Acetyl-11-keto-beta-boswellic acid promotes sciatic nerve repair after injury: molecular mechanism

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Abstract
Previous studies showed that acetyl-11-keto-beta-boswellic acid (AKBA), the active ingredient in the natural Chinese medicine Boswellia, can stimulate sciatic nerve injury repair via promoting Schwann cell proliferation. However, the underlying molecular mechanism remains poorly understood. In this study, we performed genomic sequencing in a rat model of sciatic nerve crush injury after gastric AKBA administration for 30 days. We found that the phagosome pathway was related to AKBA treatment, and brain-derived neurotrophic factor expression in the neurotrophic factor signaling pathway was also highly up-regulated. We further investigated gene and protein expression changes in the phagosome pathway and neurotrophic factor signaling pathway. Myeloperoxidase expression in the phagosome pathway was markedly decreased, and brain-derived neurotrophic factor, nerve growth factor, and nerve growth factor receptor expression levels in the neurotrophic factor signaling pathway were greatly increased. Additionally, expression levels of the inflammatory factors CD68, interleukin-1β, pro-interleukin-1β, and tumor necrosis factor-α were also decreased. Myelin basic protein- and β3-tubulin-positive expression as well as the axon diameter-to-total nerve diameter ratio in the injured sciatic nerve were also increased. These findings suggest that, at the molecular level, AKBA can increase neurotrophic factor expression through inhibiting myeloperoxidase expression and reducing inflammatory reactions, which could promote myelin sheath and axon regeneration in the injured sciatic nerve.

Key Words: AKBA; axon; genomics; inflammatory; injury and repair; myelin sheath; myeloperoxidase; neurotrophic factor; peripheral nerve; phagosome pathway; regeneration; Sprague-Dawley rat

Introduction Peripheral nerve injury (PNI) is caused by mechanical, physical, chemical, or biological factors, and it results in partial or complete peripheral nerve loss, motor function impairment, and neuropathic pain (Vijayavenkataraman, 2020). A series of pathological changes occurs after PNI. Proximal neurons and ganglia may change in addition to the damaged part and its distal area. The inflammatory response to phagocyte recruitment occurs first after PNI. A phagosome is formed when the specific receptors on the phagocyte surface recognize ligands on the particle surface. The main participant in this process is myeloperoxidase (MPO). Changes in MPO levels and activity represent the presence of immature neuron cell bodies, dendrites, axons, and axon ends, which thereby indicates the drug’s capability to regenerate axons (Li et al., 2020). Acetyl-11-keto-beta-boswellic acid (AKBA) is a natural small-molecule compound that is extracted from the natural Chinese medicine Boswellia. AKBA is widely used to inhibit inflammation (Abdel-Tawab et al., 2011; Meka et al., 2017; Khan et al., 2019) and the nuclear factor-kappa B signaling pathway (Takada et al., 2006; Ranzato et al., 2017). Its therapeutic effect on nerves can promote nerve damage repair by reducing oxidative stress (Sadeghnia et al., 2017) and protecting ischemic neurons (Ding et al., 2014). Our team members’ previous results showed that Boswellia serrata extract and AKBA can promote the repair of rat sciatic nerve injury by regulating Schwann cells and the extracellular regulated protein kinases signaling pathway (Jiang et al., 2016, 2018, 2020). However, peripheral nerve regeneration is a complex pathophysiological process that involves numerous changes at different levels, from molecules and cells to biological organisms, and it is affected by many factors. Therefore, we used high-throughput genomics and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis to explore the mechanisms associated with the repair of sciatic nerve damage and to elucidate the mechanisms related to AKBA that promote peripheral regeneration. 

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nerve damage and repair. In this article, we show that AKBA acts on the repair of sciatic nerve injury mainly through the phagosome pathway and that the phagosome is closely related to anti-inflammation, thus further demonstrating the anti-inflammatory effect of AKBA; and we also discuss the ability of AKBA to promote myelin and axons by increasing the neurotrophic factor expression level. This study could represent a new area or a new type of investigation using AKBA and traditional Chinese medicine.

