 ± 2°C, relative humidity 50%-60%. All experimental procedures were conducted in accordance with the principles of laboratory animal welfare and ethics.

Preparation of Jiawei Qiwei Baizhu powder (JQBP) decoction

The formula of JQBP consists of 15g of Puerariae Radix, 12g of Pogostemonis Herba, 6 g of Aucklandiae Radix, 9g of Pseudostellariae Radix, 12g of Poria, 12g of Atractylodis Macrocephalae Rhizoma, 6 g of Psoraleae Fructus, and 3 g of Glycyrrhizae Radix et Rhizoma, which were accurately weighed and mixed. All herbal products were purchased from the Pharmacy of the First Affiliated Hospital of Guangzhou University of Chinese Medicine and were identified by the Department of Pharmacy as complying with the Chinese Pharmacopeia standards. The decoction was prepared following the Technical Specifications for Standardized Decoction Preparation of Chinese Herbal Medicine (T-CACM 1572—2024) to ensure the reproducibility and scientific validity of the experimental data. The mixture was placed in 4 times its volume of pure water and soaked for 2 hours to allow the herbs to soften fully. Subsequently, decoction was performed twice, each lasting 30 minutes. The decoctions from both cycles were combined and initially filtered through gauze to remove herb residues. The filtrate was then passed through a G4 fritted glass filter to obtain a clear solution. This filtrate was concentrated in an 80°C constant temperature water bath until the concentration reached 1.5 grams of crude herbs per milliliter. The concentrated decoction was aliquoted into sterile centrifuge tubes and stored at 4°C.

Preparation of drug-containing serum

After three days of acclimatization, 10 adult SD rats were randomly divided into two groups (n=5 per group): (1) Normal Saline (NS) group: administered an equal volume of normal saline by gavage; (2) JQBP group: administered JQBP concentrated decoction by gavage at twice the clinical equivalent dose (1 mL/100g body weight). Gavage was performed once daily at a fixed time in the morning for 7 consecutive days. 1 hour after the last administration, rats were anesthetized with an intraperitoneal injection of freshly prepared sodium pentobarbital (40 mg/kg). Anesthetized rats were secured in a supine position on a surgical board. The abdominal area was shaved and disinfected. An incision was made along the midline to open the abdominal cavity. Tissues and mesentery were bluntly dissected to fully expose the abdominal aorta. A blood collection needle was rapidly inserted at a 30-degree angle into the abdominal aorta, and blood was collected into vacuum blood collection tubes. Approximately 5-6 mL of blood was collected—samples were placed at 4°C for 60 minutes to allow natural clotting, then centrifuged for 20 minutes. The upper, clear, pale-yellow serum was carefully aspirated and transferred to sterile containers. Serum from the same group of rats was pooled, inactivated in a 56°C water bath for 30 minutes to complement, and then filtered through a 0.22 μm disposable needle filter for sterilization. The final serum was aliquoted into sterile EP tubes, labeled, and stored at -80°C for subsequent cell experiments.

Isolation, culture and identification of rat BMSCs

Primary Isolation and Culture: Primary bone marrow-derived cells were isolated using the whole bone marrow adherent method (Heidari *et al*., 2024; Rahimi *et al*., 2025). Five Sprague-Dawley rats weighing 80–100 g were euthanized by cervical dislocation, soaked in 75% ethanol for 5 minutes and the femurs and tibiae were aseptically dissected without exposing the bone marrow cavity. The bones were immersed in PBS containing 1% penicillin-streptomycin in a centrifuge tube and transferred to a biosafety cabinet. After careful removal of residual muscle tissue, the epiphyses at both ends were trimmed. The bone marrow cavity was flushed with α-MEM solution containing 1% penicillin-streptomycin using a 1 mL sterile syringe, and the flushed fluid was collected into a 50 mL sterile centrifuge tube. The cell suspension was pipetted to dissociate the cells, centrifuged at 1000 rpm for 5 minutes and the supernatant was discarded. The pellet was resuspended in an appropriate volume of pre-prepared complete medium (10% fetal bovine serum + 1% penicillin-streptomycin + α-MEM) and evenly distributed across five 25 cm² culture flasks. The flasks were incubated at 37°C in a humidified atmosphere with 5% CO_2. The medium was changed after 48 hours and refreshed every 2–3 days thereafter. Cell morphology and growth were observed under a microscope and photographed.

