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Anti-inflammatory, Analgesic, and Motor Improvement Effects of Bee Venom Acupuncture on Freund's Complete Adjuvant-Induced Spinal Degeneration in Rats

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ABSTRACT

Spinal degeneration (SD) is a common condition among the elderly, causing pain and reduced mobility. Prompt detection, monitoring, and treatment of degenerative conditions is crucial to minimize serious complications. In this experiment, rats with Freund's complete adjuvant (CFA)-induced SD received bee venom acupuncture (BVA) at doses of 0.5, 1.0, and 1.5 mg/kg or Mobic (meloxicam) acupuncture (MA) at 1.0 mg/kg. All treatments were administered twice weekly for three weeks. The anti-inflammatory, analgesic, and motor improvement effects of BVA were evaluated via behavioral tests, white blood cell counts, and histological analysis. Results showed significant reductions in white blood cell count and the inflammatory marker interleukin-1β (IL-1β) in both BVA and MA groups compared to the control SD group. Additionally, the BVA and MA treatment groups showed increased hotplate paw-withdrawal latency, indicating reduced pain sensitivity, and longer times on the accelerating rotarod, indicating improved motor function, compared to the control group. These findings suggest that BVA has promising anti-inflammatory, analgesic, and motor-improving effects for the symptomatic treatment of SD.

Keywords: Spinal degeneration, Acupuncture, Bee venom, Analgesic, Motor improvement.

Introduction

Spinal degeneration (SD) is a common age-related condition affecting the musculoskeletal system. Studies using magnetic resonance imaging (MRI) on people without back pain symptoms have shown surprisingly high rates of disc abnormalities. These abnormalities include reduced disc signal intensity (hypointensity) in 20–83% of cases, bulging discs (10-81%), disc protrusions (3–63%), and spinal stenosis (narrowing of the spinal canal) in 3–56%.[1,2] While these abnormalities may not cause immediate symptoms, they can worsen over time, leading to impaired movement and disability. Lower back pain, a common consequence of SD, was identified as a leading cause of disability in a recent report, affecting 83 million people globally in 2010.[3] Therefore, early and effective treatment for SD is crucial to improve patients' quality of life and reduce the economic burden on individuals and society.

Currently, SD is mainly treated by non-steroidal anti-inflammatory drugs (NSAIDs). Although these drugs have anti-inflammatory and analgesic effects and can improve motor function markedly in patients with SD, they can cause complications such as peptic ulcer, gastric bleeding, and even gastrointestinal perforation.[4]

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Therefore, therapeutic measures that have both anti-inflammatory and analgesic effects and few adverse effects have been a focus of numerous studies.[5–10] One of the methods employed in traditional medicine is bee venom acupuncture (BVA),[11] in which a bee venom solution is injected into acupuncture points on the body. It has been widely applied in the treatment of pain and inflammation in skeletal, cardiovascular, digestive, respiratory, systemic and immune, endocrine and metabolic, neurological, psychiatric, ear, nose, and throat, and dermatological conditions.[12]

In this study, rats were induced with SD by applying Freund's complete adjuvant (CFA) to the L4-L5 intervertebral disc. After 2 weeks, the rats were treated with BVA at doses of 0.5, 1.0, and 1.5 mg/kg. The study results show that the preparation containing bee venom has anti-inflammatory and analgesic effects, improving motor function as evidenced by variation in local temperature, paw withdrawal latency on the hot plate, locomotion in an open field, motor coordination on a rotatod, changes in white blood cell (WBC) count, and serum concentrations of certain cytokines.

Materials and Methods

Materials

The acupuncture drug product containing bee venom, Apitoxin (GUJU Pharmaceutical Co., Ltd., Korea), was provided by the Faculty of Pharmacology, University of Medicine and Pharmacy. South-Korea Joint School. Mobic (meloxicam, 15 mg/1.5ml) was provided by Boehringer Ingelheim (Germany).

