

Original Article

Shangke Jiegu tablet enhances callus formation during fracture repair by promoting angiogenesis-osteogenesis coupling via PTGS2-PGE2/EP4 axis

Lin Chen^{a,1}, Chu Zhang^{a,1}, Xiaojie Li^{a,b,1}, Jingyuan Zhang^a, Jie Zou^a, Suyu Zhang^a,
Jing Zhang^c, Xueting Liu^a, Yudong Liu^a, Zhaochen Ma^a, Ying Liu^a, Na Lin^a,
Jigang Wang^{a,*}, Yanqiong Zhang^a

^a State Key Laboratory for Quality Assurance and Sustainable Use of Dao-di Herbs, Institute of Chinese Materia Medica and Artemisinin Research Center, China Academy of Chinese Medical Sciences, Beijing 100700, China

^b Science and Technology Innovation Center, Guangzhou University of Chinese Medicine, Guangzhou, Guangdong 510405, China

^c School of Pharmacy, Zhejiang Chinese Medical University, No 548 Binwen Road, Hangzhou, Zhejiang 310053, China

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ABSTRACT

Background: Bone fractures greatly limit patients' mobility and quality of life. Shangke Jiegu tablet (SJT), a Chinese patent drug, shows significant efficacy in fracture treatment, yet its molecular mechanism remains unclear.

Purpose: This study aimed to elucidate SJT's therapeutic effects and mechanisms in fracture repair.

Methods: A rat tibial fracture model was first established to evaluate the therapeutic effects of SJT. Subsequently, HPLC-QTOF/MS and ADMET rules were utilized to identify the candidate bioactive constituents in SJT, and the corresponding putative targets were predicted via the TCMIP v2.0 platform. An interaction network was constructed to link fracture-related differentially expressed genes and SJT's putative targets, and to screen the key targets using CytoScape, which were verified through a series of *in-vivo* experiments.

Results: SJT significantly promoted the early-mid tibial fracture healing by restoring limb mobility, enhancing bone microstructure remodeling, and reducing inflammatory responses. It increased levels of bone formation and angiogenesis markers while decreasing bone resorption indicators. Chemical profiling identified 184 constituents in SJT, 34 of which met the optimal ADMET criteria. Additionally, PTGS2 was identified as one of hub nodes playing a crucial role in the network. Notably, SJT markedly upregulated the expression of PTGS2, PGE2 and EP4 protein, which in turn activated CXCL12 and CXCR4, improving the coupling of angiogenesis and osteogenesis to promote the early-mid fracture repair.

Conclusions: SJT accelerates the early-mid fracture repair by enhancing osteogenesis-angiogenesis coupling through regulating the PTGS2-PGE2/EP4 signaling axis. This study is expected to offer a novel approach for exploring the effect of SJT on bone fractures.

Introduction

The issue of bone fractures represents a significant global public health concern, characterized by disruption to the continuity and

integrity of bone tissue caused by trauma or pathology. The fracture injuries frequently lead to impaired limb function, reduced productivity, and disability (Alarkawi et al., 2024; Wang et al., 2021). In 2019, the global incidence included approximately 178 million new fracture cases

Abbreviations: BALP, bone-specific alkaline phosphatase; BGP, osteocalcin; BMP-2, bone morphogenetic protein-2; CD31, platelet endothelial cell adhesion molecule-1; CXCL12, C-X-C X Chemokine Ligand 12; CXCR4, C-X-C chemokine receptor type 4; DEGs, differentially expressed genes; DL, drug-likeness; EP4, Prostaglandin E Receptor 4; GAPDH, Glyceraldehyde-3-Phosphate Dehydrogenase; IL-1 β , interleukin-1 beta; IL-6, interleukin-6; micro-CT, micro-computed tomography; OB, oral bioavailability; OPG, osteoprotegerin; PGE2, prostaglandin E2; PINP, procollagen type I N-terminal propeptide; PTGS2, Prostaglandin-Endoperoxide Synthase 2; SJT, Shangke Jiegu Tablet; TGF- β , transforming growth factor-beta; TRACP-5b, tartrate-resistant acid phosphatase 5b; VEGF, vascular endothelial growth factor; β -CTX, beta C-terminal telopeptide.

* Corresponding authors.

E-mail addresses: jgwang@icmm.ac.cn (J. Wang), yqzhang@icmm.ac.cn (Y. Zhang).

¹ These authors offer the equal contributions to this study.

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and 445 million prevalent cases, imposing substantial economic burdens due to prolonged recovery, delayed healing, non-union complications, and associated healthcare costs (2021; Panteli et al., 2022; Zura et al., 2016). Current therapeutic approaches primarily involve the surgical intervention and the conservative management. While surgery effectively stabilizes severe fractures, it carries risks of infection, implant failure and other procedure-related complications (Dong, 2024; Gillespie and Walenkamp, 2010). Pharmacological treatments, such as non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics, are routinely used to manage the pain and the inflammations but lack of direct bone repair-promoting effects. Certain evidences showed they may also induce gastrointestinal or cardiovascular side effects (Baertl et al., 2021; Bible and Mir, 2015; Elsevier and Cannada, 2020; Vuolteenaho et al., 2008). Consequently, safe and efficacious strategies to enhance fracture healing, particularly those addressing both structural repair and biological regeneration, remain urgently needed.

Shangke Jiegu tablet (SJT), listed in the 2020 edition of the Chinese Pharmacopoeia, is a well-established Chinese patent medicine widely used in clinical fracture management (Wang et al., 2022). It is composed of 12 herbal ingredients, including *Carthami Flos* (HongHua, Safflower), *Eupolyphaga Steleophaga* (TuBieChong, Ground Beetle), *Cinnabaris* (ZhuSha, Cinnabar), *Strychni Semen* (MaQianZi, Nux Vomica), *Myrrha* (MoYao, Myrrh), *Notoginseng Radix et Rhizoma* (SanQi, Panax Notoginseng), *Asterias* (HaiXing, Starfish), *Os Galli* (JiGu, Chicken Bone), *Borneolum Syntheticum* (BingPian, Synthetic Borneol), *Pyrimum Usta* (ZiRanTong, Calcined Pyrite), *Olibanum* (RuXiang, Frankincense) and *Melo Semen* (TianGuaZi, Bitter Gourd Seed). The prescription exerts its therapeutic effects through coordinated actions: promoting blood circulation, resolving stasis, reducing swelling and pain and supporting bone regeneration. Our previous meta-analyses have shown that SJT significantly enhances clinical efficacy in fracture healing, exhibiting analgesic, anti-inflammatory properties, and promoting callus formation (Xueting et al., 2024). While the well-documented clinical benefits of SJT are clear, the specific pharmacological mechanisms by which it accelerates bone repair, including its interactions with the molecular pathways that govern osteogenesis, angiogenesis and inflammatory resolution, remain incompletely understood.

We herein adopted a multistep approach to clarify the uncertainties of the field. First, we established a rat tibial fracture model to completely evaluate the therapeutic effects of SJT in accelerating bone repair and to determine its pharmacological characteristics. After that, we employed HPLC-QTOF/MS and ADMET rules to identify the candidate bioactive constituents within SJT. These constituents were then mapped to their corresponding putative targets using the TCMIP v2.0 platform. To understand the relationship between SJT and fracture-related processes, we consequently constructed an interaction network connecting fracture-related differentially expressed genes and SJT's putative targets. Through the use of CytoScape, we screened for key targets within this network, which were verified using a series of *in vivo* experiments.

Material and methods

Establishment of tibial bone fracture rat model

Seventy-two male Sprague-Dawley rats (8 weeks, 240±10 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (License No.: SXYK (Beijing) 2021–0017). The animals were housed under specific pathogen-free (SPF) conditions with a controlled humidity of 50–70 %, temperature of 24 ± 1 °C, a standard 12 h light/dark cycle, and free access to food and water. All experimental procedures strictly adhered to the Guidelines for the Care and Use of Laboratory Animals provided by the Experimental Animal Welfare Committee of the China Academy of Chinese Medical Sciences (Ethics Approval No.: IBTCMCACMS21–2402–02, approval date: February 2, 2024).

After a one-week acclimatization period, all rats were fasted for 12 h with free access to water. The tibia fracture rat model was then

established as previously described (Zhang et al., 2025). Following the isoflurane anesthesia, the right lower limb was shaved and disinfected with iodophor. Subsequently, a 3 cm longitudinal incision was made along the midshaft of the right tibia, and the underlying subcutaneous tissue, the fascia, and the muscle were dissected layer-by-layer to fully expose the bone. Thereafter, a complete osteotomy was performed at the point of maximal tibial curvature of the right tibia shaft (the junction of the proximal and middle thirds) using an electric saw. To stabilize the fracture, a 1.0 mm Kirschner wire was retrogradely inserted into the proximal tibia and advanced anterogradely across the fracture site via an electric drill. Following this, the incision was sterilized and sutured. Finally, to prevent postoperative infection, penicillin sodium solution (40,000 U/kg; Hongbao Veterinary Medicine Co., Ltd.) was administered intramuscularly to the contralateral limb daily for three consecutive days.

