

# Synergistic effects of *Boswellia serrata* and *Acmella oleracea* extract combination for treating neuropathic pain in a preclinical model of spared nerve injury

Dear Editor in Chief,

We would like to submit a Letter detailing our recent findings on the pharmacological effect of a combination of *Boswellia serrata* and *Acmella oleracea* (code 9100135 dry extract 25% triterpenic acids for the *Boswellia serrata* and code 1136/45/A dry extract 3% alclaylamides for the *Acmella oleracea*) extracts in a mouse model of spared nerve injury-induced neuropathic pain. The spared nerve injury was induced as previously described by our group and others (Boccella, Cristiano, et al., 2019). Our study aimed to assess the potential synergistic effects of these extracts at different dosages and evaluate their antiallodynic efficacy in a peripheral neuropathic pain model. Neuropathic pain is a debilitating condition resulting from damage or dysfunction of the nervous system (Guida et al., 2020). The tactile allodynia, defined as a painful sensation perceived following an innocuous stimulus, is the major symptom that limit the quality of life of patients suffering from neuropathic pain. Current pharmacological interventions often provide limited relief and are associated with undesirable side effects. Because of their analgesic qualities and excellent safety profiles, natural compounds have drawn interest as viable add on to pharmaceuticals for the treatment of neuropathic pain.

For millennia, folk medicine has made use of the plant *Boswellia serrata*. Among consumers, it has grown in popularity as a treatment for a variety of chronic inflammatory disorders, including inflammatory bowel disease, asthma, arthritis, particularly osteoarthritis and pain. *Boswellia serrata* contains monoterpenes, diterpenes, triterpenes, tetracyclic triterpene acids, and pentacyclic triterpene acids, called boswellic acids (BAs)(Riva et al., 2017).

*Acmella oleracea* (L.) is the most common cultured species of the *Acmella* genus belonging to the *Asteraceae* family. It is native to South America and has been used all over the world for several purposes including pain management (Spinozzi et al., 2022). This plant contains a wide array of secondary metabolites, the N-alkylamides, including spilanthol, being mainly responsible for its biological and pharmacological properties. Intriguingly, recent report showed that the alkylamides contained in the plant could also act through cannabinoid and TRPV1 receptors (Yien et al., 2022).

To our knowledge, there are no data in the literature showing a potential synergistic effect of these two natural compounds.

We have used the up and down method of the von Frey filament test (Boccella, Guida, et al., 2019) to assess the allodynia. The analgesic activity of each substance was calculated as the percentage

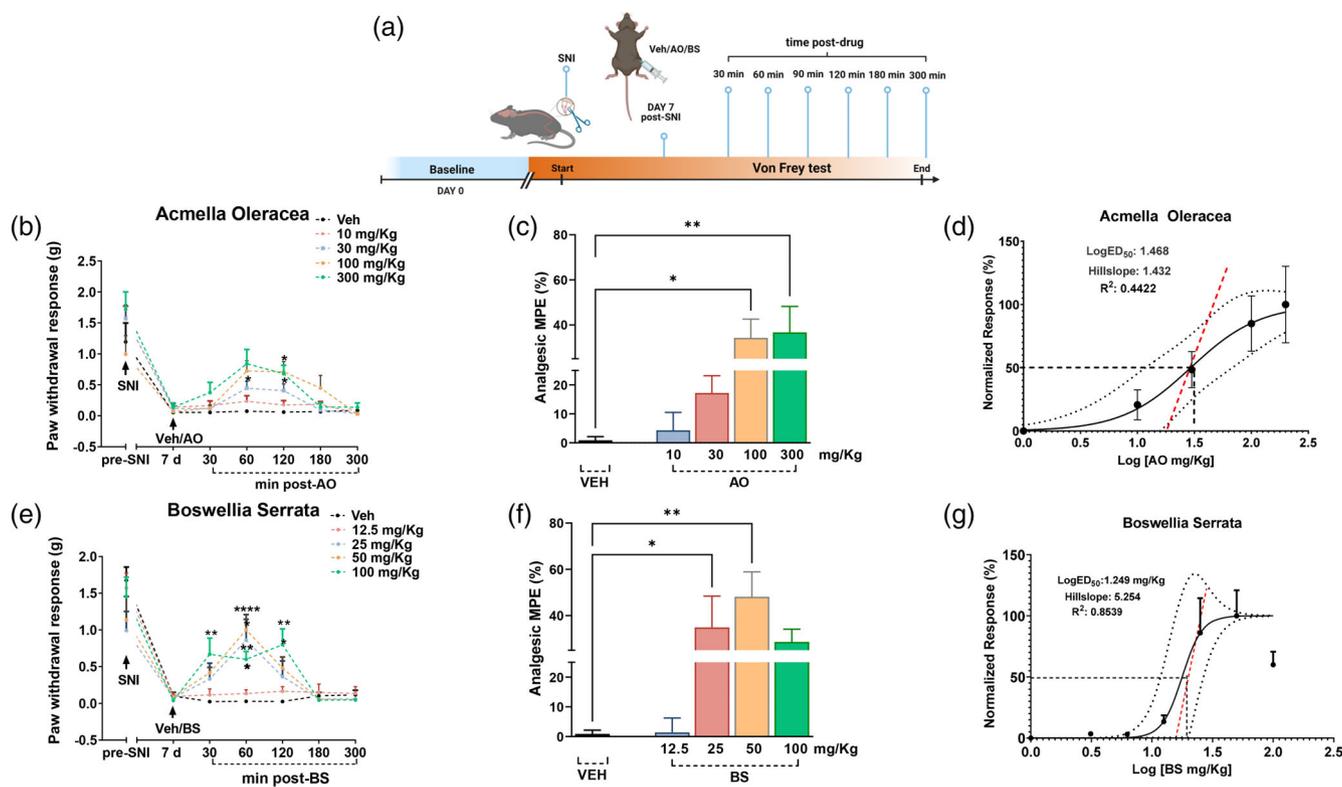
inhibition of maximal possible effect (%MPE) and a dose-response curve, through a non-linear regression analysis, was constructed to determine the dose that produced 50% of the MPE (ED50) (Tallarida, 2000). Finally, an isobolographic analysis was performed, combining the ED50 values of each substance, to obtain the line of additivity, as previously described (Miranda et al., 2006; Tallarida, 2000). As a first study we have used the male mice in order to avoid, at this stage, the estrous effect on pain detection. Further study in different models of pain will include female mice.