Animals

Adult Wistar rats (n = 90, 180 – 230 g body weight) were housed in a room with controlled temperature and 12 h light/dark cycles. The animals were randomly divided into 6 groups:

+ Group 1 (Control, n = 15) non-SD, treated with saline;
+ Group 2 (SD, n = 15) treated with saline on SD rats;

+ Group 3 (n = 15) treated with 0.5 mg/kg BVA on SD rats;
+ Group 4 (n = 15) treated with 1.0 mg/kg BVA on SD rats;
+ Group 5 (n = 15) treated with 1.5 mg/kg BVA on SD rats; and
+ Group 6 (MA, n = 15) treated with 1.0 mg/kg Mobic on SD rats.

The experimental protocol was approved by the Vietnam Military Medical University, Hanoi, Vietnam (ethical permission number IACUC-063/19 issued on February 15, 2019).

Method to Induce SD in Rats

The rats were SD-induced by injecting CFA (Freund's complete adjuvant, Sigma, USA) into the L4-L5 intervertebral disc according to the method of Jiang *et al*. (2017).[13] Specifically, after spinal surgery, the back and paravertebral muscles were dissected to expose the L4-L5 intervertebral disc. CFA solution (10 µl) was injected into the intervertebral disc of each rat and sutured layer by layer. The rats were monitored until they were awake and were then placed in the care cage for further monitoring.

Method of Acupuncture on Rats

The rats were treated with 0.1 ml of a saline injection, BVA, or MA at the paravertebral position equivalent to the 4th and 5th lumbar intervertebral space (the position of the paravertebral acupoint was about equivalent to intervertebral space L4-L5), twice per week for 3 weeks.[14]

Local Temperatures in Rats

Local and systemic inflammation are manifestations of SD. The temperature around the L4-L5 spinal segment where CFA was injected was measured to evaluate local inflammation.[15] The rats were kept comfortably in the hand, the ambient temperature in the room was maintained at about 26 °C, and the temperature at the L4-L5 spinal segment was measured using an infrared thermometer for 3 seconds. This parameter was measured before treatment and 1 week and 2 weeks after treatment.

Hot Plate Test

The rats were placed in a plastic chamber with a glass bottom and acclimated for 5 minutes. Then, the heat source was placed under the floor, just below the hind paws. The period between initial thermal stimulation and paw withdrawal was recorded for both hind paws of the rats.[16]

Open Field Test

The rats were put in a box of about 60 cm × 60 cm × 100 cm (width × length × height, respectively) and acclimated for 5 minutes. Then, over 5 minutes, the entire movement of the rats were recorded as a video clip by a CCD camera connected to a computer, and the motor parameters of the rats were analyzed automatically by commercial software ANY-maze (Stoelting, USA).[17,18]

Rotarod Test

Motor performance improvement was assessed through the rotarod performance test. Animals were placed on a rotarod apparatus with the horizontal rod rotated at 10 times/min. The latency to fall was measured from when the rat was placed onto the rod to the fall. If the latency was more than 300 seconds and the rat still clung to the crossbar, the test was stopped and the latency of the test was recorded as 300 seconds.[17,18]

Measurements of WBC Counts and Serum Concentrations of Cytokines

Before and after one week of SD induction, 2 ml blood sample was obtained from the tail veins of the rats.[19] WBC count was measured by an automatic hematology analyzer (XN1000, Sysmex, Japan). Serum concentrations of IL-1β, IL-10 and tumor necrosis factor alpha (TNF-α) were measured by ELISA kits (Wuhan Fine Biotech Co.).

Histology

After 3 weeks of treatments, the animals were euthanized. The L4-L5 intervertebral joint were removed, and histological images of this spinal cord area was determined following hematoxylin and eosin (HE) staining.[20]

Statistical Analysis

Data were analyzed by two-way repeated measures ANOVA followed by Bonferroni tests. The difference was statistically significant with $p < 0.05$. All data are expressed as the mean ± SD. The software for data analysis was SPSS version 22.0.