Drug treatment

According to the SJT prescribing information, the recommended adult clinical dosage is 4 tablets taken three times daily, with each tablet containing 0.33 g of extract, resulting in a total of 3.96 g/day for a 60 kg adult. Dose conversion from human to rat was performed using the body surface area (BSA) normalization method, according to the following formula: Rat dose (g/kg) = (3.96 g / 60 kg) × 0.018 / 0.2 kg ≈ 0.42 g/kg/d. This value was designated as the clinical-equivalent dose for rats. A total of 72 rats were randomly assigned into six groups (*n* = 12 per group): Normal control (CON), tibial fracture model (MOD), tibial fracture model with SJT low-dose (SJT-L, 0.21 g/kg/day, 0.5× clinical-equivalent dose), medium-dose (SJT-M, 0.42 g/kg/day, 1× clinical-equivalent dose), high-dose (SJT-H, 0.84 g/kg/day, 2× clinical-equivalent dose), and Jiegu Qili Capsule (JQC, 0.10 g/kg/day, positive control, a well-established traditional Chinese patent medicine extensively used in the field of traumatic fractures). Starting from the first postoperative day, the CON and MOD groups received an equivalent volume of saline via oral gavage, whereas the drug treatment groups were administered corresponding doses daily for 23 consecutive days.

Assessment of swelling degree

The diameter of the affected limb at the fracture site was measured and recorded using an electronic digital caliper at baseline (pre-modeling) and on post-modeling days 3, 6, 9, 12, 15, 18, and 21.

Behavioral assessment

Mechanical pain threshold measurement: Mechanical hyperalgesia of rats were detected in different groups according to our previous study (Chen et al., 2024). Briefly, the BME-404 electronic mechanical analgesiometer was used to apply force via its needle tip, which was aligned perpendicularly to the central plantar surface of the rat's right hind paw. The force was increased gradually and continuously until the rat exhibited a paw withdrawal response (defined as a rapid lifting or flicking of the hind paw). The instantaneous force value was recorded as the paw withdrawal threshold. Each rat underwent three measurements with 5-minute intervals.

Inclined plate test: The inclined plane test was used to measure the strength of the limb muscles in rats both before and after fracture modeling. Each rat was placed on a standardized inclined plane, and the angle of inclination was measured using a digital inclinometer that was securely attached to the plane's surface. The plane was started at 0° and raised in 5° increments at a constant speed until the rat could barely stay on the inclined surface for 5 s without slipping down, after which the inclination angle was recorded. Each rat performed three repeated measurements.

Hindlimb weight-bearing test: On days 0, 1, 8, 15, and 21, all rats were placed on the testing platform of the YLS-11A dynamic paw force

measurement system, with both hind limbs positioned individually on the left and right pressure sensor plates. The forelimbs rested on an occlusion panel, ensuring that the hind limbs supported the majority of the body weight. The weight-bearing difference was recorded as the weight on the contralateral (left) limb minus that on the fracture (right) limb. Each rat performed three repeated measurements at one-hour intervals.

CatWalk XT gait analysis: On the day before the end of the experiment, rats were placed at the starting point of the CatWalk XT runway, and a successful gait recording was defined as a trial in which the rat traversed the runway at a constant speed without pausing or turning back. Each rat was examined three times. The following gait metrics were analyzed: the paw print area, the mean print intensity, the stand time, the swing time, the swing speed and the duty cycle.

X-ray scanning and micro-computed tomography (micro-CT) analysis

To evaluate the success of fracture modeling and monitor the progression of fracture healing, serial X-ray scans were performed at 24 h postoperatively and on days 7, 14, and 21 after surgery, using a small-animal *in vivo* imaging system with the following parameters: tube voltage, 48 kV; tube current, 100 mA; exposure dose, 10 mAs; and exposure time, 0.06 s. Successful fracture modeling was defined by the following radiographic criteria: (1) a clear transverse or oblique fracture line; (2) absence of displacement or rotational deformity of the fracture fragments; and (3) proper positioning of the intramedullary Kirschner wire. Representative radiographs obtained within 24 h post-surgery for all fractured rats were presented in **Supplementary Figure S1**. Fracture healing was quantitatively assessed using the modified Radiographic Union Score for Tibial fractures (RUST).

Upon tissue harvest, the afflicted tibiae were fixed and scanned with a high-resolution micro-CT system (GE Healthcare, USA; resolution: 15.6 μm ; voltage: 70 kV; current: 114 μA ; power: 8 W). A region of interest (ROI) encompassing the central callus area was selected for analysis. Bone microarchitecture was evaluated using the manufacturer's software, and the following parameters were measured: bone mineral density (BMD), bone surface to volume ratio (BS/BV), structure model index (SMI), bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp).

Viscera index detection

Following sacrifice and necropsy, the liver, kidneys, thymus, spleen, and brain were harvested and weighed. Organ-to-brain mass ratios (liver-to-brain, kidney-to-brain, thymus-to-brain, and spleen-to-brain) were calculated to assess systemic toxicity and organ-specific effects. Furthermore, body weight was constantly measured throughout the experiment to evaluate general physiological health.

Histopathological analysis

After micro-CT scanning, tibial specimens were decalcified, embedded in paraffin, and sectioned into 4 μm slices for histological staining. Hematoxylin and eosin (H&E) and Masson-stained sections were examined under a light microscope at 40 $\times$ magnification to assess the overall morphology of newly formed bone within the callus. TRAP-stained slices were observed at 100 $\times$ magnification to determine the distribution and activity of osteoclasts. For quantitative analysis, three representative fields encompassing the entire callus region were captured. The percentage of mineralized mature bone area and TRAP-positive area were calculated using Image-Pro Plus 6.0 software.

HPLC-QTOF-MS analysis and admet assessment

Chemical profiling of the constituents in SJT was performed using a HPLC-QTOF-MS system. The analysis was carried out utilizing an

Alliance e2695 HPLC equipment (Waters, USA) together with an Agilent 6520 quadrupole time-of-flight mass spectrometer (Agilent Technologies, USA). MS and MSn spectra provided reliable mass values for constituent identification. The drug-likeness and pharmacokinetic properties of the identified SJT constituents were evaluated using ADMETlab 3.0 (Fu et al., 2024). The following parameters were considered: physicochemical properties (molecular weight, MW; logarithm of aqueous solubility value, logS; topological polar surface area, TPSA), medicinal chemistry characteristics (quantitative estimate of drug-likeness, QED), excretion profiles (half-life, $T_{1/2}$), and toxicology indicators (FDA maximum recommended daily dose, FDAMDD). Constituents that met the ideal requirements were regarded potential bioactive candidates of SJT based on the ADMETlab3.0 endpoints explanation.

Data collection and network pharmacology analysis

All chemical constituents of SJT and their putative targets were systematically retrieved from the Traditional Chinese Medicine Integrated Pharmacology platform (<http://www.tcmip.cn/TCMIP/index.php/Home/Login/login.html>, TCMIP v2.0 (Zhang et al., 2023)). Concurrently, fracture-related transcriptome data were retrieved from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/gse/query/acc.cgi?acc=GSE494>). Identification of differentially expressed genes (DEGs) in healthy controls versus fracture patients employed $|\log_2 \text{fold change}| \geq 1$ with adjusted p-value < 0.05 as significance criteria. Subsequently, the interaction network between SJT-predicted targets and fracture-related DEGs was constructed collected from STRING database (<https://string-db.org/>). Core target genes in this network were identified based on topological parameters, including degree centrality, closeness centrality, and betweenness centrality, calculated by CytoHubba plug-in of Cytoscape (<https://cytoscape.org/>, version 3.8.2). Furthermore, Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses (KEGG) were conducted using the Metascape software to elucidate the biological processes and signaling pathways mediated by the identified core targets.

ELISA detection

Blood samples were collected from the abdominal aorta using non-anticoagulant tubes and centrifuged at 3500 rpm for 15 min to isolate the supernatant. Serum levels of bone-specific alkaline phosphatase (BALP, Cat No ml003415), osteocalcin (BGP, Cat No E20230906–30405A), osteoprotegerin (OPG, Cat No E20230906–30411A), transforming growth factor-beta (TGF- β , Cat No ml952806), bone morphogenetic protein-2 (BMP-2, Cat No ml102832), procollagen type I N-terminal propeptide (PINP, Cat No ml038224), beta C-terminal telopeptide (β -CTX, Cat No ml038228), tartrate-resistant acid phosphatase 5b (TRACP-5b, Cat No ml590485), interleukin-1 beta (IL-1 β , Cat No E20230906–30206A), and interleukin-6 (IL-6, Cat No E20230906–30219A) and vascular endothelial growth factor (VEGF, Cat No ml064294) were determined using commercially available ELISA kits (Shanghai Enzyme-linked Biotechnology Co., Ltd.) following the manufacturer's instructions.

Serum biochemical indexes analysis

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine (Cr) were measured to assess the effects of SJT in hepatic and renal function. All assays were performed following the manufacturer's protocols (Nanjing Jiancheng Bioengineering Institute, Nanjing, China; kits #C009–2–1, #C010–2–1, #C013–2–1, #C011–2–1)

Immunohistochemistry (IHC)

Paraffin-embedded tissue sections were deparaffinized and rehydrated, followed by antigen retrieval using enzymatic and heat-induced epitope retrieval methods. Sections were then incubated with primary antibodies, including CD31 (A19014, 1:1000), PTGS2 (a3560, 1:200), and EP4 (A20295, 1:200) purchased from ABclonal (Wuhan, China) at 4 °C overnight, subsequently treated with species-matched secondary antibodies, and visualized using 3,3'-diaminobenzidine (DAB) chromogenic substrate. Stained sections were examined and imaged under a light microscope. For quantitative analysis, three random fields of view were taken using CaseViewer software, and the percentage of positive area was quantified across all fields using ImageJ software.