Subculture and identification: After 5–7 days of culture, when cell confluence reached 80–90%, subculture was performed as follows: The medium was aspirated from the 25 cm² culture flask, and the cells were washed twice with 2 mL of sterile PBS. An appropriate volume of 0.25% Trypsin-EDTA was added to cover the cell layer and the flask was placed in the incubator for 1–2 minutes. Upon microscopic observation of cell retraction and rounding, the trypsin was aspirated and 2–3 mL of complete medium was added to terminate digestion. The cells were gently pipetted to detach, collected into a 15 mL centrifuge tube and centrifuged at 1000 rpm for 3 minutes. After discarding the supernatant, the pellet was resuspended in complete medium, split 1:2 into 100 mm culture dishes and incubated continuously. Some cells at passage 3 were collected for flow cytometric identification and subsequent experiments.

Cryopreservation and recovery: Due to the rapid growth of BMSCs and the extended experimental timeline, passage 2 cells in good condition were cryopreserved for sustainable use. Cryopreservation was performed following the same digestion procedure as subculture (Chithambaran and Al Harbi, 2025). An appropriate amount of serum-free cell freezing medium was added to resuspend the cells, which

were then aliquoted into several 2 mL cryovials. After sealing with parafilm, the vials were placed in a freezing container and directly transferred to a –80°C freezer without programmed cooling. For recovery, the cryovial was quickly retrieved and thawed in a 37°C water bath. The thawed suspension was transferred to a biosafety cabinet, pipetted into a 15 mL centrifuge tube containing pre-warmed complete medium, and centrifuged at 1000 rpm for 3 minutes. The supernatant was discarded, the pellet was resuspended and the cells were seeded into culture dishes for further incubation.

Flow cytometry assay

Cells were detected with TransDetect® Annexin V-FITC/PI Kit (TransGen Biotech Co.). Added 5 μL of the Annexin V-FITC in a 6-well plate, then cultured in the dark. The NovoCyte Advanteon B4 Flow Cytometer and the NovoSampler Q software were used to measure.

Cell proliferation assay (CCK-8)

Third-generation BMSCs were collected and adjusted to a concentration of 2,000 cells per well, then seeded into a 96-well plate. The cells were divided into the following groups: a blank control group (10% FBS), normal saline (NS) control groups containing 1% (1% NS serum + 9% FBS), 2% (2% NS serum + 8% FBS), 4% (4% NS serum + 6% FBS), 8% (8% NS serum + 2% FBS) and 10% (10% NS serum), as well as drug-containing serum (ZY) groups prepared with Jiawei Qiwei Baizhu San (JWQWBZ) serum at 1% (1% JWQWBZ serum + 9% FBS), 2% (2% JWQWBZ serum + 8% FBS), 4% (4% JWQWBZ serum + 6% FBS), 8% (8% JWQWBZ serum + 2% FBS) and 10% (10% JWQWBZ serum). Each group was set up with four replicate wells, each containing 100 μL of cell suspension. After 24 hours of incubation for cell adhesion, the medium was replaced with fresh medium containing the respective serum concentrations. Cell proliferation was assessed at 0, 1, 3, 5, and 7 days. At each time point, 10 μL of CCK-8 solution was added to the corresponding wells, and the plate was incubated in the dark for 2 hours. Absorbance was measured using a microplate reader with the following settings: low-speed shaking for 5 seconds, single-wavelength mode with high precision, and a detection wavelength of 450 nm. Cell proliferation rates were calculated according to the manufacturer's instructions. Statistical analysis was performed using SPSS version 22.0 and bar graphs were generated with GraphPad Prism version 8.

Osteogenic induction alkaline phosphatase staining

Based on the observed optimal proliferative effect, the corresponding drug-containing serum concentration was selected for alkaline phosphatase (ALP) staining. Third-passage BMSCs were seeded. After 24-48 hours of culture, the medium was replaced with osteogenic induction medium containing different sera for intervention. The groups were: Induction group, Normal Saline (NS) serum induction group, and Jiawei Qiwei Baizhu Powder (ZY) serum induction group. On day 7 of osteogenic induction, ALP staining was performed. The stained cells were photographed, and the images were converted to grayscale in ImageJ 1.53 (Schneider e*t al*., 2012).