Results and Discussion

Local Temperature

The results in Table 1 indicate that rats in the control group (non-SD) had lower local temperatures than all other groups before treatment. After treatment, local temperature remained unchanged in the control and untreated SD groups. Interestingly, BVA and MA treatment significantly lowered local temperatures in SD rats after 1 and 3 weeks (compared to before treatment). The most significant decrease in local temperature occurred with the lowest BVA dose (0.5 mg/kg), which was comparable to the effect of MA treatment (1.0 mg/kg).

Hot Plate Test

Before treatment, all SD rat groups (including BVA and MA) had lower paw withdrawal times from the hot plate compared to the control group, indicating increased pain sensitivity. After treatment, paw withdrawal times remained unchanged in the untreated SD group. Conversely, paw withdrawal times significantly increased in all BVA and MA treatment groups compared to before treatment. The most significant improvement in pain sensitivity occurred with the lowest BVA dose (0.5 mg/kg), again mirroring the effect of MA treatment (1.0 mg/kg) (Table 2).

Table 1: Temperature before and after treatment (°C).

Group	Time of study			p
	Before treatment (1)	After 1 week (2)	After 3 week (3)	
Control (a)	34.43 ± 1.13	33.87 ± 3.48	34.71 ± 0.95	$p_{1\text{-}2,3} > 0.05$
SD (b)	37.76 ± 0.94	37.91 ± 1.19	37.86 ± 0.97	$p_{1\text{-}2,3} > 0.05$
BVA 0.5 mg (c)	37.78 ± 0.98	36.40 ± 0.76	35.22 ± 0.65	$p_{1\text{-}2,3} < 0.01$
BVA 1.0 mg (d)	37.75 ± 0.85	36.53 ± 0.75	36.11 ± 0.79	$p_{1\text{-}2,3} < 0.01$
BVA 1.5 mg (e)	37.71 ± 0.88	36.53 ± 0.67	36.21 ± 0.74	$p_{1\text{-}2,3} < 0.01$
Mobic 1.0 mg (f)	37.75 ± 1.01	36.42 ± 1.12	34.86 ± 2.79	$p_{1\text{-}2,3} < 0.05$
p	$p_{a\text{-}b,c,d,e,f} < 0.001$	$p_{b\text{-}a,c,d,e} < 0.05$	$p_{b\text{-}a,c,d,e,f} < 0.05$	

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Table 2: Paw withdrawal latency of rats on hot plate test (s).

Group	Time of study			p
	Before treatment (1)	After 1 week (2)	After 3 week (3)	
Control (a)	32.33 ± 6.51	34.67 ± 7.19	32.67 ± 5.30	$p_{1\text{-}2,3} > 0.05$
SD (b)	13.67 ± 3.81	13.67 ± 3.06	14.33 ± 4.72	$p_{1\text{-}2,3} > 0.05$
BVA 0.5 mg (c)	14.20 ± 3.73	20.73 ± 3.47	31.67 ± 4.73	$p_{1\text{-}2,3} < 0.001$
BVA 1.0 mg (d)	13.67 ± 9.74	17.67 ± 4.67	25.07 ± 10.99	$p_{1\text{-}2,3} < 0.05$
BVA 1.5 mg (e)	14.13 ± 4.47	19.73 ± 6.34	30.47 ± 4.90	$p_{1\text{-}3} < 0.05$
Mobic 1.0 mg (f)	14.47 ± 5.05	20.93 ± 3.47	32.73 ± 3.55	$p_{1\text{-}2,3} < 0.001$
p	$p_{a\text{-}b,c,d,e,f} < 0.001$ $p_{b\text{-}c,d,e,f} > 0.05$ $p_{c\text{-}d,e,f} > 0.05$	$p_{a\text{-}b} < 0.001$ $p_{a\text{-}c,d,e,f} < 0.01$ $p_{b\text{-}c,d,f} < 0.05$ $pc_{,d,e,f} > 0.05$	$P_{b\text{-}c,d,e,f} < 0.05$	

Open Field Test
After treatment with BVA at doses of 0.5, 1.0, and 1.5 mg/kg, and with MA at a dose of 1.0 mg/kg, the mean locomotor speeds and distances were higher than those in the SD groups before treatment ($p < 0.05$ for groups 2-1, 3, 4, 5, 6) (Table 3).