Quantitative real-time PCR

Total RNA extracted from callus tissue was reverse-transcribed into cDNA using the Thermo Fisher Scientific RNA reverse transcription kit according to the manufacturer's instructions. The expression levels of CXCL12, CXCR4, and VEGF were measured using quantitative real-time PCR on an Applied Biosystems QuantStudio system. Gene-specific primers were created and tested for amplification efficiency. The relative mRNA expression was normalized to GAPDH and evaluated using the $\Delta\Delta CT$ method.

Molecular docking analysis

The crystal structure of PTGS2 (PDB: 5F19) was retrieved from the RCSB PDB database. Candidate SJT compounds were acquired in SDF format from PubChem, converted to PDB format using Open Babel, and prepared with AutoDockTools by removing solvent molecules, adding polar hydrogens, and assigning partial charges. The constructed structures then underwent molecular docking utilizing AutoDock Vina with appropriate grid parameters. Finally, PyMOL was employed to visualize and analyze the docked complexes.

Surface plasmon resonance (SPR) assay

The binding affinity between human PTGS2 protein (Sino biological, 12,036-H08B) and drug-like small constituents was evaluated by SPR technique using a Biacore T200 instrument (Cytiva) at 25 °C. The running buffer was 1× HBS-EP (1 mM HEPES, 150 mM NaCl, 3 mM EDTA, and 0.05 % surfactant P20, pH 7.4). Flow-cell 2 of a CM5 chip was activated with a 1:1 mixture of 50 mM NHS and 200 mM EDC, then immobilized with PTGS2 diluted in 10 mM acetate 4.0. Remaining reactive groups were blocked with 1 M ethanolamine. The chip was equilibrated at 30 μ l/min. Serially diluted small molecules (100–0.78 μ M) were injected at 30 μ l/min with association and dissociation phases monitored for 90 s and 420 s, respectively. Following each cycle, the chip surface was regenerated with 10 mM glycine-HCl (pH 4.5) for 90 s. Kinetic parameters (K_a , K_d , and K_D) were collected from sensorgrams using Biacore T200 evaluation software (version 3.2).

Statistical analyses

Statistical analyses were performed using the GraphPad Prism software (version 8.0; San Diego, CA, USA). Student's *t*-test or One-way ANOVA, followed by Newman-Keuls multiple comparison tests, were performed for statistical analysis. A *p*-value < 0.05 was considered statistically significant.

Results

SJT promotes recovery of muscle strength and motor function in tibial fracture rats

The mid-shaft tibial fracture model in rats was successfully established and verified through a series of behavioral assessments to evaluate the efficacy of SJT in promoting the early-mid fracture healing. In terms of body weight, compared to the control rats, the body weight of the tibial fracture rats increased significantly on days 8 and 15, which were reduced by the SJT-M intervention (Fig. 1A). Swelling degree analysis showed that the diameter of the affected limb in the MOD group increased notably. The swelling gradually subsided over the experimental period and became non-significant by day 9. SJT treatment significantly reduced limb swelling on days 18 and 21 (Fig. 1B). Mechanical pain threshold, inclined plane angle, and hind limb weight-bearing difference all demonstrated significant improvements in the SJT-treated groups compared with the MOD group (Fig. 1C-E). Specifically, SJT administration resulted in a higher paw withdrawal threshold, an increased inclined plane angle and a reduced hind limb gravity difference. Further gait analysis revealed that compared to the MOD group, SJT administration significantly increased foot intensity, stand time, swing speed, print area, and duty cycle, while markedly decreasing swing time (Fig. 1G-L). These changes indicated substantial improvements in limb function and gait parameters. Moreover, there were no significant differences in thymus, kidney, spleen, and liver indices between SJT-treated and tibial fracture rats (Fig. 1F), suggesting that SJT treatment did not cause adverse effects on these visceral organs. In addition, serum biochemical parameters, including alanine aminotransferase—ALT, aspartate aminotransferase—AST, blood urea nitrogen—BUN, and creatinine, showed no significant differences between SJT-treated and control group (Fig. S2). These findings indicate that SJT, at the administered dosage, did not induce detectable hepatic or renal toxicity.

SJT ameliorates tibial fracture-induced bone structural damage and enhances bone quality in rats

To evaluate the effect of SJT on the early-mid fracture healing, X-ray scanning was carried out to observe the closure of the fracture line and the formation of callus in fracture rats. In contrast to the control group, rats in the model group exhibited minimal new bone callus at the fracture site (Fig. 2A). Their mRUST scores were significantly lower initially but gradually increased (Fig. 2B), indicating severe bone damage at the fracture site and ongoing self-healing activity. With the SJT intervention, more new bone callus was formed and the fracture line gradually became less distinct over time. In addition, the micro-CT was employed to examine the microstructure changes of the tibia, aiming to evaluate the quality and strength of the bone repair. In model rats, the fracture line in the tibia was widened, accompanied by extensive structural damage and substantial bone loss at the fracture site (Fig. 2C-E). Consistent with these observations, quantified data demonstrated that in the tibiae of fractured rats, bone mineral density (BMD), bone volume fraction (BV/TV), and trabecular thickness (Tb.Th) were significantly decreased, while the structure model index (SMI) and bone surface to bone volume ratio (BS/BV) was markedly increased (Fig. 2F-I and Supplementary Figure S3A). These alterations were reversed by medium and high doses of SJT, with pharmacological effects comparable to those of JQC. Overall, the microscopic findings confirmed that SJT effectively expedited the early-mid fracture healing by alleviating microstructural destruction and bone loss and improving bone quality.