Materials and Methods

Animals

Because estrogen in female rats may affect test results (Acosta et al., 2017), we uniformly used male rats to avoid affecting the test result accuracy. Fifty-five male Sprague-Dawley rats (provided by Harbin Medical University, China) were housed in the Animal House of the Neurosurgery Department of Nanjing Medical University (Nanjing, China). The rats were 4–6 months old and weighed 240–300 g. Prior to surgery, the rats were starved for 12 h and allowed access to water ad libitum. The experiment was conducted in compliance with the requirements of the National Research Council Guide (1996) and the Animal Welfare Committee of Heilongjiang Province, China (revised 2016). All experiments were designed and reported in accordance with the journal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines (Percie du Sert et al., 2020). The study was approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University (approval No. NEAU-[2018]-009; approval date: January 5, 2018).

Sciatic nerve injury model

Ten rats were randomly selected as the blank (control) group (without any surgical treatment). The remaining 45 rats were randomly and equally divided into the following three groups: Sham group (exposed nerves only); Model group (exposed nerves and a crush injury); and AKBA group (exposed nerves, crush injury, and AKBA).

The rats were anesthetized via an intraperitoneal injection of Zoletil 50 (zolazepam + tiletamine) (30 mg/kg; Virbac, Nice, France). Each rat’s right sciatic nerve was exposed surgically and in the Model and AKBA groups, the nerve was squeezed with vascular forceps (Shanghai Medical Devices (Group) Co., Ltd., Shanghai, China) to the second button to provide the same force and help to create the sciatic nerve injury model (Jiang et al., 2018; Remacle et al., 2018). Each nerve was marked with sutures near the distal end of the clamp, and the wound was closed layer by layer using sutures. In the sham group, only nerve exposure was performed without inflicting nerve damage.

Water and food were withheld for 24 hours after the model was created. Rats in the AKBA group received AKBA intragastrically (6 mg/kg; Shanghai Yuanye Biological Co., Ltd., Shanghai, China; dissolved in 1 ml normal saline) every 2 days for 30 days beginning on day 1 after surgery (Jiang et al., 2018). Each AKBA administration time was the same, and the Sham and Model group rats received 1 ml normal saline. The study timeline is shown in Figure 1.

Genomics detection in sciatic nerve

On day 30, the rats were anesthetized via intraperitoneal injection of Zoletil 50 (30 mg/kg) and sacrificed by cervical dislocation. To investigate the mechanism of AKBA in sciatic nerve injury repair, rats in the AKBA and Model groups underwent genomics analysis. Rats in the Sham group underwent necropsy analysis to exclude the effect of surgical stress. The four groups (n = 5/group) in the three groups were obtained surgically. After the samples were quick-frozen in liquid nitrogen, the genome was sequenced by Tianhao Biotechnology (Group) Co., Ltd. (Shanghai, China). Briefly, Trizol (Thermo Fisher, Waltham, MA, USA) was used for total RNA extraction. Invitrogen Qubit 3.0 Spectrophotometer (Thermo Fisher) was used to test the accuracy of RNA detection. A Nanodrop 2000 (Thermo Fisher) was used to detect the RNA concentration, and an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) was used to detect the degree of RNA degradation. The total RNA library was prepared in accordance with the TruSeq Stranded Total RNA Library Prep Kit instructions (Illumina, San Diego, CA, USA). The library was sequenced on the Illumina HiSeq 2500 (Illumina, San Diego, CA, USA) according to the manufacturer’s instructions. After sequencing, the Illumina RNA-Seq reads were analyzed using the Bioinformatics Analysis Platform for Bioclinical Research (Beckman Coulter, Carlsbad, CA, USA). The library was sequenced using the high-throughput sequencing platform (Illumina) with a 2 × 150 bp pair-end sequencing strategy.

The quality of raw sequencing data was evaluated using Fast QC software (Babraham Institute, Cambridge, UK) and R software (Free Software Foundation, Boston, MA, USA). The original sequence contained some low-quality reads with connectors. The raw reads were filtered using the Trim Galore method (Li et al., 2017) (https://ccb.jhu.edu/software/trim_galore/index.shtml) to obtain clean reads for subsequent analysis and to ensure the quality of information analysis. The clean reads obtained after filtering were compared with the reference database annotations (the Rn6 version of the rat genome was selected) using HISAT2 software (https://ccb.jhu.edu/software/hisat2/index.shtml). Differential expression analysis was performed using Cuffdiff software (University of Washington, Washington, WA, USA), and two criteria were used for screening differentially expressed genes. Specifically, a gene was considered to be differentially expressed when P < 0.05 and |log2fold change| > 1, where log2fold change = -1 was marked as an upregulated gene and log2fold change < -1 was marked as a downregulated gene.