Rotarod Test
After treatment, the latencies to fall in the control and sham control groups were not significantly different from those before treatment ($p > 0.05$). However, after 1 and 3 weeks of treatment, the latencies in the BVA groups and the MA group increased significantly compared to before treatment (Table 4).

WBC Count
In groups treated with BVA (0.5, 1.0, and 1.5 mg/kg) and MA, the WBC counts after 2 weeks of treatment were significantly lower than before treatment ($p < 0.001$). The BVA 0.5 mg/kg group showed the most significant decrease in WBC count ($p < 0.05$) (Table 5).

Table 3: Changes in motor ability of rats between before and after treatment.

Group	Motor ability	
	Mean locomotor speed (m/gy)	Mean locomotor distance (m)
Control (a)	0.020 ± 0.012	5.93 ± 3.19
SD (b)	0.008 ± 0.008	3.10 ± 2.54
BVA 0.5 mg (c)	0.020 ± 0.012	5.96 ± 2.88
BVA 1.0 mg (d)	0.020 ± 0.010	5.76 ± 2.85
BVA 1.5 mg (e)	0.019 ± 0.010	5.70 ± 2.40
Mobic 1.0 mg (f)	0.020 ± 0.015	5.88 ± 3.55
p	$p_{b\text{-}a,c,d,e,f} < 0.05$	$p_{b\text{-}a,c,d,e,f} < 0.05$

Table 4: Latency falling down from the accelerating rotarod (s).

Group	Time of study			p
	Before treatment (1)	After 1 week (2)	After 3 week (3)	
Control (a)	10.47 ± 7.18	11.40 ± 6.39	10.00 ± 5.83	$p_{1\text{-}2.3} > 0.05$
SD (b)	4.13 ± 1.73	4.20 ± 1.47	4.07 ± 1.03	$p_{1\text{-}2.3} > 0.05$
BVA 0.5 mg (c)	4.27 ± 1.22	10.60 ± 2.38	12.67 ± 6.72	$p_{1\text{-}2.3} < 0.01$
BVA 1.0 mg (d)	4.20 ± 2.01	10.33 ± 6.99	11.80 ± 9.96	$p_{1\text{-}2.3} < 0.05$
BVA 1.5 mg (e)	4.33 ± 1.49	10.40 ± 4.15	10.40 ± 3.79	$p_{1\text{-}2.3} < 0.01$
Mobic 1.0 mg (f)	4.27 ± 1.91	11.93 ± 8.85	12.07 ± 8.70	$p_{1\text{-}2.3} < 0.05$
p	$p_{b\text{-}c.d.e.f} > 0.05$	$p_{b\text{-}c.d.e.f} < 0.05$	$p_{b\text{-}c.e.f} < 0.05$	

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Table 5: White blood cell counts before and after treatment (10^9/l).

Group	Time of study		p
	Before treatment (1)	After 2 weeks (2)	
Control (a)	7.23 ± 1.54	7.28 ± 1.72	$p_{1-2} > 0.05$
SD (b)	12.55 ± 2.68	13.54 ± 5.09	$p_{1-2} > 0.05$
BVA 0.5 mg (c)	13.68 ± 3.69	7.68 ± 1.43	$p_{1-2} < 0.001$
BVA 1.0 mg (d)	13.32 ± 3.20	8.01 ± 1.74	$p_{1-2} < 0.001$
BVA 1.5 mg (e)	13.42 ± 2.95	8.73 ± 1.29	$p_{1-2} < 0.001$
Mobic 1.0 mg (f)	13.42 ± 3.52	8.08 ± 1.06	$p_{1-2} < 0.001$
p	$p_{a-b,c,d,e,f} < 0.001$	$p_{b-a,c,d,e,f} < 0.05$	

Serum Concentrations of IL-1β
Before treatment, the IL-1*β* serum concentration in the SD groups was significantly higher than in the control group ($p < 0.001$). After 1 week of treatment, the IL-1*β* serum concentration in the SD group was not significantly different from that in the control group before treatment ($p > 0.05$). However, after 1 week of treatment, IL-1*β* serum concentrations in all the BVA groups (0.5, 1.0, and 1.5 mg/kg) decreased significantly compared to before treatment ($p < 0.001$). The mean serum concentration of IL-1*β* decreased the most in the BVA 0.5 mg/kg group (Table 6).