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) 22.0 (IBM, Armonk, NY, USA) was used to analyze the experimental results. If the measurement data from multiple independent samples were normally distributed and homoscedastic, the Bonferroni method was used in a one-way analysis of variance. If the distribution did not meet the normality assumption or the variance was unequal, the Kruskal-Wallis test was used to perform stepwise reduction for multiple comparisons and to generate a uniform subset table. There were significant differences between groups in the different columns, with a significance level of 0.05.

RESULTS

Preparation of drug-containing serum and culture & identification of BMSCs

The gavage and abdominal aorta blood collection procedures were performed smoothly. None of the rats experienced complications such as aspiration or esophageal perforation, and their mental state remained good. The obtained sera were transparent, clear and earth-yellow in color, without any signs of hemolysis, indicating high-quality serum suitable for subsequent cell experiments. The results of detecting the active ingredients in drug-containing serum by liquid chromatography-mass spectrometry (LC-MS) were puerarin, psoralen and atractylolactone III.

Rat BMSCs were successfully isolated using the whole bone marrow adherence method. At 24 hours post-seeding of primary cells, many round, highly refractive cells were observed in suspension, representing a heterogeneous population (Fig. 1). After the first medium change at 48 hours, most non-adherent hematopoietic cells were removed, revealing a small number of adherent cells with irregular morphology and processes (Fig. 1). After 5 day, adherent cells had increased significantly and their morphology gradually transitioned to typical long spindle or fibroblast-like shapes, nearly covering the flask bottom (Fig. 1). After passaging, cell purity improved, showing a homogeneous, fibroblast-like morphology and growing in characteristic swirling or radial patterns when confluent (Fig. 1). Flow cytometric identification showed that passage 3 BMSCs highly expressed the mesenchymal stem cell surface markers CD29 and CD90, with positive rates of 98.5% and 97.8%, respectively. In contrast, they expressed low levels of the hematopoietic stem cell marker CD45 and the monocyte/macrophage marker CD11b, with positive rates of only 1.2% and 0.8%, respectively (Fig. 2).

These results confirmed that the cultured cells were high-purity BMSCs suitable for subsequent experiments.

Effects of JQBP-containing serum on BMSC proliferation

The CCK-8 assay systematically evaluated the dynamic effects of different concentrations of drug-containing serum on BMSC proliferation at various time points (Fig. 3). Results showed that after 24 hours of intervention, compared to the 10% FBS blank control group, all concentrations of both NS and JQBP serum groups demonstrated proliferative effects ($P<0.01$). More importantly, at the same concentrations, the proliferation rates in the JQBP-containing serum groups (except the 2% concentration group) were notably higher than those in the corresponding NS control groups. At this time point, no significant differences in pro-proliferative effects among the JQBP concentration groups were observed, suggesting that during early intervention, the pro-proliferative effect of JQBP might have plateaued and not increased further with increasing concentration. After 3 days of intervention, the pro-proliferative effects of the 1%, 2% and 4% JQBP-containing serum groups remained markedly higher than 10% FBS blank control group ($P < 0.01$). However, at this time point, no statistical differences were observed between the JQBP and NS groups at the same concentrations. On days 5 and 7, the proliferation rates in the 1% and 2% JQBP-containing serum groups, as well as the 1% and 2% NS control groups, were still markedly higher than 10% FBS blank control group ($P < 0.05$). Notably, the proliferation rates in the 10% high-concentration NS and JQBP groups were lower than those of the 10% FBS blank control group ($P < 0.05$), suggesting that excessively high serum concentrations (whether normal or drug-containing) may exert inhibitory or toxic effects on cells during long-term culture. Throughout the experimental period, the 1% concentration of JQBP-containing serum demonstrated the most stable and sustained pro-proliferative effect on BMSCs.

Effects of JQBP-containing serum on early osteogenic differentiation (ALP activity) of BMSCs