SJT promotes bone formation and inhibits bone resorption at the fracture site

In the model group of tibial fracture rats, HE staining showed that the

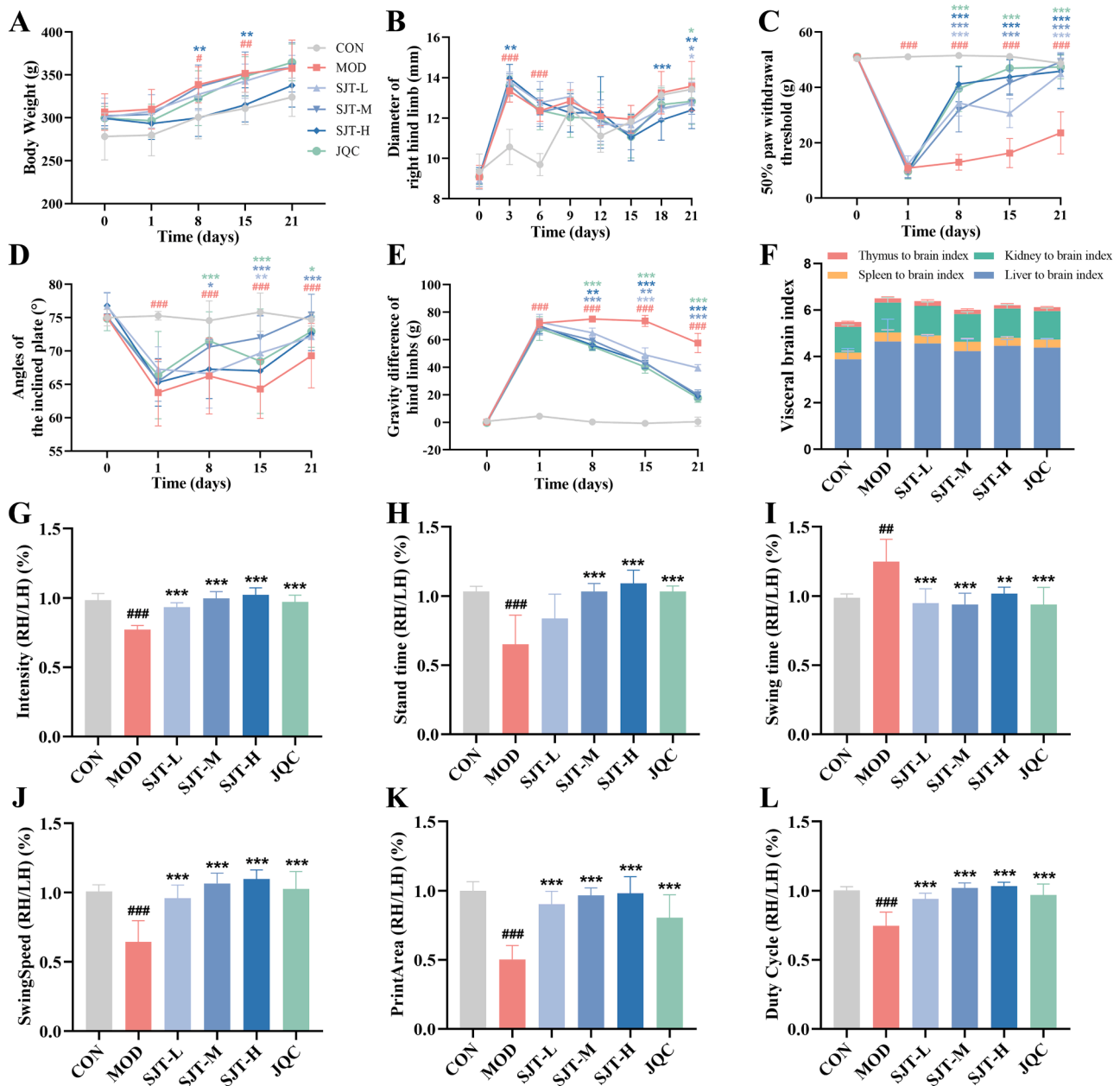


Fig. 1. SJT promotes recovery of muscle strength and motor function in tibial fracture rats. (A) Changes in body weight of rats in each group on days 1, 8, 15, and 21, $n = 9$. (B) Degree of swelling, $n = 9$. (C) Mechanical pain threshold, $n = 9$. (D) Inclined plane test, $n = 9$. (E) Hindlimb weight-bearing difference, $n = 9$. (F) Organ indices (organ-to-brain mass ratio) of rats in each group, including thymus-to-brain, kidney-to-brain, spleen-to-brain, and liver-to-brain indices, $n = 9$. (G-L) Gait analysis parameters of rats in each group, including intensity, standing time, swing time, swing speed, footprint area and duty cycle, $n = 6$. Data are expressed as the mean $\pm$ SD. Compared with the CON group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$; compared with the MOD group, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

newly formed bone callus in the fracture-healing tissue failed to completely fill the fracture gap. However, this was remarkably rectified by SJT treatment (Fig. 3A). Masson staining further demonstrated that the early-mid fracture healing was impaired in the model group, manifested as an overabundance of collagen fibers (blue-stained areas) and restricted bone matrix deposition (red-stained areas) (Fig. 3A, B). In contrast, SJT intervention significantly stimulated osteogenesis, leading to the formation of mature bone tissue. To evaluate the role of osteoclasts in the early-mid fracture healing with or without SJT, TRAP staining was carried out. Compared to the control group, the tibial fracture rats exhibited a substantial increase in TRAP-positive regions within the fracture-healing tissue (Fig. 3A, C). This increase was reversed by SJT administration, suggesting that SJT diminishes bone resorption activity at the fracture site. ELISA analysis indicated that,

compared to control rats, tibial fracture rats had significantly lower serum levels of osteogenic factors such as BALP, BGP, TGF- β , BMP-2, and PINP (Fig. 3D-H). Conversely, they had higher levels of bone resorption factors, including TRACP-5b and β -CTX (Fig. 3I-J). SJT treatment demonstrated its pro-osteogenic and anti-resorptive potential by restoring these biomarker levels to near-normal values.

Collectively, these findings suggested that SJT may enhance the early-mid fracture healing by promoting neocallus mineralization and maturation, upregulating osteogenic mediators and reducing the osteoclast-mediated bone resorption.

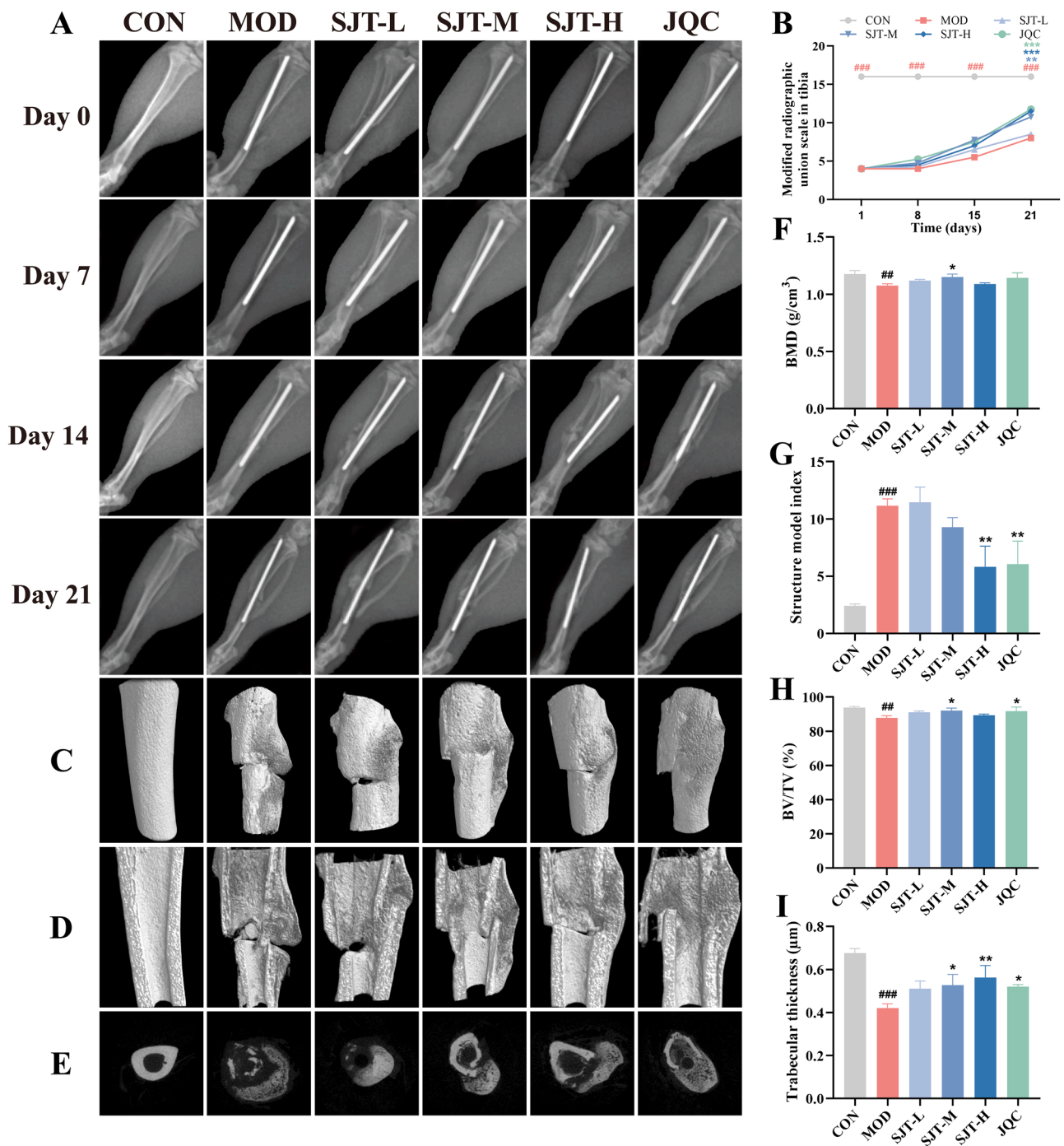


Fig. 2. Effects of SJT on tibial morphometry in tibial fracture rats. (A) X-ray scan results of fractured tibiae on days 0, 7, 14, and 21 for each group. (B) Changes in mRUST scores of fractured tibiae based on X-ray scans, $n = 4$. (C-E) Representative micro-CT scan images of tibiae in each group, $n = 3$. (F-I) Analysis of bone mineral density (BMD), structure model index (SMI), bone volume fraction (BV/TV), and trabecular thickness (Tb.Th) based on micro-CT scan results, $n = 3$. Data are expressed as the mean $\pm$ SD. Compared with the CON group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; compared with the MOD group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

SJT promotes angiogenesis-osteogenesis coupling and suppresses inflammatory responses in the fracture region

Angiogenesis is pivotal in bone fracture healing. Blood vessels not only supply essential nutrients and oxygen but also stimulate new blood vessel formation in the healing area, thereby supporting bone repair and regeneration. Immunohistochemical analysis revealed that CD31 expression in the callus of the model group was lower compared to the control group. In contrast, SJT administration led to a remarkable

upregulation of CD31 expression (Fig. 4A-B). Subsequently, PCR and ELISA assays demonstrated that SJT intervention significantly increased VEGF levels in both the callus and serum of fracture-affected rats (Fig. 4C-D). These results clearly indicated that SJT could effectively enhance angiogenesis at the fracture site. Additionally, SJT exerted an inhibitory effect on the inflammatory response by downregulating the expression of IL-6 and IL-1 β in the serum of fracture rats (Fig. 4E-F). Given that SJT facilitates fracture healing by regulating vascular development and bone homeostasis, we further investigated the