Real-time polymerase chain reaction

Total RNA from the sciatic nerve in four groups (Blank, Sham, Model, and AKBA groups; n = 3/group) on day 30 after injury was extracted as previously described. Briefly, the extracted RNA can be used for reverse transcription at the ratio of A260/A280 between 1.8 and 2.0 as detected by NanoDrop 2000 (Thermo Fisher). Moreover, cDNA was synthesized using the HiScript III RT SuperMix for real-time polymerase chain reaction (RT-qPCR) (+gDNA wiper) kit (Novozan Biotechnology Co., Ltd., Nanjing, China). The target mRNA primers are shown in Table 1. The test was conducted in accordance with the PCR strategy that was provided in the 2× SYBR Green RT-qPCR Master Mix (Bimake, Houston, TX, USA) dye manual. RT-qPCR was performed using a Light Cycler® 480 System (Roche, Basel, Switzerland). PCR reaction conditions are shown in Table 2. The relative mRNA level was calculated using the 2-ΔΔCT method (Qianru et al., 2021). Glyceroldehyde-3-phosphate dehydrogenase was used as an endogenous control for standardization.

Table 1 Primer sequence list

| Genes     | Primer sequence |
|-----------|-----------------|
| NGFR      | Forward: 5′-CAT CC TGG CTG CTG TGG TTG TG-3′; Reverse: 3′-TGG CTC TCT GCT GTC TCT G-5′ |
| NGF       | Forward: 5′-CAA CAG GAC TCA CAG GAG CAA GC-3′; Reverse: 3′-GAT GTC CGG TTC GCT CTT ATC-5′ |
| BDNF      | Forward: 5′-TGG AAC TCA TCG TCA GAC AGA C-3′; Reverse: 3′-TTT CTA TGA ACC GGC ACG CAA TTC-5′ |
| MPO       | Forward: 5′-CCG AGC ATG CAA GAT TGG CTC AC-3′; Reverse: 3′-GCC ACC TCA CAG ACC TCC-5′ |
| GAPDH     | Forward: 5′-GGG GAT TAC TGG CTC GCT-3′; Reverse: 3′-GAC TCA TGG TAC TCT-5′ |

BDNF: brain-derived neurotrophic factor; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; MPO: myeloperoxidase; NGF: nerve growth factor; NGFR: nerve growth factor receptor.

Table 2 Polymerase chain reaction conditions

| Step     | Time | Temperature (°C) |
|----------|------|------------------|
| Denature | 10 min | 95                |
| Anneal   | 30 s  | 55                |
| Extend   | 30 s  | 55                |
| MelTcurve| 15 s  | 55                |
| 95°C pre-denature | 10 min | 95 |
| Denature  | 15 s  | 95                |
| Anneal    | 60 s   | 95                |
| Melt curve | 15 s  | 95                |
*There were 40 cycles.

Western blot assay

To verify the genomics results, we performed western blot tests on MPO, BDNF, NGF, and other relative proteins (NGF, interleukin-1β, pro-interleukin-1β, and tumor necrosis factor-α) in the four groups (n = 3/group) after 30 days were subjected to total protein extraction in accordance with Hu et al.’s method (Hu et al., 2019). Subsequently, 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed, and the samples were then transferred to a polyvinylidene difluoride membrane. The polyvinylidene difluoride membrane was blocked with 5% skimmed milk (Biozharp, Heifei, China) at room temperature for 2 hours and then placed into a solution with diluted primary antibody. The primary antibody information is shown in Table 3. The sample was incubated overnight in a refrigerator at 4°C. Then, the membrane was incubated with horseradish peroxidase-conjugated goat anti-rabbit antibody (1:5000, Bios, Beijing, China, Cat#: bs-0295G-HRP, RRID: AB_10923683) at 37°C for 2 hours. Image acquisition was performed using the Tanon 5200 gel imaging system (Tanon Bio., Shanghai, China). Image analysis was performed using ImageJ software v1.53c (Schneider et al., 2012), and relative protein expression was presented as the optical density (OD) ratio of the target protein to glyceraldehyde-3-phosphate dehydrogenase/β-actin.