Serum Concentrations of TNF-α
The TNF-*α* serum concentrations in the control group and the SD group after 1 week of treatment were not significantly different from those before treatment ($p > 0.05$). However, the TNF-*α* serum concentrations in all the BVA groups (0.5, 1.0, and 1.5 mg/kg) decreased significantly compared to before treatment ($p < 0.001$). After 1 week of treatment, the BVA 0.5 mg/kg group showed the greatest reduction in mean TNF-*α* serum concentration compared to the other two dose groups (Table 7).

Serum Concentrations of IL-10
There were no significant differences in IL-10 serum concentrations in the control group and the SD group after treatment compared to before treatment ($p > 0.05$). In the BVA groups (0.5, 1.0 and 1.5 mg/kg) and the MA group, IL-10 serum concentrations after treatment were significantly higher than before treatment ($p < 0.001$). In particular, the IL-10 serum concentration in the BVA 0.5 mg/kg group increased the most after 1 week and was significantly higher than in the BVA 1.0 and 1.5 mg/kg groups ($p < 0.05$) (Table 8).

Table 6: Serum concentrations of IL-1*β* before and after treatments.

Group	Time of study		p
	Before treatment (1) (pg/ml)	After 1 week (2) (pg/ml)	
Control (a)	343.43 ± 110.83	337.82 ± 148.76	$p_{1-2} > 0.05$
SD (b)	1632.74 ± 622.99	1662.62 ± 416.33	$p_{1-2} > 0.05$
BVA 0.5 mg (c)	1682.73 ± 572.84	336.09 ± 167.19	$p_{1-2} < 0.001$
BVA 1.0 mg (d)	1658.02 ± 538.33	387.06 ± 154.96	$p_{1-2} < 0.001$
BVA 1.5 mg (e)	1660.67 ± 510.69	424.24 ± 147.24	$p_{1-2} < 0.001$
Mobic 1.0 mg (f)	1652.31 ± 508.34	351.73 ± 129.31	$p_{1-2} < 0.001$
p	$p_{a-b,c,d,e,f} < 0.001$	$p_{b-a,c,d,e,f} < 0.001$	

Table 7: Serum concentrations of TNF-*α* before and after treatments.

Group	Time of study		p
	Before treatment (a) (pg/ml)	After 1 week (b) (pg/ml)	
Control (a)	20.74 ± 13.68	19.81 ± 12.79	$p_{1-2} > 0.05$
SD (b)	165.80 ± 111.98	167.30 ± 121.17	$p_{1-2} > 0.05$
BVA 0.5 mg (c)	168.12 ± 101.29	30.03 ± 11.99	$p_{1-2} < 0.001$
BVA 1.0 mg (d)	169.20 ± 109.82	28.63 ± 11.41	$p_{1-2} < 0.001$
BVA 1.5 mg (e)	168.97 ± 113.33	19.76 ± 13.55	$p_{1-2} < 0.001$
Mobic 1.0 mg (f)	170.22 ± 78.52	23.92 ± 14.26	$p_{1-2} < 0.001$
p	$p_{a-b,c,d,e,f} < 0.01$	$p_{b-a,c,d,e,f} < 0.05$	

Table 8: Serum concentrations IL-10 before and after treatments.

