Annals of Agricultural and Environmental Medicine 2024, Vol 31, No 4, 506–512
www.aaem.pl

ORIGINAL ARTICLE

Influence of bee venom on antinociceptive activity of selected analgesic drugs in hot plate test in mice

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Zagaja M, Zagaja A, Szala-Rycaj J, Szewczyk A, Maruszewska A, Łuszczki J, Andres-Mach M. Influence of bee venom on antinociceptive activity of selected analgesic drugs in hot plate test in mice. Ann Agric Environ Med. 2024; 31(4): 506–512. doi: 10.26444/aaem/195108

Abstract

Introduction and Objective. The aim of the study was to investigate the effect of bee venom on the activity of two analgesics: ketoprofen (a non-steroidal anti-inflammatory drug) and tramadol (an opioid drug) in the acute thermal pain model (hot-plate test) in mice.

Materials and Method. Linear regression analysis was used to evaluate the dose-response relationship between logarithms of drug doses and their resultant maximum possible anti-nociceptive effects in the mouse hot-plate test. Doses that increased the anti-nociceptive effect by 20% (ED_{20} values) for bee venom, ketoprofen and tramadol, and their combination were calculated from linear equations. The interaction between bee venom and the selected anaglesics was evaluated using isobolographic analysis.

Results. The study showed that all compounds produced a definite anti-nociceptive effect, and the experimentally-derived ED_{20} values for bee venom, ketoprofen and tramadol, when applied indivisually, was 3.64 mg/kg, 79.88 mg/kg and 13.26 mg/kg, respectively. Isobolographic analysis revealed that the combination of bee venom and ketoprofen at a fixed ratio of 1:1 was supra-additive (synergistic). The experimentally-derived $ED_{20\,mix}$ was 26.33 mg/kg, which significantly differed from the $ED_{20\,add}$ of 41.76 mg/kg ($p < 0.5$). The experimentally-derived $ED_{20\,mix}$ of bee venom and tramadol was 2.90 mg/kg, and differed significantly from the theoretically estimated $ED_{20\,add}$ of 8.45 mg/kg ($p < 0.5$), also indicating a synergistic interaction in the hot-plate test in mice. Moreover, none of the tested combinations indicated any adverse effects in the chimney test and the grip-strength test in mice.

Conclusions. Overall, the obtained results demonstrated that bee venom significantly increased the anti-nociceptive activity of ketoprofen and tramadol in the hot-plate model of nociceptive pain in mice.

Key words

tramadol, ketoprofen, hot-plate test, bee venom

INTRODUCTION

Pain is a prevalent medical concern linked to the majority of medical conditions [1]. Finding a successful, pharmacological way to manage pain is a huge challenge for modern medicine and pharmacology. Despite the fact that a wide range of drugs have been available on the pharmaceutical market for decades, an ongoing search continues for novel remedies that are as effective as currently used analgesics while avoiding adverse effects [2, 3].

Many new compounds derived from animals, plants, and microorganisms have been examined for analgesic properties, and many of them demonstrate a wide range of biological activities, suppressing nociceptive responses in experimental pain models [4–8]. One such promising substance is bee venom (BV) from *Apis mellifera* – the honey bee [9]. Although honey bee stings cause local swelling and pain [10], the venom from this useful insect has historically been utilized for the treatment of inflammatory disorders and reduction of pain [11, 12].

Apitoxinotherapy is a fairly common form of BV treatment and involves the use of direct injections with bee stings. The venom is additionally used in the form of ointments and injections, and this therapy has been used since ancient times in Egypt, Greece, and China for rheumatism and arthritic patients. The method is still used to treat a variety of disorders throughout Asia, Eastern Europe, and increasingly, in Western Europe and the United States, for among others rheumatic arthritis, bursitis, tendonitis, shingles, multiple sclerosis, wounds, gout, burns, and infections. Bee venom therapy in people suffering from the afore-mentioned disorders aims to relieve pain and inflammation, restoring normal body functions [11, 13].

Bee venom (BV) consists of at least 18 active compounds, including enzymes, peptides, and biogenic amines, which are responsible for the analgesic properties of the venom. It also includes a wide range of peptides, including melittin (a major component of BV), phospholipase A2, apamin, adolapin, and mast cell degranulating peptide [14].