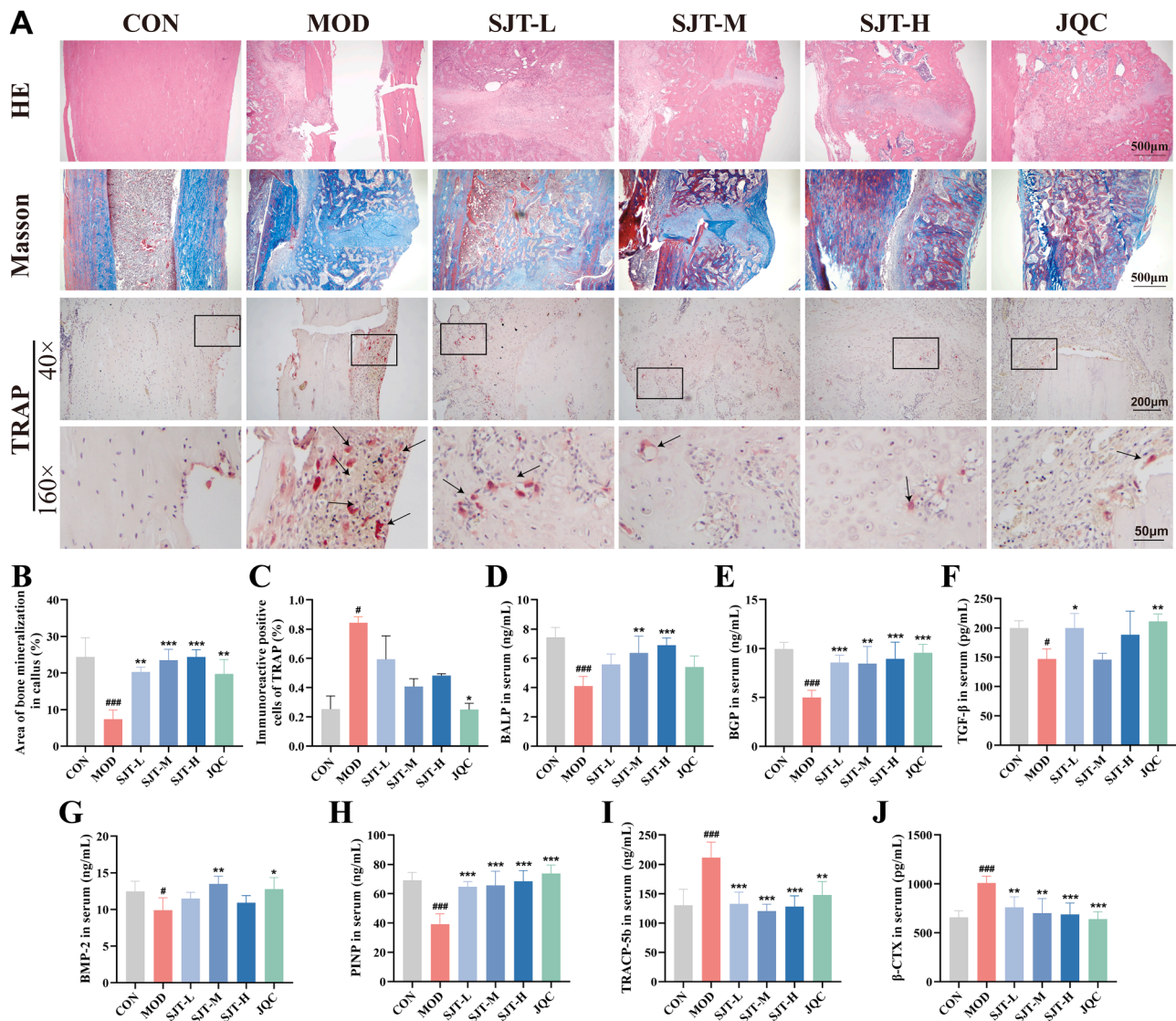


Fig. 3. SJT promotes bone formation and reduces bone resorption at the fracture site. (A) Representative images of HE staining, Masson staining, and TRAP staining in callus tissues of rats in each group, $n = 3$. (B) Quantitative analysis of mature bone tissue area based on Masson staining, $n = 3$. (C) Quantitative analysis of TRAP-positive area in callus tissues, $n = 3$. (D-H) Expression of osteogenesis-related factors, including BALP, BGP, TGF- β , BMP-2, and PINP, in serum of rats in each group detected by ELISA, $n = 5$. (I-J) Expression of TRACP-5b and β -CTX in serum of rats in each group detected by ELISA, $n = 5$. Data are expressed as the mean $\pm$ SD. Compared with the CON group, # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$; compared with the MOD group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

correlations between angiogenesis-related markers and bone metabolism indicators. The results showed that the angiogenesis markers CD31 and VEGF were positively correlated with bone formation markers such as BALP, BGP, BMP-2, and PINP (Fig. 4G-H). Conversely, negative correlations were observed between angiogenesis markers and bone resorption markers, namely TRACP-5b and β -CTX (Fig. 4I). Collectively, SJT promoted the early-mid fracture healing by maintaining the equilibrium of angiogenesis-osteogenesis coupling and suppressing inflammatory responses, thus fostering bone healing and regeneration.

Chemical profiling and putative target identification for SJT

A comprehensive HPLC-QTOF-MS analysis identified a total of 180 chemical constituents in SJT (Fig. 5A). These constituents encompassed 37 saponins, 26 flavonoids, 23 alkaloids, 20 terpenoids, 16 amino acids, 15 phenolic acids, 12 steroids, and 31 constituents belonging to other diverse categories (Fig. 5B). Through drug-likeness evaluation and ADMET (absorption, distribution, metabolism, excretion, and toxicity) profiling, 34 candidate bioactive constituents were carefully screened,

as shown in Fig. 5C. These selected constituents exhibited favorable physicochemical properties, desirable pharmacokinetic profiles, and were predicted to possess low toxicity, making them potential key players in SJT's pharmacological actions. In addition, a total of 224 putative targets of SJT were predicted using the TCMIP v2.0 platform for further mechanism investigation. The detail information is attached in **Supplementary Tables S1 and S2**.

SJT promotes angiogenesis-osteogenesis coupling via modulation of the PTGS2-PGE2/EP4-CXCL12/CXCR4 signaling axis

The interaction network was constructed using the gene-gene interaction data among fracture-related genes and SJT putative targets. Topological analysis of this network revealed 28 hub nodes, with PTGS2 demonstrating the highest degree of centrality (Fig. 6A). This finding implies that PTGS2 may play a crucial regulatory role within the network. Functional enrichment analysis showed that these key targets were significantly enriched in various pathways that regulate immune-inflammatory responses and osteoclast differentiation (Fig. 6B).