Immunohistochemical staining

Nerve injuries are accompanied by damage to the myelin sheaths and neurons (Gordon, 2020), so myelin basic protein as well as β3-tubulin was detected. Nerve samples from the four groups (n = 3/group) after 30 days were fixed in 4% polyvinyl alcohol and then sectioned in paraffin. The paraffin sections were deparaffinized and washed with distilled water (Jia et al., 2021). Then, the tissue sections were placed in a repair box filled with citric acid antigen retrieval buffer antigen retrieval solution (pH 6.0, G1202, Servicebio, Wuhan, China) in a microwave oven to retrieve the antigens followed by incubation with goat serum for 30 minutes. Then, the samples were then incubated with primary antibody (rabbit anti-MBP antibody, 1:100, Servicebio, Cat#: GB111226, RRID:AB 2895013; rabbit anti-β3-tubulin antibody, 1:100, Servicebio, Cat#: GB11139, RRID:AB 2895013) in a humidified incubator overnight at 4°C. After washing, the slides were dried slightly and incubated with secondary antibody (1:200, horseradish peroxidase-conjugated goat anti-rabbit, Servicebio, Cat#: GB23303, RRID:AB2811189) for 50 minutes at room temperature.


### Results

Differential gene expression analysis showed that 258 genes were significantly differentially expressed at the mRNA level in the AKBA group compared with the Model group (Figure 2A1). These genes included 189 upregulated genes and 69 downregulated genes. Three hundred fifty-five genes, including 194 upregulated genes and 161 downregulated genes, were significantly differentially expressed at the mRNA level in the AKBA group compared with the Sham group (Figure 2B1). In addition to the genes in the Model group (Figure 2A1), the genes in the Sham group (Figure 2B1), the genes in the AKBA group, among which 171 were upregulated and 239 were downregulated, were significantly differentially expressed between the Sham and Model groups (Figure 2A2). Significant differences in the expression of different genes are shown in Table 3.

### Statistical analysis

No statistical methods were used to predetermine sample sizes; however, our sample sizes are similar to those reported in a previous publication (Liu et al., 2022b). The statistical analysis of all data was performed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA) and graphed using GraphPad Prism software version 8.0 (GraphPad Software, San Diego, CA, USA, www.graphpad.com). All data are expressed as the mean ± standard deviation (SD). A one-way analysis of variance was used to test for differences between the data in each group using the least significant difference and Duncan method of multiple comparisons. *P < 0.05 was considered to be a significant difference.

### Enrichment analysis of differentially expressed genes in the injured rat sciatic nerve after AKBA treatment

KEGG results showed that significantly differentially expressed genes in the AKBA group, Model groups were mainly enriched in 193 pathways. The ten pathways with the highest enrichment included the phagosome pathways (Figure 2B1). The following ten genes were involved in the phagosome pathway: (�g1, Cbb, Mpo, Thbs2, Cd209, Stx7, R1, M3, C3, LOC100363064, and RGD1564571) (Figure 2B1). Among these genes, Mpo, Cd209, LOC100363064, and RGD1564571 were downregulated, and the rest were upregulated in the AKBA group compared with the genes in the Model group (Figure 2B1). In addition to the ten most enriched items, the neurotrophic factor pathway was also enriched, in which the upregulated gene NGFR plays a role (Figure 2B4) in the AKBA group compared with that in the Model group. The ten pathways with the highest enrichment in the AKBA and Sham groups are shown in Figure 2B5. The neurotrophic factor signaling pathway, in which the upregulated gene NGFR plays a role, was another enriched pathway (Figure 2B4) in the AKBA group compared with that in the Sham group. The ten pathways with the highest enrichment in the Model and Sham groups are presented in Figure 2B6.

We also performed an overall KEGG enrichment analysis of the up- and downregulated genes. The results are shown in Additional Figure 1. Compared with the genes in the Model group, the up-regulated genes in the AKBA group were mainly enriched in myocardial contraction (Additional Figure 1A) and the main down-regulated genes were enriched in the peroxisome proliferators-activated receptors and phagosome signaling pathway (Additional Figure 1B). Compared with the genes in the Sham group, the up-regulated genes in the AKBA group were mainly enriched in cardiac muscle contraction (Additional Figure 1C), and the main down-regulated genes were enriched in adenosine 5'-monophosphate activated protein kinase signaling pathway (Additional Figure 1D). Compared with the genes in the Model group, the up-regulated genes in the AKBA group were mainly enriched in cell adhesion molecules (Additional Figure 1F), and down-regulated genes were mainly enriched in the glucagon signaling pathway (Additional Figure 1E).