To investigate whether the drug-containing serum, beyond promoting proliferation, could guide BMSCs toward osteogenic differentiation, the effect of 1% drug-containing serum on ALP activity—an early marker of osteogenic differentiation—was assessed under osteogenic induction conditions. Microscopic observation (Fig. 4) revealed that cells in the 1% JQBP serum induction group were filled with deep blue cytoplasmic precipitates, and the staining intensity was markedly higher than that observed in the induction-only and 1% NS serum induction groups. Macroscopic observation (Fig. 5) revealed a deep, intense blue-purple color at the bottom of the wells in the 1% JQBP serum induction group, indicating higher ALP activity. Grayscale value analysis of the microscopic images yielded results highly consistent with the morphological observations (Table 1). Significant differences in mean grayscale values were observed among the three groups ($F = 11.705$, $P = 0.008$). Further Bonferroni post-hoc tests found: The grayscale value of the 1% NS serum induction group was notably lower than that of the induction-only group, suggesting that under basic induction conditions, even the addition of a small amount of normal rat serum might have a slight effect on ALP activity. Most crucially, the grayscale value in the 1% JQBP serum induction group was significantly lower than that in the that of the 1% NS serum induction group. Lower grayscale values indicate darker ALP staining and stronger enzyme activity. This demonstrates that, under the same osteogenic induction environment, 1% JQBP-containing serum significantly enhances the early osteogenic differentiation capacity of BMSCs, and this effect is superior to that of osteogenic induction alone or intervention with normal serum.

DISCUSSION

For the first time, research on JQBP has focused on its intervention for children with short stature. The potential cellular mechanisms of action of JQBP in treating this condition were investigated using *in vitro* experiments. Using the serum pharmacology method, it was confirmed that JQBP-containing serum significantly promotes BMSC proliferation and enhances their early osteogenic differentiation capacity. This provides key evidence for elucidating the modern cytobiological mechanisms by which this spleen-fortifying and kidney-tonifying formula regulates bone metabolism and promotes linear bone growth to treat growth retardation.

Regarding cell proliferation, a hormetic effect characterized by "low-concentration promotion and high-concentration inhibition" was observed with JQBP-containing serum (Calabrese *et al.,* 2024; Wang *et al.,* 2018). Low concentrations (1%-4%) of drug-containing serum, particularly 1%, showed significant proliferative-promoting effects, whereas a high concentration (10%) showed an inhibitory effect. This complex concentration-response relationship reflects the multi-component, multi-target characteristics of Chinese herbal formulas. Active components such as ginsenosides from Panax Ginseng and polysaccharides from Atractylodis Macrocephalae Rhizoma within the formula have been confirmed by numerous studies to possess cell proliferation-stimulating activities (Faghani *et al*., 2022; Iwabuchi *et al*., 2024; Zheng and Wang, 2022).

However, the formula may also contain other components that exert effects at high concentrations, potentially inducing mild endoplasmic reticulum stress or altering the osmotic pressure and acid-base balance of the culture medium, thereby counteracting the proliferative effects at high concentrations. This suggests that precise dose control is crucial in TCM research and clinical application.