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Received: 25.09.2024; accepted: 21.10.2024; first published: 22.11.2024

Research results over the last few years have demonstrated the effectiveness of BV in various inflammatory pain models, including Freund's complete adjuvant-induced arthritis model in rats and mice [15–20], arthritic pain induced by collagen [21], in a carrageenan-induced inflammation [22, 23], formalin test in mice and rats (a reduction in nociceptive behaviour) [24–26], as well as in a visceral pain model (writhing test) induced by acetic acid in mice [24, 27, 28], or in the thermal analgesia hot plate testin mice [9]. Moreover, in the mouse model of paclitaxel-induced neuropathic pain, BV exerts strong suppressive effects [29]. BV also alleviates thermal hyperalgesia, mechanical and cold allodynia in rat neuropathic pain models, induced by sciatic nerve injury [30, 31], as well as chemotherapy-induced neuropathy in mice [32, 33]. In a migraine mouse model, repetitive BV therapy reduced nitroglycerin-induced face and hind paw hypersensitivities and c-Fos levels [34].

Currently, there are two main types of analgesics available on the pharmaceutical market: non-steroidal anti-inflammatory drugs (NSAIDs), and opioids, which are the most often used analgesics in the treatment of acute and chronic pain. It is commonly accepted that these medications have both peripheral and central effects on pain modulation [35].

Due to the adverse effects of painkillers, particularly opioids, they are frequently used as combination therapy which provides a better analgesic effect while limiting negative side- effects [36]. Such polytherapy is used, among others, for post--operative pain treatment, in which opioid medications are combined with NSAIDs [36, 37]. Combinations of drugs with analgesic effects and synergistic with each other should reduce the required dose and, consequently, reduce the incidence of side-effects [38].

Considering recent research findings about the anti-nociceptive features of BV in several experimental models of both acute and chronic pain, it seemed justified to examine the interactions between this substance and analgesics applying the hot plate test in mice. This standard model which allows determination of the analgesic effect of the tested substances, will allow assessment of the impact of BV on the analgesic properties of ketoprofen (KET) and tramadol (TRA).

MATERIALS AND METHOD

Animals and experimental conditions. The male adult albino Swiss mice used in the experiments weighed between 22–26 g. The animals were kept in standard laboratory conditions in colony cages, with a 12-hour natural light-dark cycle, a temperature range of 20–24 °C, air humidity of 45–65%, and free access to food (chow pellets) and tap water. Following a week of acclimation to lab conditions, the animals were randomized into 8 mouse groups for the experiments. The experimental procedures used were approved by the Local Ethics Committee in Lublin, Poland (Approval No. 23/2014) and were conducted in accordance with EU Directive 2010/63/EU for animal experiments.

Drugs. The drugs used in the current study were: ketoprofen (Ketonal® forte, Sandoz GmbH, Kundl, Austria; KET), tramadol (Tramal® Grünenthal GmbH, Aachen, Germany; TRA) and bee venom from *Apis mellifera* (Sigma-Aldrich, St. Louis, *MO*, *USA; BV*). KET and TRA were suspended in a 1% aqueous solution of Tween 80 (Sigma-Aldrich, St. Louis, MO, USA) in distilled water, while BV was directly dissolved in distilled water only. Drugs were injected intraperitoneally (*i.p.*), and BV subcutaneously (*s.c.*) 30 min. before the hot-plate test, administered with 1 mL syringes as a single injection in a volume of 10 mL/kg. BV was administered at doses ranging from 0.5–10 mg/kg, KET at 50–250 mg/kg, and TRA from 5–30 mg/kg. These pre-treatment times were selected on the basis of biological activity information obtained from the literature [9, 39, 40].

Hot-plate (HP) test. The HP test is a common model to examine the anti-nociceptive effectiveness of drugs in relation to acute heat nociception [41]. The device used was an electrically heated surface and an open Plexiglas tube to confine the animals to a temperature of 55.0 ±0.1 °C. (Ugo Basile, Varese, Italy). The absence of a nociceptive response in mice exposed to heat stimuli was defined as the maximum possible anti-nociceptive effect (MPAE), and the percentage of MPAE was calculated using the method published by Schmauss and Yaksh [42]. Then, using the linear dose-response equation, a dose of the drug that raised the AE by 20% (ED_{20} value) was calculated. A more detailed description of this experimental method is included in previous studies by the authors of the current study [6, 41, 43].

Isobolographic analysis (IA). The interaction of KET or TRA (both analgesic drugs) with BV, with respect to the antinociceptive effect (AE) produced by these drugs in the HP test, were investigated using the IA, as detailed in other studies [6, 41, 43].