In summary, the phagosome pathway was the main enriched pathway that played a role in AKBA's effects on sciatic nerve damage and repair (Figure 2B2 and 3), and NGFR and BDNF were significantly differentially expressed genes that were involved in nerve damage repair (Figure 2B4).

### Table 3 | Primary antibodies

| Antibody | Concentration | Species | Catalog No. | RRID No. | Supplier |
|----------|---------------|---------|-------------|----------|----------|
| BDNF     | 1:500         | Rabbit  | DF6387      | AB_2833850 | Affinity Biosciences (Beijing, China) |
| NGFR     | 1:500         | Rabbit  | DF6821      | AB_2838781 | Affinity Biosciences (Beijing, China) |
| MPO      | 1:500         | Rabbit  | A1374       | AB_2760590 | Abclonal Technology (Wuhan, China) |
| NGF      | 1:500         | Rabbit  | A14216      | AB_2761076 | Abclonal Technology (Wuhan, China) |
| GAPDH    | 1:1000        | Rabbit  | Bs-2188R    | AB_11056564 | Bios Biotechnology (Beijing, China) |
| IL-1β    | 1:400         | Rabbit  | WtH19030    | AB_2894981 | Wanleibio |
| TNF-α    | 1:1000        | Rabbit  | WtO1225     | AB_2894987 | Wanleibio |
| β-Actin  | 1:500         | Rabbit  | WtO1518     | AB_2894992 | Wanleibio |
| IFN-γ    | 1:500         | Rabbit  | Wt0061R     | AB_10855480 | Bios Biotechnology (Beijing, China) |
| IFN-γ    | 1:400         | Rabbit  | WtO1225     | AB_2894987 | Wanleibio |
| IFN-γ    | 1:400         | Rabbit  | WtO1518     | AB_2894992 | Wanleibio |

BDNF: Brain derived neurotrophic factor; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; IL-1β: interleukin 1 beta; MPO: myeloperoxidase; NGF: nerve growth factor; NGFR: nerve growth factor receptor; Pro-IL-1β: pro interleukin 1 beta; TNF-α: tumor necrosis factor-alpha.

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Previous studies (Jiang et al., 2018, 2020) have shown that AKBA can promote Schwann cell proliferation through the extracellular regulated protein kinases signaling pathway to accelerate the repair of sciatic nerve damage. However, the specific mechanism of this effect is unclear. We used whole transcriptomics to sequence and compare all genes in different groups to obtain differentially expressed genes and then performed KEGG enrichment analysis on the differentially expressed genes. We found that AKBA could promote phagosome formation, inhibit inflammatory reactions, and participate in sciatic nerve damage repair. Additionally, AKBA could improve BDNF, NGF, and NGFR protein expression. This provides a theoretical basis for further development of AKBA as a new veterinary drug to treat PNI in small animals.

KEGG analysis aggregated differentially expressed genes into pathways, thus identifying pathways that promote damage repair. KEGG enrichment analysis revealed that AKBA showed differential regulation between the AKBA and Model groups were most enriched in the phagosome pathway. Phagocytosis is the process where cells ingest relatively large particles, and it is a central mechanism in inflammation (Brown and Neher, 2014). Phagosomes are formed when specific receptors on the phagocyte surface recognize ligands and a particle’s surfaces. The following 10 genes are involved in phagocytosis: Tcirg1, Cybb, MPO, Thbs2, Cd209, Stx7, RT1-M2, C3, LOC100363064, and RGDI564571. The results of this study showed that among these genes, MPO and Cd209 were downregulated, while the rest were upregulated after nerve injury, and thus, genes are jointly involved in the repair of sciatic nerve injury. Therefore, it is speculated that AKBA could promote phagosome formation, inhibit inflammatory reactions, and participate in sciatic nerve damage repair. Through detecting MPO gene downregulation, AKBA was shown to reduce MPO mRNA and protein expression. This result is consistent with previous results that showed a reduction in MPO can effectively reduce peripheral nerve lesions, protect peripheral nerve cells, and reduce oxidative stress (Chen et al., 2020). AKBA has been shown to reduce CD68 and inflammatory protein expression, such as tumor necrosis factor-a, interleukin-1β, and pro-interleukin-1β, which suggest that AKBA could inhibit inflammation. It remains unknown if inhibiting the inflammatory response is directly mediated by MPO, which will be studied in the future.