Group	Time of study		p
	Before treatment (1) (pg/ml)	After 1 week (2) (pg/ml)	
Control (a)	91.94 ± 7.62	91.60 ± 6.35	$p_{1-2} > 0.05$
SD (b)	45.60 ± 7.89	46.78 ± 6.68	$p_{1-2} > 0.05$
BVA 0.5 mg (c)	46.39 ± 7.79	76.79 ± 11.44	$p_{1-2} < 0.001$
BVA 1.0 mg (d)	46.72 ± 8.42	83.81 ± 11.59	$p_{1-2} < 0.001$
BVA 1.5 mg (e)	46.83 ± 8.72	91.72 ± 10.64	$p_{1-2} < 0.001$
Mobic 1.0 mg (f)	46.66 ± 15.55	91.37 ± 12.59	$p_{1-2} < 0.001$
p	$p_{a-b,c,d,e,f} < 0.001$	$p_{b-a,c,d,e,f} < 0.001$	

Histological Images

In the control group, the spinal images appeared normal with the following characteristics. The articular cartilage consisted of cartilage cells situated in cartilage cavities, the cartilage cells showed no signs of degeneration, and the matrix was clear. The medullary cavities contained blood cells. In contrast, the SD groups exhibited signs of inflammation and degeneration of the spine, with the following features. Inflammatory foci were present adjacent to the spinal bones within the striated muscle tissue and fibrous connective tissue. These foci included granulomatous inflammatory cells such as lymphocytes, macrophages, epithelial cells, giant cells with multiple cytoplasmic nuclei, bright cavities, and proliferating fibrous cells. The joint surfaces had a thin layer of cartilage, with degenerated cartilage cells and a translucent matrix. Inflammation was most pronounced in the control group treated with saline, whereas the groups treated with bee venom and Mobic exhibited only mild inflammation (Figure 1).

In this study, the anti-inflammatory effect of bee venom was assessed by measuring changes in white blood cell count and serum concentrations of cytokines (IL-1β, IL-10, and TNF-α). The results show that in CFA-induced SD rats, there were significant increases in WBC count and serum concentrations of IL-1β and TNF-α, along with a decrease in IL-10 serum concentration, compared to normal rats in the control group. After treatment with BVA at doses of 0.5, 1.0, and 1.5 mg/kg, there were significant decreases in WBC count and serum concentrations of IL-1β and TNF-α, and an increase in IL-10 serum concentration. These findings indicate that BVA treatment at the specified doses significantly reduced inflammation in the experimental animals.

White blood cells play an important role in the immune response, protecting the body.[21] An increased WBC count is an indicator of inflammation in the body. The results of our study are consistent with previous studies that evaluated the anti-inflammatory effect in terms of changes in WBC count. Petchi *et al.* induced inflammation in rat's paws using CFA and found an increase in WBC count.[22] When treated with a multi-herbal formulation, the WBC count decreased. Similarly, Kamarudin *et al.* used subcutaneous collagen to induce inflammation and observed that WBC counts gradually increased after 2−4 weeks of induction.[23] When rats were administered curcumin orally, there was a statistically significant decrease in WBC count in the curcumin group compared to the control group.[23]

Proinflammatory cytokines such as IL-1β, IL-6, and TNF-α, produced mainly by active macrophages, upregulate the inflammatory response. Meanwhile, anti-inflammatory cytokines, including IL-10, IL-11, and IL-13, regulate and control this response. In inflammation, pro-inflammatory cytokines are activated, leading to increased levels of these cytokines in the serum. Conversely, anti-inflammatory cytokines are inhibited, resulting in decreased plasma levels of these cytokines. During the process of reducing inflammation, the action of cytokines reverses, causing a decrease in pro-inflammatory cytokines and an increase in anti-inflammatory cytokines.[24,25] The study results show that, in the inflammatory SD group before BVA treatment, there was a significant increase in proinflammatory cytokines (IL-1β and TNF-α) and a decrease in the anti-inflammatory cytokine IL-10. The elevation of proinflammatory cytokines and the decrease in anti-inflammatory cytokines indicated increased inflammation in the rats injected with inflammatory and degenerative substances, validating the success of the experimental model.