Briefly, C57BL/6J male mice (18–20 g, Envigo) were used and each animal was subjected to the Von Frey test the day before the induction of neuropathy (basal threshold). For acute therapeutic protocol, mechanical allodynia was repeated 7 days after the surgery, before and after the intra-peritoneal administration of the single substances or their combinations. The paw withdrawal response (PWT) was monitored in each group of animals, at different time intervals, up to 300 min (5 h) post-dosing, as shown in Figure 1a. For chronic therapeutic regimen, drug combination was daily administered for 25 days starting from the day after the surgery and mechanical allodynia was weekly monitored up to 30 days post-SNI (Figure 2a).

SNI mice were treated with a single administration of increasing doses of *Acmella oleracea* (AO: 10, 30, 100 e 300 mg/kg, i.p.) or *Boswellia serrata* (BS: 12.5, 25, 50, and 100 mg/kg, i.p.) at day 7 post-SNI. The treatment groups were compared with the respective vehicle. The vehicle per se did not exert any significant changes.

Single administration of AO induced a significant reduction of mechanical allodynia at highest doses in SNI mice, as compared with the **vehicle-treated group** (AO at 100 and 300 mg/kg were  $0.72 \pm 0.16$  g ( $p = 0.04$ ) and  $0.84 \pm 0.23$  ( $p = 0.049$ ), respectively, 60 min post-administration, Figure 1b). The corresponding %MPE were  $34.25 \pm 8.32\%$  and  $36.6 \pm 11.57\%$ , respectively (Figure 1c). On the contrary, lowest doses of AO (10 and 30 mg/kg) showed a trend of increasing mechanical threshold to  $0.23 \pm 0.09$  g ( $p = 0.84$ ) and  $0.44 \pm 0.1$  g ( $p = 0.84$ ), respectively, in SNI mice (Figure 1b). The effective dose value that produced 50% reduction of mechanical allodynia was ED50: 29.37 mg/kg (slope factor = 1.43) with a corresponding 95% confidence interval of (12.04–60.65), as shown in Figure 1d.

In BS-treated animals, an important reduction of mechanical allodynia was observed at doses of 25 ( $0.86 \pm 0.28$  g,  $p < 0.05$ ), 50 ( $1.0 \pm 0.20$  g,  $p = 0.01$ ) and 100 mg/kg ( $0.6 \pm 0.1$  g,  $p = 0.009$ ), with the maximum effect at 60 min after treatment (Figure 1e). The