The $ED_{20\,add}$ indicates the total additive dose of used drugs in the combination, which theoretically enhances the AE by 20% in the HP test in mice. The $ED_{20\,mix}$ is an experimentally estimated total dose of a combination of 2 drugs at a constant proportion of 1:1, resulting in a 20% increase in AE in animals in the HP test.

The current study established the ED_{20} values for KET, TRA and BV, as well as the combination of BV and KET or TRA, which correlated with the doses of drugs and their combination and resulted in a 20% AE in the HP test conducted on mice. In the HP test, the anti-nociceptive effect observed in mice could not reach a 100% effect because the animals would be unable to fulfill and respond to the thermal stimulus, which would be destructive and harmful for the animals. On the other hand, a 20% anti-nociceptive effect for BV, KT and TRA was strong enough to detect the anti-nociceptive properties of drugs and their mixture in animals, without any acute adverse effects produced by the drugs at doses corresponding to their ED_{20} values. Furthermore, in other studies, the maximum BV dose used was 10 mg/kg [9], therefore, the ED_{50} values were not evaluated in the current study.

Chimney Test. The chimney test was used to evaluate the effects of KET and TRA administered alone and in combination with BV (at doses that matched their ED_{20} values from the HP test) on mice's motor coordination, as described elsewhere [44, 45].

Grip-Strength Test. The effects of KET and TRA administered alone, and in combinations with BV (in doses reflecting their ED_{20} values from the HP test), on muscular strength

of forelegs in mice were determined with the grip-strength test, also described elsewhere [45, 46].

Statistical analysis. The ED_{20} values with SE were computed using least-squares linear regression analysis. According to Tallarida [47], the isobolographic interaction between chosen drugs and BV was statistically evaluated using a Student's t-test with Welch's correction to detect differences between experimentally derived ($ED_{20\ mix}$) and theoretical additive ($ED_{20\ add}$) values [47]. Results of the chimney test were compared using Fisher's exact probability test. The grip-strength test results were statistically verified using one-way ANOVA with Bonferroni's *post-hoc* test. Statistical analyses were performed using the commercially available GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

Effects of BV on the anti-nociception in the mouse HP test. BV administered 30 min. before the HP test delayed the first painful reaction in mice in a dose-related way. The experimentally determined MPAE values for BV ranged from 8.4% – 28.0%. The dose-response equation for BV, as determined by least-squares linear regression, was y = 1.544x + 14.37 (R^2 = 0.764). The ED_{20} value for BV in the HP test was 3.64 mg/kg (Fig. 1, 2).

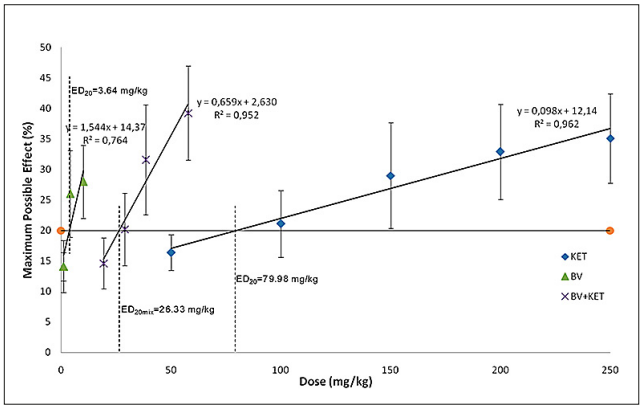

Figure 1. Dose-response effects of KET, BV and the combination of the 2 substances at a fixed ratio of 1:1 in the HP test in mice.
y – max. possible effect value (in %); x – log dose (mg/kg) of KET or BV administered alone, or in combination with KET at a fixed ratio of 1:1; R^2 – coefficient of determination

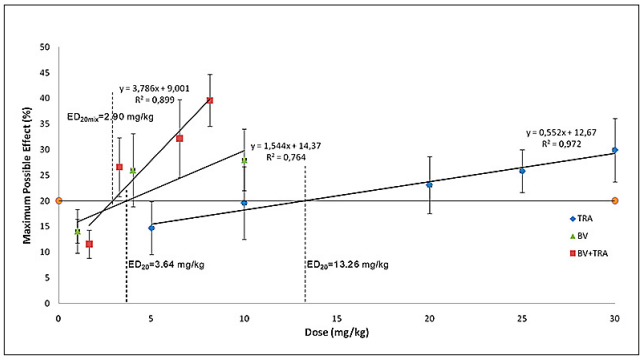

Figure 2. Dose-response effects of TRA, BV and combination of the two substances at a fixed ratio of 1:1 in the HP test in mice.
y – maximum possible effect value (in %); x – log dose (mg/kg) of TRA or BV administered alone, or in combination with TRA, at a fixed ratio of 1:1; R^2 – coefficient of determination