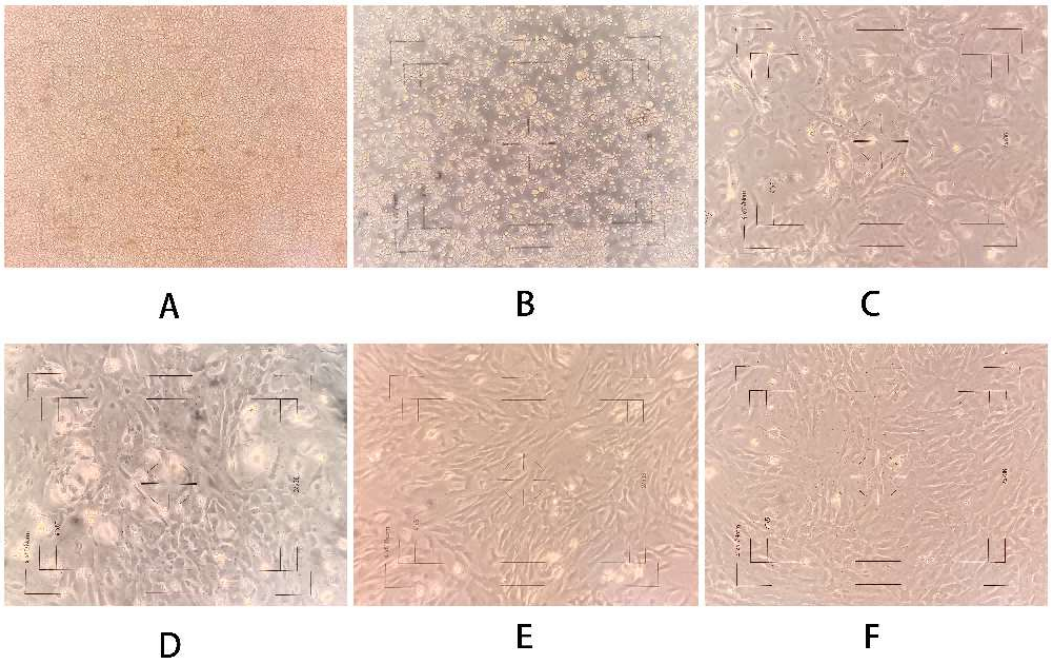

Fig. 1: Morphological characteristics of rat bone marrow mesenchymal stem cells (BMSCs) during primary culture and passaging (10× magnification). (A)(P0, day1)Primary cells at 24 h after seeding, showing numerous round, highly refractive cells suspended in the culture medium; (B) (P0, day3) 48 h after seeding, following the first medium change, non-adherent hematopoietic cells were removed and a small number of adherent cells with irregular morphology and cytoplasmic processes were observed; (C) (P0, day5) Day 5 of culture, adherent cells increased markedly and gradually displayed typical long spindle-shaped or fibroblast-like morphology; (D-F) Passage cells, exhibiting a homogeneous fibroblast-like morphology and characteristic swirling or radial growth patterns when reaching confluence.

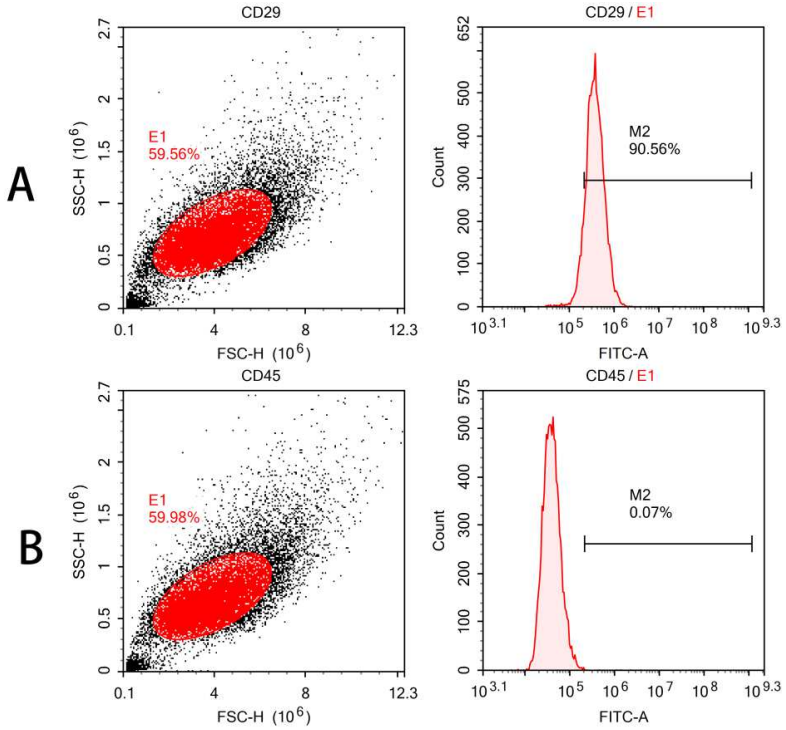

Fig. 2: Flow cytometry identification result graph of BMSCs: (A) CD29 piositive cell population Scatter plot (59.56%), CD29 /E1 piositive cell population cytometry plot (90.65%); (B) CD45 piositive cell population Scatter plot (59.98%), CD45/E1 piositive cell population cytometry plot (0.07%)