Consistent with the theory of cytokines roles in inflammation, our study results demonstrated that rats in all the BVA groups (0.5, 1.0, and 1.5 mg/kg) showed significantly decreased plasma levels of TNF-α and IL-1β, along with increased plasma IL-10 levels. Notably, BVA 0.5 mg/kg appears to be more efficacious than BVA 1.0 and 1.5 mg/kg, a finding consistent with previous results. Huh *et al.* showed that the anti-inflammatory and analgesic effects of BVA depend on the dose. In particular, low doses had better anti-inflammatory and analgesic effects than high doses.[26] Bee venom components such as melatonin have anti-inflammatory effects but can also stimulate the body's immune response, even releasing some substances from mast cells such as histamine. At higher doses, these immune responses also increase, thereby reducing the anti-inflammatory and analgesic effects of BVA. Similar results were observed in rats treated with MA at a dose of 1.0 mg/kg (Tables 6-8). This evidence supports the conclusion that BVA at the tested doses has a significant anti-inflammatory effect in experimental animal models of inflammation and degeneration. In both BVA and MA-treated rats, the concentration of pro-inflammatory cytokines decreased significantly after 4 weeks of treatment, while the concentration of anti-inflammatory cytokines increased markedly after 3 weeks of treatment.

The hot plate is a widely used test to assess nociception in rodents. In SD rats, the rats will retract their paws more quickly due to increased nerve sensitivity to thermal stimulation.[27] Measuring the latency from stimulation to the rat's response to the thermal stimulus reveals the pain level. This parameter was measured before treatment, and 1 and 3 weeks after treatment. The results show that the latency for the rats to withdraw their paws from the hot plate decreased after inflammation induction and increased after BVA and MA treatment.

CFA-induced neuropathic pain is characterized by high neuroexcitatory, proinflammatory cytokine release, and the activation of nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) in the spinal dorsal horn, increasing an animal's sensitivity to heat.[28] BVA has been shown to have an activating effect on adenosine 3 receptor (A3R) and spinal α2-adrenergic receptor, inhibiting NF-κB and MAPK signal pathways, thus leading to a decreased release of pro-inflammatory TNF-α and IL-1β, an increased release of anti-inflammatory IL-10, and an increased sensitivity to heat.[29] In BVA and MA-treated rats, there was an increased paw-withdrawal latency, indicating a decreased pain response. These results are consistent with previous studies showing that BVA treatment has an analgesic effect on experimental animals.[30-32]

The motor improvement effect of BVA in this study was evaluated using several tasks. First, rat locomotor activity in the open field test was measured by locomotor speed and distance traveled. In the SD group, the locomotor speed and distance traveled were lower than those in the control group. Inflammation and pain restricted the locomotor ability of the CFA-induced SD rats. However, in BVA-treated rats, the motor speed and distance traveled increased markedly, indicating that bee venom effectively reduced inflammation and pain, thereby improving motor ability impaired by inflammation induced around the L4-L5 spinal segment. Secondly, motor improvement was measured using the rotarod test, assessing the motor coordination of rats before and after BVA treatment. This indicator was evaluated by the latency of the rats to fall from the rotating rod. In this behavioral test, the rod is a horizontal bar placed at a certain distance from the ground, designed so that the rats cannot obtain an external grip on the rod. Our findings

align with previous studies that evaluated the therapeutic efficacy of medicinal herbs and substances in SD animal models. For instance, Kim *et al.* developed an SD-induced experimental animal model by inducing damage to the joints and discs of rats, and assessed the motor coordination of the rats using the rotarod was assessed. The results revealed a reduction in the latency to fall, indicating diminished motor coordination in the CFA-induced SD rats.[33] Therefore, by employing behavioral tests in our study, the impact of BVA injection on the motor ability and motor coordination of CFA-induced SD rats has been illustrated.