Effects of KET, TRA, and their combination with BV on the anti-nociception in the mouse HP test. The administration of KET before the HP test increased the latency to the first pain reaction in mice in a dose-dependent way. The experimentally obtained values of the MPAE for KET ranged between 16.4% – 35.1%. The equation for the KET dose-response relationship was: y = 0.098x + 12.14 (R^2= 0.962). Therefore, the experimentally estimated ED_{20} value in the HP test was 79.88 mg/kg (Fig. 1). The combination of KET and BV at a fixed ratio of 1:1 improved the delay of the onset pain reaction in the HP test in a dose-related manner. The empirically determined MPAE values for the combination, given at doses ranging from 19.23–57.70 mg/kg, varied from 14.6% – 57.7%. Least-squares linear regression found that the experimentally obtained equation for the KET/BV combination was: y = 0.659x + 2.63 (R^2 = 0.952). $ED_{20\ mix}$ value corresponding to the mixture's dose of 26.33 mg/kg (Fig. 1).

Identically, TRA given before the mouse HP test, increased the delay to the first pain response in a dose-related manner. The experimentally determined MPAE values for TRA varied from 14.7% – 29.9%. The equation for the TRA dose-response relationship was: y = 0.552x + 12,67 (R^2= 0.972). Therefore, the empirically calculated ED_{20} was 13.26 mg/kg (Fig. 2). In mice, a 1:1 combination of TRA and BV delayed the latency to the first pain reaction in the HP test in a dependent on dose way. The experimentally determined MPAE values for the combination, given at doses ranging from 1.63–8.14 mg/kg, varied from 11.6% – 39.6%. The experimentally derived equation for the TRA/BV combination was y = 3.786x + 9,00 (R^2 = 0.959), according to least-squares linear regression. Consequently, experimentally obtained $ED_{20\ mix}$ in the HP test corresponded to the mixture's dose of 2.90 mg/kg (Fig. 2).

IA of KET and BV combination in the mouse HP test. When KET and BV were combined at a constant proportion of 1:1, the results of an unpaired Student's t-test followed by Welch's correction showed that the combination was supra-additive (synergistic) in the HP test (Tab. 1; Fig. 3). The experimentally calculated $ED_{20\ mix}$ was 26.33 mg/kg, which significantly differed from the ED_{20add} of 41.76 mg/kg ($p < 0.5$; Tab. 1; Fig. 3). Table 1 shows the separate doses of KET and BV in the combination at a constant ratio of 1:1, determined using the $ED_{20\ add}$ and $ED_{20\ mix}$ values.

IA of TRA and BV combination in the mouse HP test. Statistical evaluation of the results indicated that the combination of TRA and BV caused a supra-additive (synergistic) interaction in the HP test (Tab. 1; Fig. 4). The experimentally derived $ED_{20\ mix}$ was 2.90 mg/kg, which significantly differed from the ED_{20add} of 8.45 mg/kg ($p < 0.5$; Tab. 1; Fig. 4). Table 1 shows the separate doses of TRA and BV in the mixture at a constant ratio of 1:1, determined via the $ED_{20\ add}$ and $ED_{20\ mix}$ values.

Behavioural test – grip strength test and chimney test. BV (1.2 mg/kg) in combinations with KET (37.15 mg/kg) and TRA (5.3 mg/kg) neither altered skeletal muscular strength in mice subjected to the grip strength test, nor impaired motor performance in the animals subjected to the chimney test (Tab. 2).

There was no significant distinction in motor performance or muscle strength in mice when KET and TRA were given alone at doses matching their ED20 values from the HP test (Tab. 2).