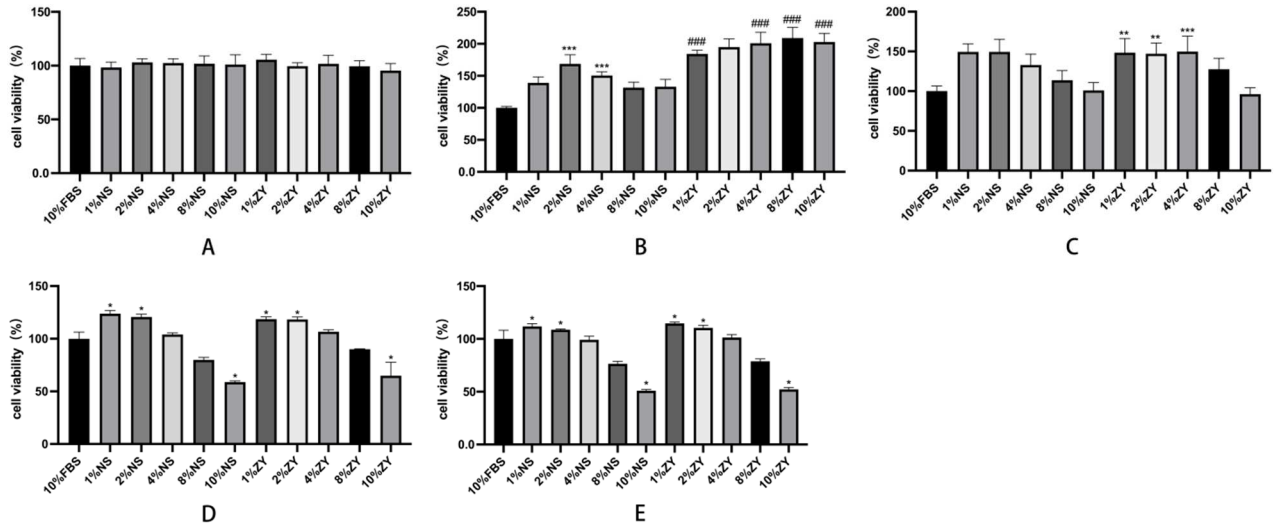

Fig. 3: (A) Bar charts of proliferation rates of BMSCs cells in each group on day 0 after culture; (B)Bar charts of proliferation rates of BMSCs cells in each group on day 1 after culture; (C)Bar charts of proliferation rates of BMSCs cells in each group on day 3 after culture; (D) Bar charts of proliferation rates of BMSCs cells in each group on day 5 after culture; (E) Bar charts of proliferation rates of BMSCs cells in each group on day 7 after culture.
Compared with the blank control group (10%FBS), *P < 0.05, **P < 0.01, ***P < 0.001; Compared with the NS group of the same concentration, P < 0.01 and P < 0.001 in the ZY group.

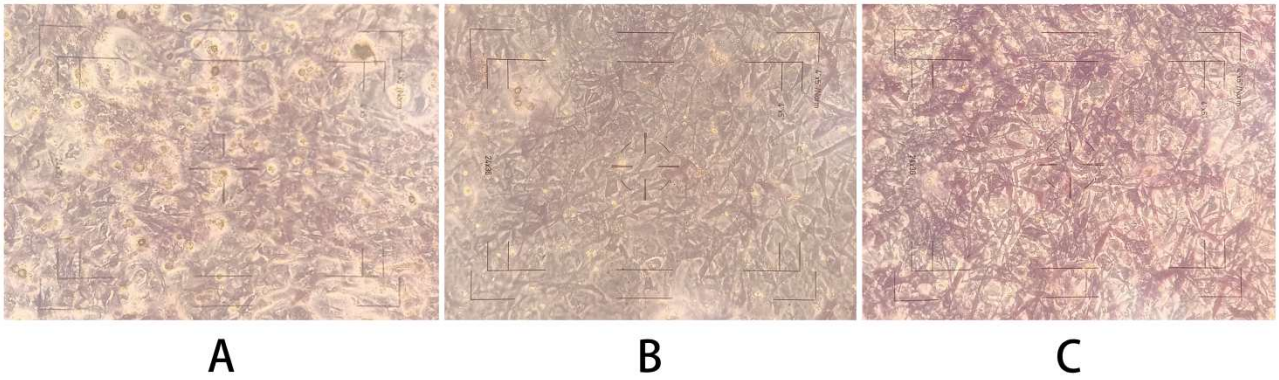

Fig. 4: Alkaline phosphatase staining light micrograph (microscopic, 10×). (A) Induction group; (B) 1% NS Induction group; (C)1%ZYInduction group