BDNF can promote peripheral nerve regeneration, protect damaged neurons and nerve cells, and maintain sensitivity to neurons (Lu et al., 2019). NGF is an endogenously produced polypeptide that can promote neuronal differentiation, survival, and repair in the central and peripheral nervous systems (Farbuddin et al., 2003; Jones et al., 2019; Nocchi et al., 2019). NGFR is a low-affinity receptor that binds to BDNF and NGF, and it can mediate nerve cell survival (Carito et al., 2014). These results demonstrated that AKBA promoted sciatic nerve injury repair by increasing NGF, NGF, and BDNF mRNA expression and thereby increasing the corresponding protein expression level.

MBP is an oligodendrocyte marker and a myelin sheath quantity and function marker. Mahall et al. (2015) reported that MBP is sufficient for specific differentiation of neuronal cell types (Reichert et al., 2019), and an increase in this marker can reflect nerve repair after injury. The experimental results illustrated that MBP expression in the AKBA group was significantly increased compared with that in the Model group. β3-Tubulin expression in the AKBA group was significantly higher than that in the Blank group. The G-ratio is an indicator that reflects the maturity of regenerated myelinated nerve fibers and is most suitable for evaluating the axon diameter, which is approximately 0.6, which is more conducive to a better conduction velocity (Philpott et al., 2017). A myelin thickness analysis also showed that the myelin thickness in the Model group was greater and the axons were smaller. The myelin sheath thickness in the AKBA group was relatively small, and the G-ratio was closer to that of the Blank and Sham groups. Thus, AKBA has the effect of repairing damage to the myelin sheath. The results of silver staining further demonstrated that AKBA exerted a good repair effect on damage to the nerve myelin sheath. The recovery of damaged nerve function and pain perception are two key clinical evaluation indicators. The SFI can be used to judge the capability of nerve regeneration after nerve injury and the degree of nerve motor function (Sang-Mam et al., 2020). If the SFI is high, then the indicators of the nerve surrounding the muscle tissue are also good. Disuse muscle atrophy occurs after PNI (Dyer et al., 2016) and affects nerve sensory function. The toe pinch test was used to assess the recovery of sensory function. The behavioral test results revealed that AKBA could accelerate damaged sciatic nerve function recovery (Figure 6A). However, it could not completely repair the damage during the experimental period. However, our research has some limitations. In vivo studies have concluded that AKBA can inhibit inflammation (Wei et al., 2020) and improve neurotrophic factor signaling pathways. However, in vitro studies are also needed, and we have not performed these studies, which is a research limitation. In this study, AKBA promotes sciatic nerve injury repair in rats by increasing BDNF, NGF, and NGFR protein expression, which thereby increases the expression of their corresponding proteins, and by decreasing MPO mRNA and protein expression in the phagosome pathway to inhibit inflammation.
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**Figure 1** | Test timeline.
Sprague-Dawley rats were used to generate a crush injury model of the right sciatic nerve. Acetyl-11-keto-beta-boswellic acid was administered on day 2 after creating the model and every other day thereafter. Behavioral tests were performed on days 10, 20, and 30. On day 30, all rats were sacrificed by cervical dislocation after anesthesia, and the injured sciatic nerve was removed. SNI: Sciatic nerve injury.