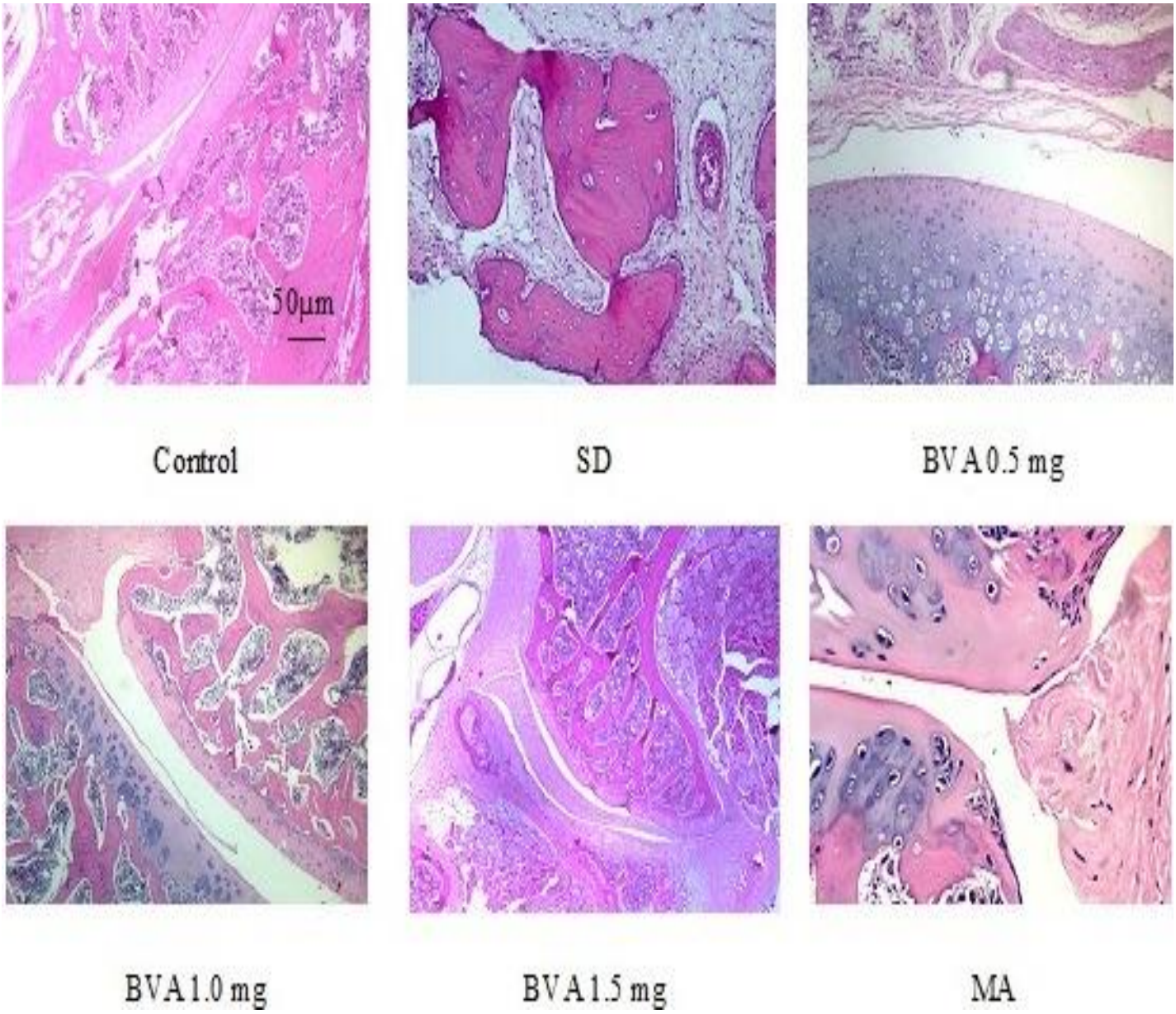

Figure 1: Histological images of spinal cord areas.

Conclusion

In this study, we investigated the anti-inflammatory, analgesic, and motor improvement effects of BVA at doses of 0.5, 1.0, and 1.5 mg/kg on CFA-induced SD rats. Our results show that BVA induced significant decreases in WBC count and serum concentrations of IL-1β and TNF-α, along with an increase in IL-10 serum concentration. In behavioral tests, BVA treatment increased paw-withdrawal latency in the hot plate test, motor ability in the open-field test, and motor coordination in the rotarod test of CFA-induced SD rats compared to saline-treated rats in the control group. These findings indicate that BVA at the specified doses exhibits potent anti-inflammatory, analgesic, and motor improvement effects on CFA-induced SD animals.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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