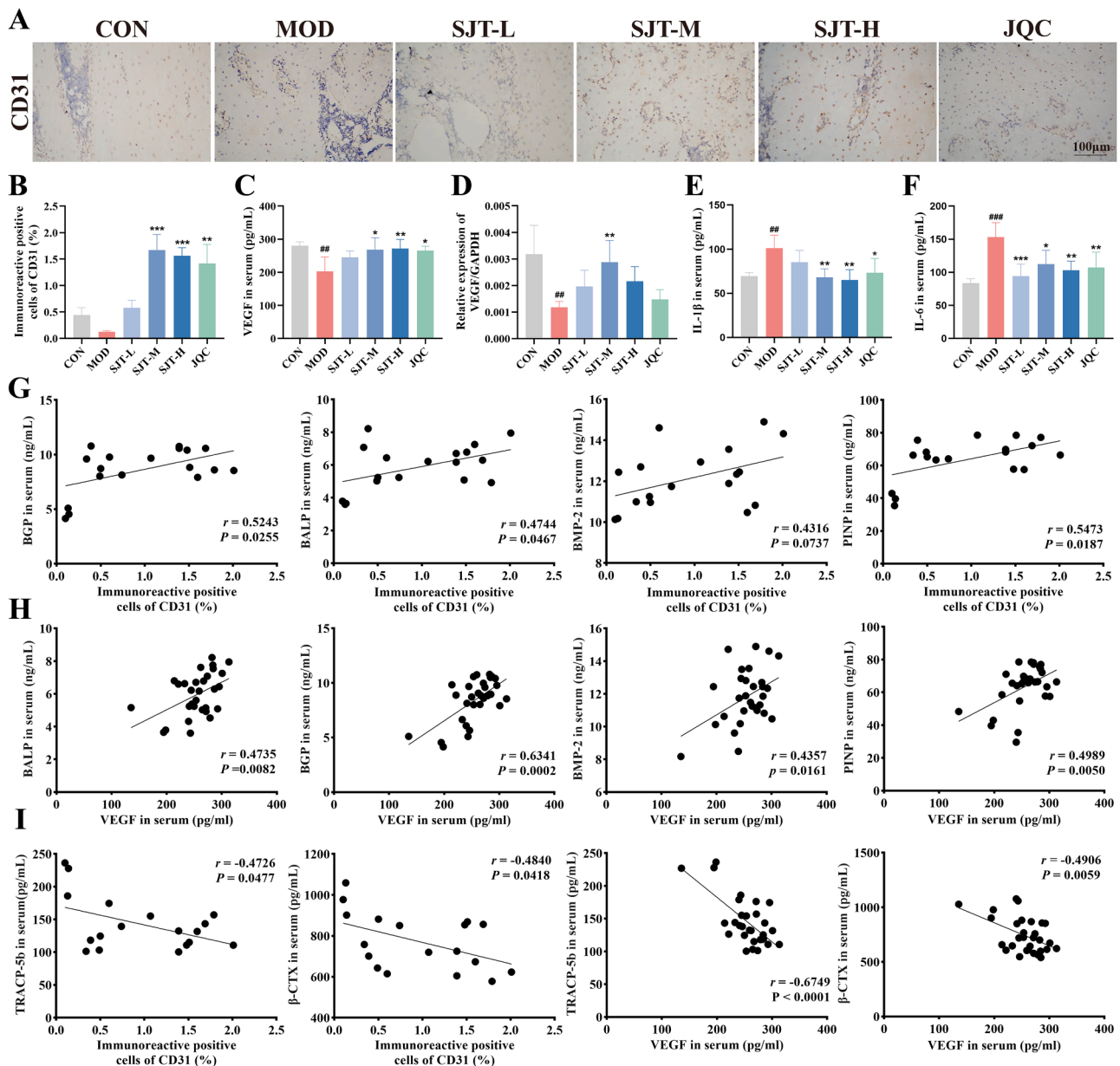


Fig. 4. SJT promotes angiogenesis-osteogenesis coupling and inhibits inflammatory responses in the fracture region. (A) Representative images of immunohistochemical staining showing CD31 protein expression in callus tissues of rats in each group, $n = 3$. (B) Quantitative analysis of CD31 expression in callus tissues of rats in each group, $n = 3$. (C) Expression of VEGF in serum of rats in each group detected by ELISA, $n = 3$. (D) The mRNA expression levels of VEGF in callus tissues of rats in each group detected by qPCR, $n = 5$. (E-F) Expression of IL-6 and IL-1 β in serum of rats in each group detected by ELISA, $n = 3$. (G-H) Correlation analysis between angiogenesis markers (CD31, VEGF) and osteogenesis-related markers (BALP, DGP, BMP-2, PINP). (I) Correlation analysis between angiogenesis markers (CD31, VEGF) and bone resorption markers (TRACP-5b, β -CTX). Data are expressed as the $mean \pm SD$. Compared with the CON group, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; compared with the MOD group, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Recent studies have demonstrated that the enzyme PTGS2 may catalyze the conversion of arachidonic acid to PGE₂, a lipid mediator that binds to the EP4 receptor and activates downstream signaling cascades. These cascades control a number of processes, including osteogenesis, tissue regeneration and bone repair (Chen et al., 2019; Minamizaki et al., 2009). Notably, pharmacological modulation of the PTGS2-PGE₂/EP4 axis can enhance fracture repair by regulating osteoblast differentiation, angiogenesis, and inflammatory response (Liu et al., 2024). Based on the findings, we hypothesized that SJT may accelerate the early-mid fracture healing through targeting the PTGS2-PGE₂/EP4 signaling pathway, thereby inhibiting inflammatory progression and enhancing angiogenesis-osteogenesis coupling.

Immunohistochemical staining and ELISA analysis demonstrated

that tibial fractures led to an upregulation of PTGS2, PGE₂, and EP4 in the callus. the administration of SJT led to a further augmentation in these levels (Fig. 6C-G). Moreover, SJT administration significantly increased the elevated mRNA expression of CXCL12 and CXCR4 in the callus of fractured rats (Fig. 6H-I). Previous studies (Liu et al., 2013; Song et al., 2018) have shown that CXCL12 and CXCR4 can drive angiogenesis and osteogenesis. This suggests that the PTGS2-PGE₂/EP4 signaling pathway may promote bone formation and vascular remodeling by activating the CXCL12/CXCR4 axis. Collectively, these results imply that SJT may enhance the early-mid fracture repair by regulating the PTGS2-PGE₂/EP4-CXCL12/CXCR4 axis.

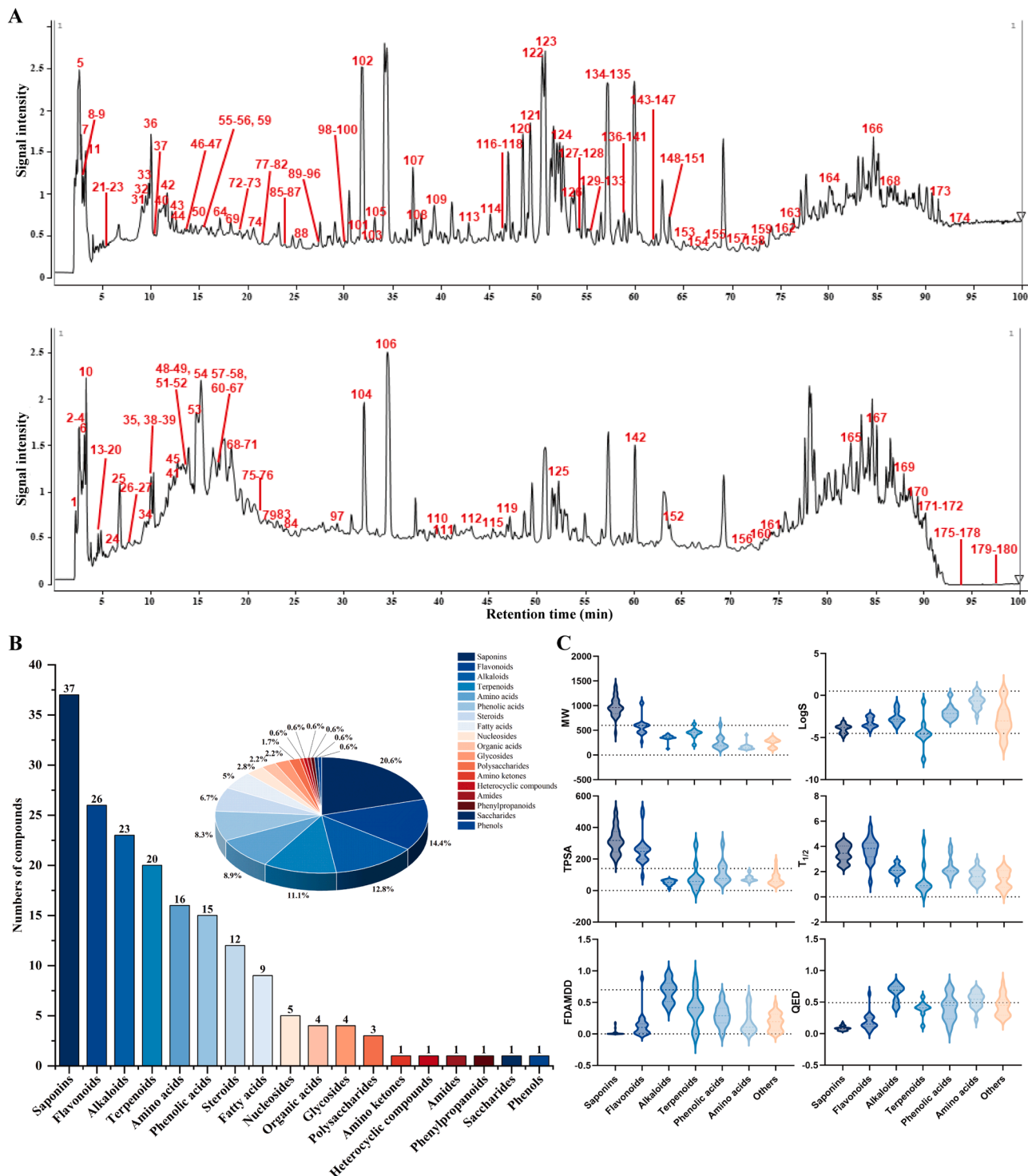


Fig. 5. Chemical profiling and candidate bioactive constituent identification for SJT. (A) Total ion chromatogram of the extract of SJT acquired in negative and positive ion mode using HPLC-QTOF-MS analysis. (B) Classification of chemical constituents identified in SJT. (C). ADMET properties distribution of the identified constituents of SJT across different chemical classes. Horizontal dashed lines represent screening thresholds for candidate bioactive constituents: Mw (100~600), logS (-4.5~0.5 log mol/L), TPSA (0~140), $T_{1/2}$ (>0), QED (> 0.49), and FDAMMD (0~0.7).

The candidate bioactive constituents of SJT exhibit direct binding to PTGS2 protein

To further identify putative active constituents of SJT in regulating the PTGS2-PGE2/EP4 axis, molecular docking and SPR assays were performed. Molecular docking revealed strong binding affinities between PTGS2 and representative SJT constituents, with binding energies

of -7.5 kcal/mol for ferulic acid (MaQianZi and HongHua, Fig. 7A), -7.1 kcal/mol for β -elemene (SanQi, RuXiang, MoYao, Fig. 7B), -9.6 kcal/mol for brucine (derived from MaQianZi, Fig. 7C), -9.4 kcal/mol for apigenin (HongHua, Fig. 7D) and -7.8 kcal/mol for trans-3-indoleacrylic acid (TuBieChong, Fig. 7E), indicating favorable interaction potentials.