More importantly, this study confirmed the definite efficacy of JQBP-containing serum in inducing the early osteogenic activation of BMSCs. Alkaline phosphatase is a key marker enzyme for early osteoblast differentiation. Its activity level directly reflects the differentiation and maturation status of osteoblasts and prepares the necessary microenvironment for subsequent matrix mineralization (Abnosi *et al*., 2023). During childhood growth and development, elevated serum bone-specific ALP levels are an important indicator of active bone turnover and vigorous growth (Zhang *et al*., 2023; Lee *et al*., 2023). On day 7, ALP activity in the experimental group treated with 1% JQBP-containing serum was notably higher than in both control groups. This not only indicates that more BMSCs were initiated into the osteogenic differentiation program but also suggests that these cells acquired a stronger capacity to initiate matrix mineralization. Interpreting this from a TCM perspective, the enhanced ALP activity can be viewed as the manifestation of the "kidney governing bones and generating marrow" function. The active components in the kidney-tonifying herbs in the formula might mimic or enhance endogenous osteogenic signals, thereby guiding BMSCs to differentiate directionally into osteoblasts, i.e., "tonifying the kidney and replenishing essence to determine their direction". This aligns with modern research findings showing that certain kidney-tonifying Chinese herbs can activate the Wnt/β-catenin signaling pathway (Duan *et al*., 2022; Wang *et al*., 2021).

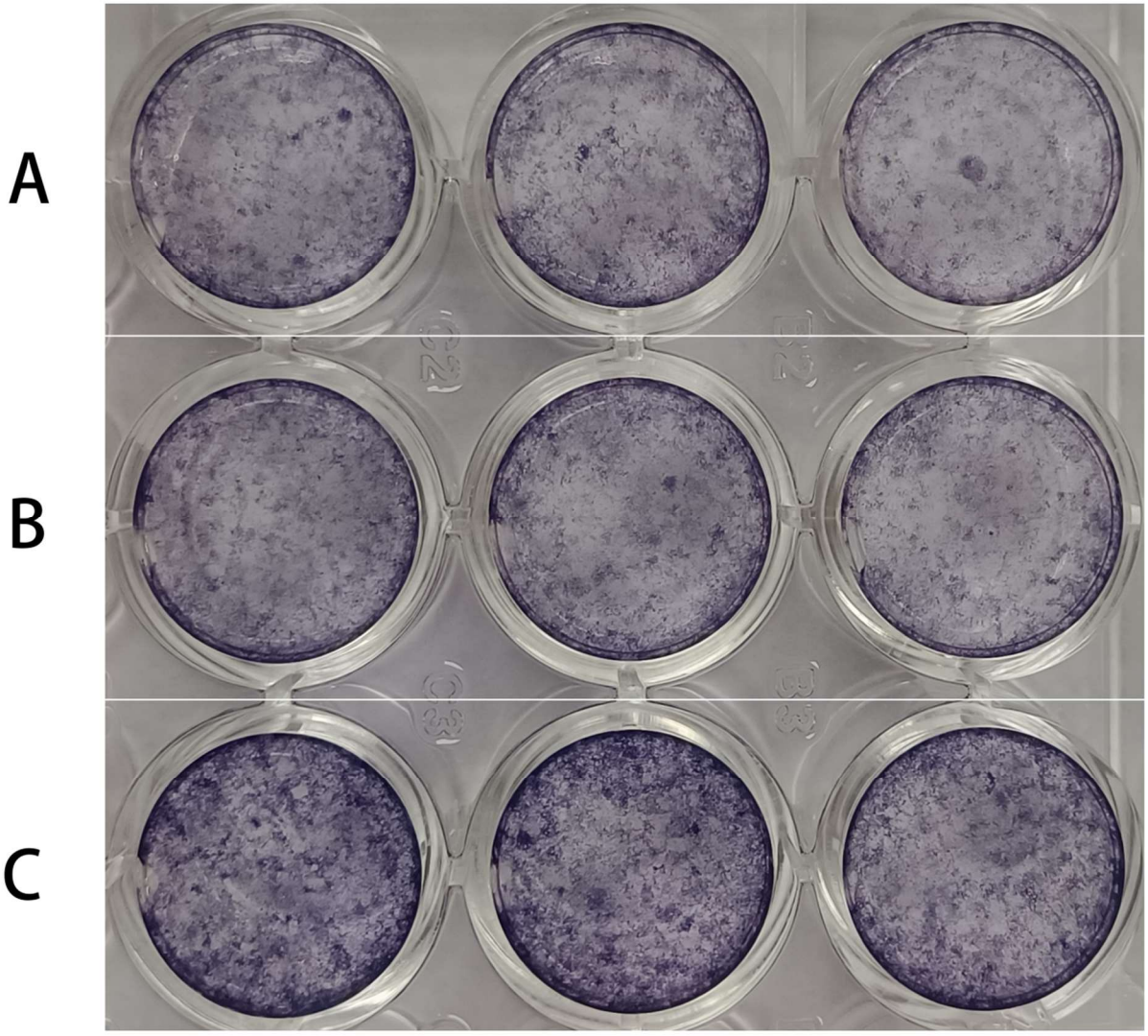

Fig. 5: Alkaline phosphatase staining (naked eye). (A) Induction group; (B) 1% NS Induction group; (C) 1% ZY Induction group