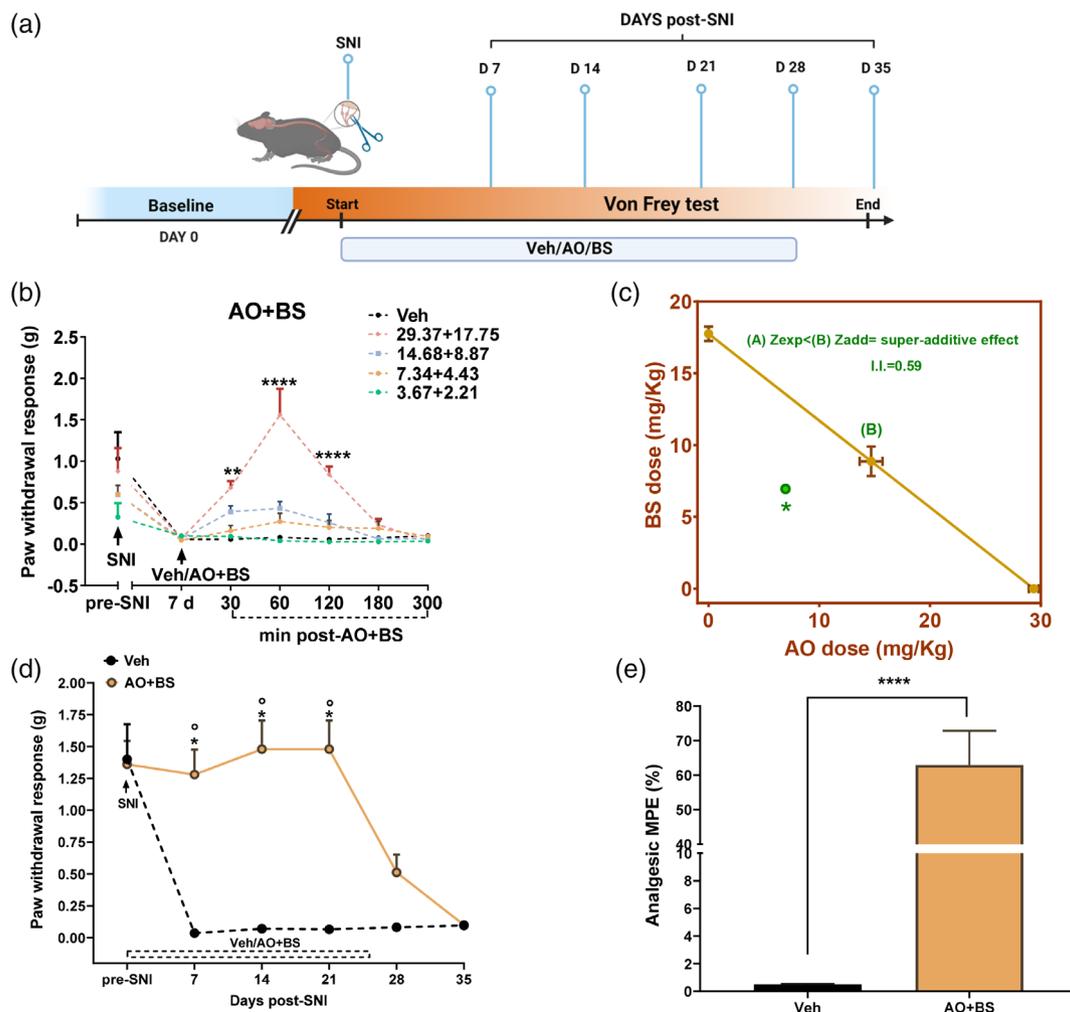


**FIGURE 1** (a) Timeline of the experimental protocol of acute treatment with *Acmella oleracea* and *Boswellia serrata*, outlining the periods of spared nerve injury (SNI) surgery, animal behavior testing and drug administration. (b) Mechanical nociceptive threshold of the ipsilateral paw to the lesion (expressed as applied force in grams), after the single injection of different doses of vehicle or of *Acmella oleracea*. (c) Percentage of the maximum possible anti-allodynic effect (%MPE) on the mechanical nociceptive threshold of the ipsilateral paw to the lesion, after single administration of different doses of vehicle or *Acmella oleracea*, 60 min post-administration. (d) Dose–response curve and ED<sub>50</sub> value for *Acmella oleracea* administration at increasing doses. (e) Mechanical nociceptive threshold of the ipsilateral paw to the lesion (expressed as applied force in grams), after the single injection of different doses of vehicle or of *Boswellia serrata*. (f) Percentage of the maximum possible anti-allodynic effect (%MPE) on the mechanical nociceptive threshold of the ipsilateral paw to the lesion, after single administration of different doses of vehicle or *Boswellia serrata*, 60 min post-administration. (g) Dose–response curve and ED<sub>50</sub> value for *Boswellia serrata* administration at increasing doses. Data are expressed as mean  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\*\* $p < 0.0001$  indicate significant differences versus vehicle-treated SNI mice ( $n = 7$ –8 per group).