SPR experiments validated direct binding of these constituents to

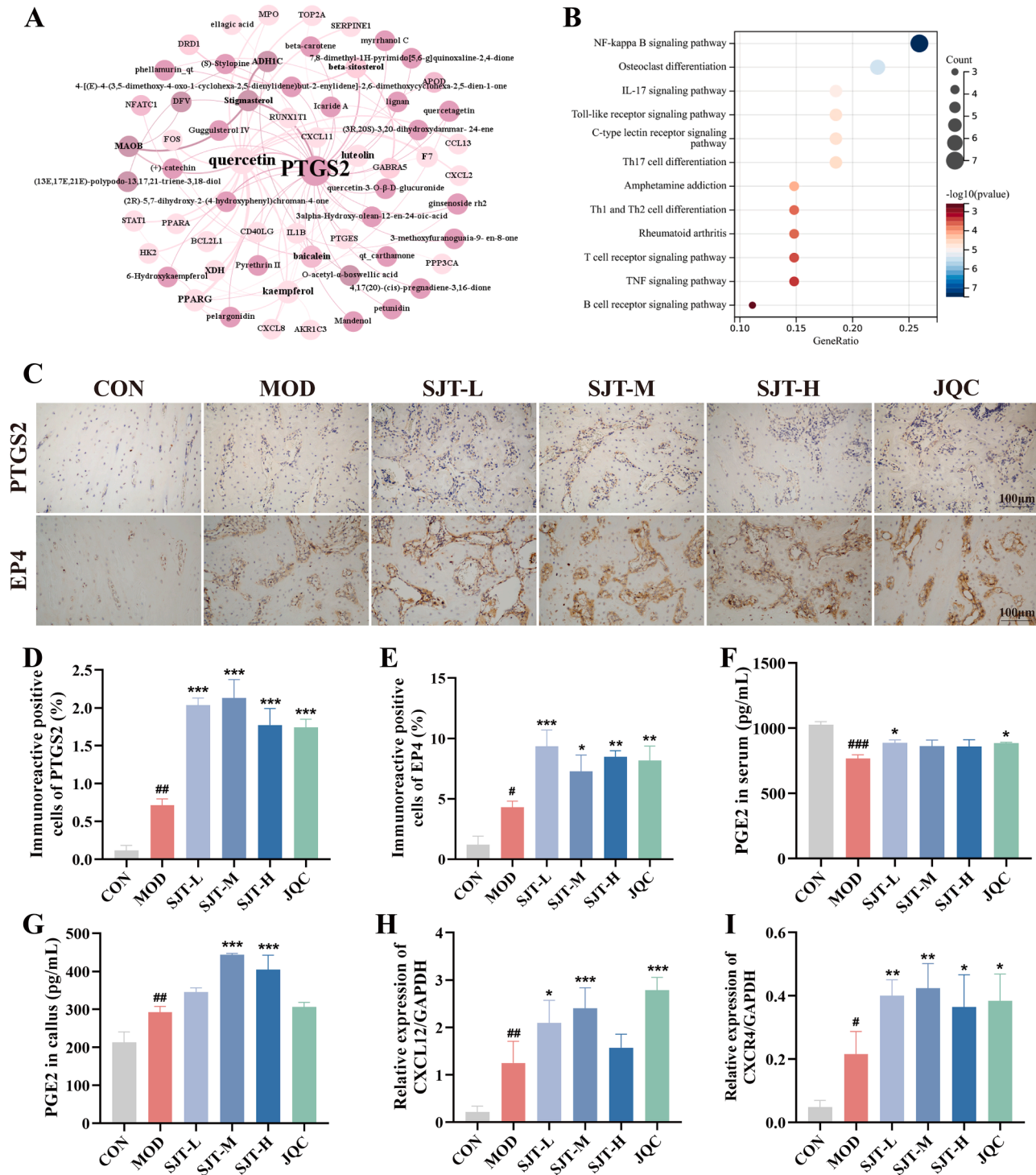


Fig. 6. SJT activates the PTGS2-PGE2/EP4-CXCL12/CXCR4 signaling axis in fracture rats. (A) Constituent-target interaction network of SJT in the treatment of bone fractures was constructed using Cytoscape. (B) KEGG pathway enrichment analysis of the potential therapeutic pathways associated with SJT in bone fractures treatment. (C) Representative images of immunohistochemical staining showing the expression of PTGS2 and EP4 proteins in callus tissues of rats in each group, $n = 3$. (D-E) Quantitative analysis of PTGS2 and EP4 protein expression in callus tissues detected by immunohistochemistry, $n = 3$. (F-G) Expression of PGE2 in the serum and callus tissues of rats in each group detected by ELISA, $n = 3$. (H-I) The mRNA expression levels of CXCL12 and CXCR4 in callus tissues of rats in each group detected by qPCR, $n = 5$. Data are expressed as the $mean \pm SD$. Compared with the CON group, $^{\#}p < 0.05$, $^{##}p < 0.01$, $^{###}p < 0.001$; compared with the MOD group, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

PTGS2, with dissociation constants (K_D) of 5.448 μM for ferulic acid, 8.096 μM for β -elemene, 11.55 μM for brucine, 29.88 μM for apigenin, and 41.51 μM for trans-3-indoleacrylic acid (Fig. 7A-E). Notably, ferulic acid exhibited the highest binding potency, followed by β -elemene and brucine, while apigenin and trans-3-indoleacrylic acid showed moderate

affinity. These results collectively demonstrate that SJT's bioactive constituents interact directly with PTGS2.

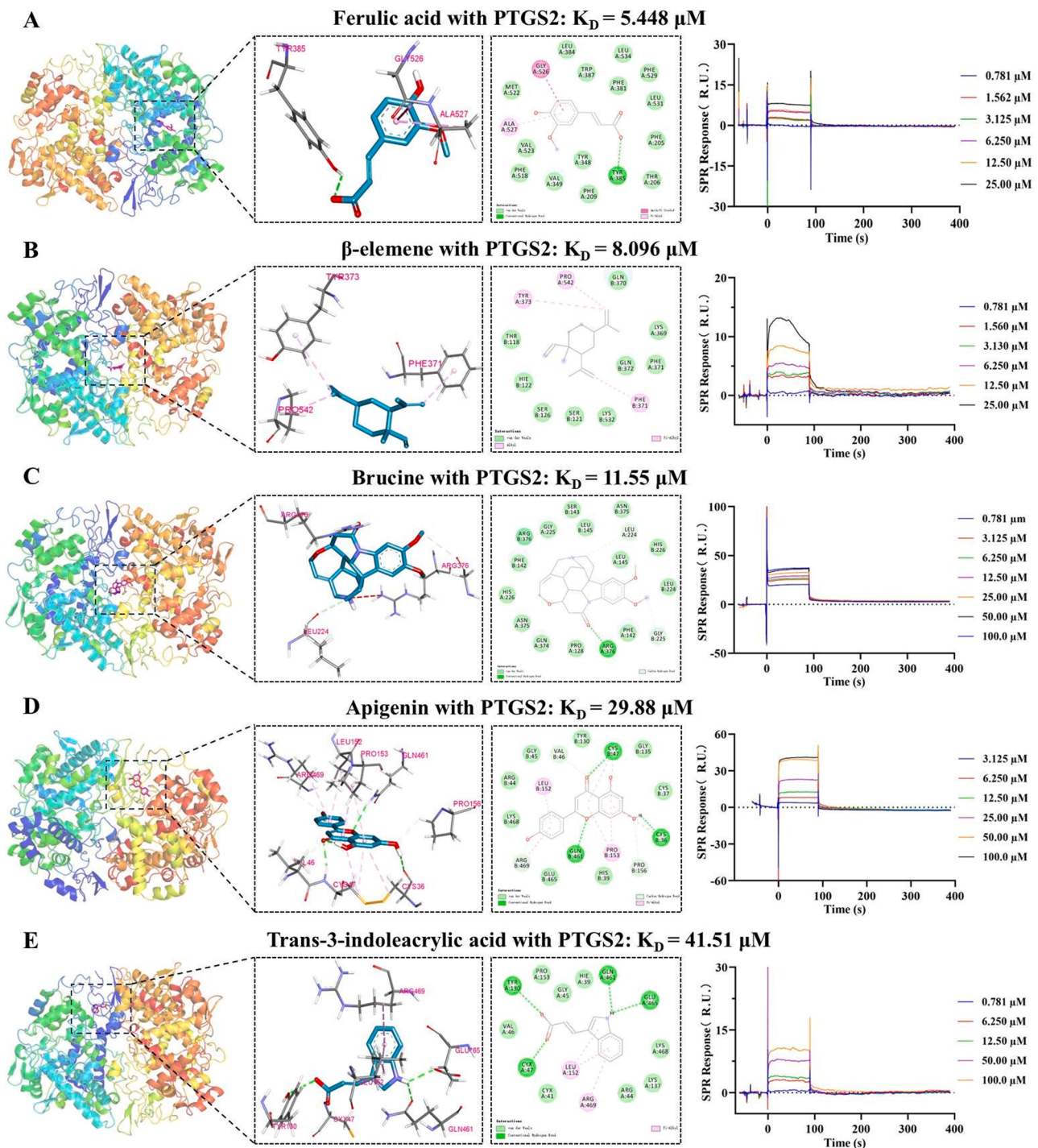


Fig. 7. Molecular docking of representative SJT constituents with PTGS2 and their biophysical validation by SPR analysis. (A) Ferulic acid, (B) β -elemene, (C) Brucine, (D) Apigenin, and (E) Trans-3-indoleacrylic acid were docked into the active site of human PTGS2 (PDB ID: 5F19) and the key interacting residues are labelled. SPR experiment indicated that these constituents bind directly to PTGS2, with K_D values of 5.448 μM , 8.096 μM , 11.55 μM , 29.88 μM , and 41.51 μM , respectively.