Table 1: Gray values of each group of staining

Group	N (Number of independent experiments)	gray value (10^6)	*F*	*P*
induction group	3	2.48	11.705	0.008
1%NS induction group	3	3.66*		
1%ZY induction group	3	9.67*#		

Therefore, JQBP might, through "concurrently treating the spleen and kidney", on the one hand, "fortify the spleen" to provide momentum for cell proliferation and, on the other hand, "tonify the kidney" to specify the direction of differentiation, synergistically promoting bone growth and maturation.

This study combines research on complex Chinese herbal formulas with cellular models of childhood growth and development, demonstrating clear innovation and clinical relevance. However, this research is still in its preliminary stage of mechanistic exploration. First, only the early differentiation marker ALP was observed; key events in bone maturation, such as the expression of late markers (e.g., osteocalcin, collagen type I) and the formation of mineralized nodules, were not assessed. Liquid chromatography-mass spectrometry (LC-MS) analysis of the drug-containing serum revealed that the active constituents were puerarin, psoralen and atractylenolide III. Psoralen has been shown to promote the proliferation of BMSCs, enhance mineralization and increase the differentiation level of osteoblasts. Given the multi-

component, multi-target characteristics of TCM compounds, a preliminary network pharmacology analysis of the potential mechanisms of Modified Qiwei Baizhu Powder in the treatment of short stature was conducted (Zhang *et al*., 2024). The results indicated that the active ingredient targets and pathways are closely associated with the proliferation and differentiation of BMSCs. The study identified quercetin, luteolin, kaempferol and naringenin as key active components of Modified Qiwei Baizhu Powder that may improve short stature. These components likely act on cellular components such as membrane rafts and membrane microdomains via core targets including AKT1, IL6, JUN, CASP3 and VEGFA. They further activate related transcription factors and DNA-binding transcription factors, thereby regulating signaling pathways such as PI3K-Akt and NF-kappa B. This promotes the proliferation and osteogenic differentiation of bone marrow mesenchymal stem cells, ultimately facilitating longitudinal bone growth (Jancevska *et al*., 2009).

Furthermore, the precise intracellular signaling pathways involved (e.g., whether it specifically activates BMP-2 or Wnt/β-catenin pathways) need to be thoroughly validated using techniques such as pathway inhibitors and small interfering RNA. Finally, results from in *vitro* cell experiments require in vivo validation in suitable animal models (e.g., growth hormone-deficient or spleen-kidney deficiency pattern rat models of short stature). Further investigation into whether synergistic effects exist between JQBP and growth hormone would provide theoretical support for its potential future clinical combination therapy.

CONCLUSION

JQBP-containing serum effectively promotes the proliferation of rat BMSCs *in vitro, with a* 1% concentration showing the most significant effect. Under osteogenic induction conditions, 1% JQBP-containing serum significantly enhances alkaline phosphatase activity in BMSCs, indicating its efficacy in promoting early osteogenic differentiation. In summary, JQBP may promote BMSCs proliferation and early osteogenic activation, thereby simulating and enhancing osteogenic activity in the growth plate region. This finding elucidates the cellular-level mechanism underlying the therapeutic intervention of TCM for children with short stature attributed to the spleen-kidney deficiency pattern. This study offers new perspectives and experimental evidence for the modern research of TCM in the field of childhood growth and development.

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Authors' contributions
YZ and HL: Designed the study and drafted the original manuscript; YZ, YC, QZ, YS and CY: Performed the experiments; CY and CK: Handled the data analysis; YZ and HL: Responsible for reviewing and editing.

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Data availability statement
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval
The study was reviewed and approved by the Animal Ethics Committee of Guangzhou University of Chinese Medicine (Approval No.: JY [2021]216). This study was performed in adherence with the ARRIVE guidelines. See supplementary file for the ARRIVE checklist.

Conflict of interests
The authors declared no conflict of interest.

Supplementary data
https://www.pjps.pk/uploads/2026/05/SUP1778146965.pdf

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