**Gene expression analysis after sciatic nerve injury treated with AKBA in rats.**
(A) Differential gene expression volcano map. (A1) AKBA group vs. Model group. (A2) AKBA group vs. Sham group. (A3) Model group vs. Sham group. (B) KEGG analysis of differential gene expression in the sciatic nerve. (B1) AKBA group vs. Model group. (B2) Heat map of KEGG enrichment analysis of differentially expressed mRNA between the AKBA and Model groups. Each row represents an enriched KEGG pathway, and each column represents differential mRNA expression. The red color indicates that the mRNA was detected in the corresponding KEGG pathway, and vice versa, and the gray color indicates that no enrichment was detected. (B3) The most significantly different gene expression was in the phagosome pathway. A redder color indicates more up-regulation of gene expression in the AKBA group than in the Model group, and a greener color indicates more down-regulation in the AKBA group than in the Model group. (B4) Expression of different genes after normalization (FRKM values). (B5) AKBA group vs. Model group. (B6) Model group vs. Sham group. Data are expressed as the mean ± SD (n = 5 rats/group). BDNF: Brain derived neurotrophic factor; Cybb: cytochrome B-245 beta chain; C3: complement C3; NGF: nerve growth factor; NGFR: nerve growth factor receptor; MPO: myeloperoxidase; RT1-M2: RT1 class Ib: locus M2; Stx7: syntaxin 7; Tcirg1: T cell immune regulator 1; Thbs2: thrombospondin 2.

**Figure 3** | Gene and protein verification in sciatic nerve injury in rats treated with AKBA.
(A) miRNA expression levels (relative to GAPDH) of NGFR (A1), NGF (A2), BDNF (A3), and MPO (A4) in the rat sciatic nerve using qPCR. (B) Protein expression levels (relative to GAPDH) of NGFR (B2), NGF (B3), BDNF (B4), and MPO (B5) in the rat sciatic nerve by western blot assay. Data are expressed as the mean ± SD (n = 3 rats/group) and were analyzed using a one-way analysis of variance followed by the least significant difference test. BDNF: Brain derived neurotrophic factor; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; MPO: myeloperoxidase; NGF: nerve growth factor; NGFR: nerve growth factor receptor; qPCR: real-time polymerase chain reaction.
Effect of AKBA on behavior of rats with sciatic nerve injury.

| SFI   | Sham | Model | Blank | AKBA |
|-------|------|-------|-------|------|
| P     | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |

- **Figures:**
  - **Figure 4** Effect of AKBA on inflammation-related factors in sciatic nerve injury in the rat.
  - **Figure 5** Effects of AKBA on myelin sheath and nerve fiber regeneration in the damaged sciatic nerve in rats 30 days after injury.
  - **Figure 6** Effect of AKBA on behavior of rats with sciatic nerve injury.

- **Tables:**
  - Data are expressed as the mean ± SD (n = 3 rats/group) and were analyzed using a one-way analysis of variance followed by the least significant difference test. AKBA: Acetyl-11-keto-beta-boswellic acid; MBP: myelin basic protein.

- **Graphs:**
  - Data are expressed as the mean ± SD (n = 3 rats/group) and were analyzed using a one-way analysis of variance followed by the least significant difference test. AKBA: Acetyl-11-keto-beta-boswellic acid; MBP: myelin basic protein.
Additional file: Additional Figure 1: KEGG directional analysis of differentially expressed genes in rat sciatic nerve after AKBA treatment

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Conflicts of interest: The authors declare that there is no conflict of interest regarding the authorship or content of this paper.

Author statement: All genomics raw sequencing data has been uploaded to GEO (accession number GSE179400). In addition, the datasets used in the project are available from the corresponding author.

Availability of data and materials: All data generated or analyzed during this study are included in this published article and its supplementary information files.

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Additional file: Additional Figure 1: KEGG directional analysis of differentially expressed genes in rat sciatic nerve after AKBA treatment.
Additional Figure 1 KEGG analysis of differentially expressed genes in the rat sciatic nerve after AKBA treatment.

(A) Overall KEGG scatter plot of up-regulated genes in the AKBA group compared with the genes in the Model group. (B) Overall KEGG scatter plot of down-regulated genes in the AKBA group compared with the genes in the Model group. (C) Overall KEGG scatter plot of up-regulated genes in the AKBA group compared with the genes in the Sham group. (D) Overall KEGG scatter plot of down-regulated genes in the AKBA group compared with the genes in the Sham group. (E) Overall KEGG scatter plot of up-regulated genes in the Model group compared with the genes in the Sham group. (F) Overall KEGG scatter plot of down-regulated genes in the Model group compared with the genes in the Sham group. AKBA: Acetyl-11-keto-beta-boswellic acid; KEGG: Kyoto Encyclopedia of Genes and Genomes.