Discussion

The fracture healing is a tightly coordinated process that relies on the spatiotemporal coupling of angiogenesis and osteogenesis (Ballesteros et al., 2024; Chen et al., 2021; Li et al., 2025; Saran et al., 2014). This interdependence ensures adequate vascular supply to support the metabolic demands of bone-forming cells and facilitates the removal of debris during fracture healing. The positive correlation between angiogenic markers such as VEGF and CD31 and bone formation

indicators, as observed in our study, underscores the critical role of this coupling in successful fracture repair. Any disruption in this delicate balance can lead to impaired healing (Grosso et al., 2017), emphasizing the significance of understanding and promoting angiogenesis-osteogenesis coupling in the context of fracture treatment.

SJT has emerged as a promising therapeutic option for fracture healing (Cheng et al., 2023; Xujia et al., 2022). Our results have clearly demonstrated its efficacy in promoting fracture repair in a rat tibial fracture model. Through radiological and behavioral assessments,

SJT-treated rats demonstrated accelerated restoration of bone integrity and limb function, accompanied by reduced inflammation and improved bone homeostasis. Notably, SJT's therapeutic superiority lies in its ability to amplify angiogenesis-osteogenesis coupling, as evidenced by the concurrent upregulation of VEGF, CD31, and osteogenic markers at fracture sites. This dual modulation distinguishes SJT from conventional therapies that often target isolated aspects of bone repair (Alsharef et al., 2023; Picelli et al., 2024; Yang et al., 2023), highlighting its holistic advantage in fracture management.

Although excessive or prolonged inflammation can impair fracture healing, an appropriate and transient inflammatory response is essential to initiate the repair process. PTGS2 is rapidly induced in osteoblasts after fracture and, beyond its pro-inflammatory role, has been shown to promote angiogenesis and support early tissue regeneration. In this context, the present study identified the PTGS2-PGE2/EP4-CXCL12/CXCR4 axis as the mechanistic linchpin underlying SJT's pro-regenerative effects. PTGS2-derived PGE2 has been established as a master regulator of bone repair, promoting both osteoblast differentiation and angiogenesis through EP4 receptor activation (Chen et al., 2019; Cheng et al., 2021; Einhorn, 2003; Minamizaki et al., 2009). EP4 are predominantly expressed in osteoblasts, osteoclasts, and chondrocyte during fracture development, with PGE2 acting on these cells via EP4 to promote osteogenic differentiation and bone healing (Claes et al., 2012; Jiang et al., 2022). Our results aligned with previous reports, demonstrating that genetic ablation of PTGS2 or EP4 impairs fracture healing, while exogenous PGE2 supplementation can rescue the bone regeneration (Hu et al., 2020a; Zhang et al., 2002). SJT treatment robustly elevated PTGS2, PGE2, and EP4 expression, suggesting its capacity to amplify endogenous repair signals. Furthermore, SJT upregulated CXCL12/CXCR4, a chemokine axis modulated by the PTGS2/PGE2/EP4 axis via cAMP-PKA signaling, which facilitates vascular remodeling and tissue regeneration (Kitase et al., 2010; Obermajer et al., 2011). The observed interdependence between PTGS2-PGE2/EP4 activation and CXCL12/CXCR4 signaling underscores SJT's multitargeted approach—stimulating PGE2-mediated osteogenesis while coordinating CXCL12-driven vascular niche development. This dual-axis regulation provides a molecular rationale for SJT's unique efficacy in synchronizing angiogenesis and osteogenesis during the

early-mid fracture repair (Fig. 8).

To further delineate SJT's bioactive constituents, we identified representative constituents (e.g., ferulic acid, beta-elemene, brucine, apigenin, and trans-3-indoleacrylic acid) that directly interact with PTGS2. Molecular docking and SPR analyses confirmed their high-affinity binding to PTGS2, validating their role as pharmacologically active agents. Specifically, ferulic acid suppresses PTGS2 enzymatic activity, thereby inhibiting inflammatory responses and angiogenesis (Bao et al., 2019; Ekowati et al., 2020). It also attenuates osteoclast differentiation through blocking the RANKL-dependent NF- κ B signaling pathway (Doss et al., 2018). The ability of beta-elemene and apigenin to significantly lower COX-2 expression enhances to their anti-inflammatory qualities (Hu et al., 2020b; Patil et al., 2016; Patra et al., 2016). Additionally, brucine suppresses COX-2 gene expression, which contributes to its pro-apoptotic effect in pathological situations (Deng et al., 2006). These constituents likely act synergistically to stabilize PTGS2 activity, thereby sustaining PGE2 production and downstream EP4 signaling. This not only provides insights into the molecular mechanisms of SJT but also paves the way for future research on developing more targeted and effective therapies for fracture healing.

However, this study has several limitations. Although we have identified the PTGS2-PGE2/EP4 axis as a crucial signaling pathway, the specific cellular origins of these targets remain to be definitively determined. Furthermore, the causal relationship between PTGS2/EP4 activation and subsequent CXCL12/CXCR4 upregulation, while supported by our *in vivo* data and literature, requires direct experimental validation. Future studies will employ cell-type-specific isolation and culture, combined with pharmacological inhibition and genetic approaches, to precisely elucidate the cellular targets and downstream signaling events. These investigations will be essential to fully decipher the mechanistic basis of SJT's pro-healing effects.

In conclusion, our findings position SJT as a promising candidate for enhancing the early-mid fracture healing through angiogenesis-osteogenesis coupling. By modulating the PTGS2-PGE2/EP4-CXCL12/CXCR4 axis, SJT not only addresses the biomechanical demands of bone repair but also rectifies the dysregulated inflammatory and metabolic microenvironment. The identification of PTGS2-targeting constituents further bridges ethnopharmacological knowledge with modern

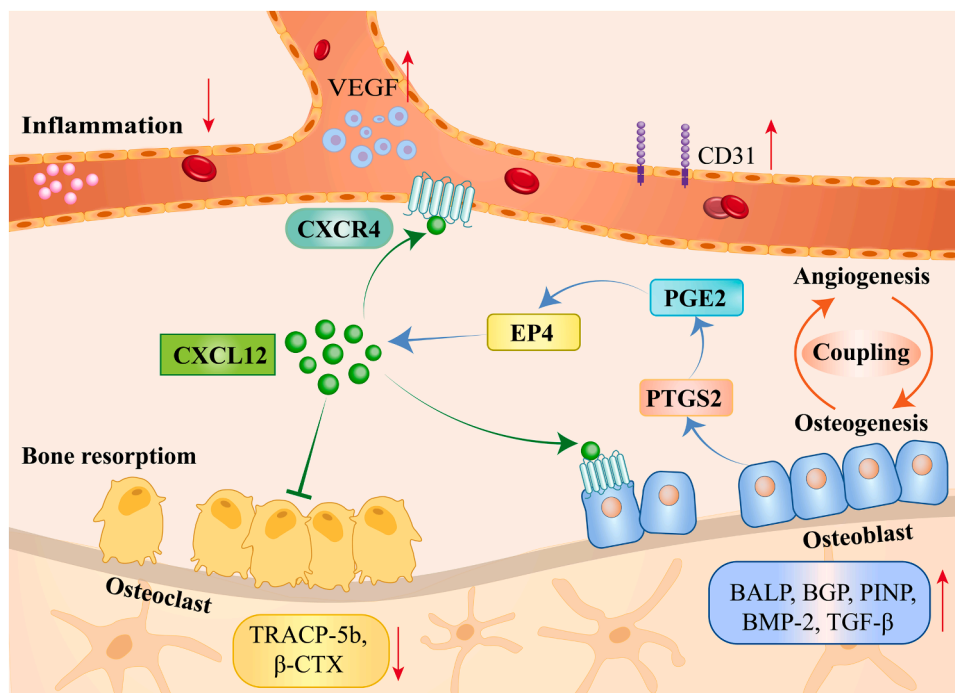


Fig. 8. Illustration of the PTGS2-PGE2/EP4-CXCL12/CXCR4 axis related to the synergistic efficacy of SJT against the early-mid bone fractures.

drug discovery. These insights reinforce SJT's clinical relevance and provide a template for developing novel bone-healing therapies grounded in the integration of angiogenesis and osteogenesis regulation.

Data availability

Data will be made available on request.

Ethics statement

All experimental procedures strictly adhered to the Guidelines for the Care and Use of Laboratory Animals provided by the Experimental Animal Welfare Committee of the China Academy of Chinese Medical Sciences (Ethics Approval No.: IBTCMCACMS21–2402–02).

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Consent for publication

All authors have provided their consent for publication.

Compliance and ethics

The authors declare that there is no conflict of interests regarding the publication of this paper.

CRediT authorship contribution statement

Lin Chen: Writing – review & editing, Validation, Methodology, Investigation. **Chu Zhang:** Validation, Methodology, Investigation. **Xiaojie Li:** Writing – original draft, Validation, Investigation. **Jingyuan Zhang:** Data curation. **Jie Zou:** Investigation. **Suya Zhang:** Investigation. **Jing Zhang:** Investigation. **Xueting Liu:** Investigation. **Yudong Liu:** Methodology. **Zhaochen Ma:** Data curation. **Ying Liu:** Supervision. **Na Lin:** Writing – review & editing, Project administration, Funding acquisition. **Jigang Wang:** Supervision, Resources. **Yanqiong Zhang:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.phymed.2025.157382](https://doi.org/10.1016/j.phymed.2025.157382).

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