corresponding %MPE induced by BS (25, 50 and 100 mg/kg), were  $34.8 \pm 13.5\%$ ,  $48.1 \pm 10.7\%$  and  $28.6 \pm 5.46\%$ , respectively (Figure 1f). In contrast, the 12.5 mg/kg dose induced no changes in mechanical threshold ( $0.13 \pm 0.05$  g,  $p = 0.79$ ) (Figure 1e). The effective dose value that produced 50% reduction in mechanical allodynia was ED<sub>50</sub>: 17.75 mg/kg (slope factor = 5.25) with a corresponding 95% confidence interval of (12.79–24.64), as shown in Figure 1g. Considering the analgesic effect following the single administration of BS or AO, the possible synergism of the co-administration of the double combination containing a fixed proportion of each substance (1:1), was determined (Figure 2b). The isobolographic analysis via non-linear regression showed that the co-administration of effective doses of AO+BS resulted in a super-additive interaction ratio, as a significant difference between Zadd and Zexp for each combination was revealed by Student's *t*-test (for unpaired data). In particular, the AO+BS combination had a Zexp of 13.91mg/kg and a Zadd of 23.55 mg/kg ( $p < 0.05$ ), with an interaction index equal to 0.59 (I.I) (Figure 2c). Our results revealed for the first time that combination of BS and AO extracts exerts a significant synergistic action in reducing

neuropathic pain symptoms in both acute and chronic regimen (Figure 2). The data clearly showed a significant anti-allodynic effect of chronic administration of AO+BA starting from day seven up to 3 weeks post-administration, compared with vehicle-treated group. In fact, the mechanical threshold 1 week after the beginning of treatment was  $1.28 \pm 0.19$  ( $p = 0.018$ ) (Figure 2d), with an inhibition percentage of the maximum possible effect (%MPE) of  $62.9 \pm 9.9\%$  compared to vehicle-treated group ( $0.51 \pm 0.05$ ) (Figure 2e)

These findings contribute to the understanding of potential treatment options for neuropathic pain management. The combination of *Boswellia serrata* and *Acmella oleracea* extracts demonstrated a synergistic effect, suggesting a potential novel approach for enhancing the analgesic effect of these natural products. Additionally, our study provides insights into the differential effects of the combination therapy in acute and chronic administration regimens, highlighting the importance of considering the timing of treatment. However, we acknowledge the need for further investigations to elucidate the precise mechanisms underlying this synergistic effect and to optimize dosing regimens for maximum efficacy and safety.



**FIGURE 2** (a) Timeline of the experimental protocol of chronic treatment with *Acemella oleracea* and *Boswellia serrata* combination, outlining the periods of spared nerve injury (SNI) surgery, animal behavior testing and drug administration. (b) Mechanical nociceptive threshold of the ipsilateral paw to the lesion (expressed as applied force in grams), after single administration of vehicle or AO + BS at increasing doses. (c) Isobolographic representation of the interaction between the AO + BS combination. Empty circles indicate  $Z_{exp}$  and full circles indicate  $Z_{add}$ . Horizontal and vertical bars indicate standard errors of the mean (SEM). (d) Mechanical nociceptive threshold of the ipsilateral paw to the lesion (expressed as applied force in grams), after repeated administrations of vehicle or AO + BS (29.37 + 17.75 mg/kg). (e) Percentage of the maximum possible anti-allodynic effect (%MPE) on the mechanical nociceptive threshold of the ipsilateral paw to the lesion, after repeated administrations of single dose of AO + BS (29.37 + 17.75 mg/kg), 60 min post-administration. Data are expressed as mean  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\*\* $p < 0.0001$  indicate significant differences versus vehicle-treated SNI mice. ° $p < 0.05$  indicate significant differences vs pre-SNI basal values ( $n = 7-8$  per group).

Because of the well-known safety profile of both plants, this letter paves the way to investigate the antiallodynic properties of this fixed combination also in patients.

#### AUTHOR CONTRIBUTIONS

**Serena Boccella:** Formal analysis; methodology. **Consalvo Mattia:** Conceptualization. **Michela Perrone:** Investigation; methodology. **Andrea Maria Morace:** Methodology. **Elif Karabacak:** Methodology. **Francesca Guida:** Writing – review and editing. **Sabatino Maione:** Conceptualization; writing – review and editing. **Livio Luongo:** Conceptualization; supervision; writing – original draft.

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#### CONFLICT OF INTEREST STATEMENT

Livio Luongo, Sabatino Maione are co-inventor of the patent number 10202000024217, owner Sanitas Farmaceutici Srl, Via Emilia 168, Tortona (AL).

#### DATA AVAILABILITY STATEMENT

Raw data will be available on request.

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