Table 1. Isobolographic characterization of interaction between KET and BV at the fixed ratio of 1:1 in the HP test in mice

KET_{add}	BV_{add}	$ED_{20\,add}$	n_{add}	$ED_{20\,mix}$	KET_{mix}	BV_{mix}	n_{mix}
39.94	1.82	41.76±3.52*	52	26.33±6.72	25.18	1.15	32
TRA_{add}	BV_{add}	$ED_{20\,add}$	n_{add}	$ED_{20\,mix}$	TRA_{mix}	BV_{mix}	n_{mix}
6.63	1.82	8.45±2.03*	44	2.90±0.46	2.28	0.62	24

ED_{20mix} - experimentally determined dose of a mixture that increased the AE by 20%; $ED_{20\,add}$ - theoretically determined from the equation of additive, dose of a mixture that enhanced the AE by 20%;BV_{add}, KET_{add}, TRA_{add} and BV_{mix}, KET_{mix} : TRA_{mix} - the doses of drugs that comprised the mixture, for both $ED_{20\,mix}$ and ED_{20add} values; n – total number of animals employed at the doses at which expected AE was larger than 16%, designated for the experimental combination of substances (n_{mix}) and theoretically estimated (n_{add}) using the additivity equation.
*p < 0.01 vs. the $ED_{20\,add}$

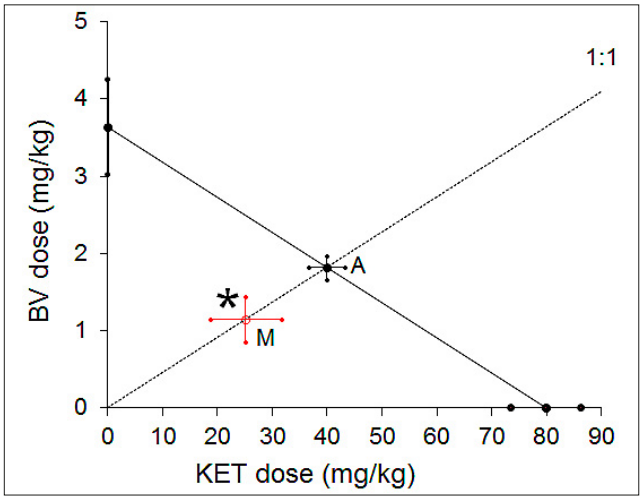

Figure 3. Isobologram illustrating the supra-additive (synergistic) interaction for the combination of BV with KET in the HP test in mice.
A – theoretical additive $ED_{20\,add}$ (± SE as error bars) for the total dose expressed as the proportion of KET and BV that produced a 20% AE; M – experimentally-derived $ED_{20\,mix}$ (± SE as the error bars) for the total dose expressed as the proportion of KET and BV produced a 20% AE. The $ED_{20\,mix}$ for the fixed ratio of 1:1 is placed significantly below the line of additivity, indicating the supra-additive (synergistic) interaction between BV and KET in the HP test in mice.
*p < 0.05

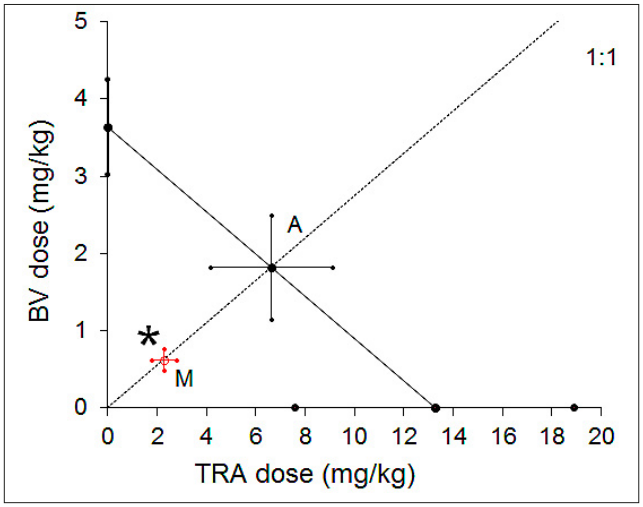

Figure 4. Isobologram illustrating the supra-additive (synergistic) interaction for the combination of BV and TRA in the HP test in mice.
A – theoretical additive $ED_{20\,add}$ (± SE as error bars) for the total dose expressed as the proportion of TRA and BV that produced a 20% AE; M – experimentally-derived $ED_{20\,mix}$ (± SE as error bars) for the total dose expressed as the proportion of KET and BV that produced a 20% AE. The $ED_{20\,mix}$ for the fixed ratio of 1:1 is placed significantly below the line of additivity, indicating the supra-additive (synergistic) interaction between BV and TRA in the HP test in mice.
*p < 0.05.

Table 2. Effects of combinations of BV with 2 anaglesic drugs on muscular strength and motor performance in mice

Drug [mg/kg]	Grip strength (gf)	Motor coordination impairment (%)
Vehicle	125.8±4,99	0
KET (39.94)	120.0±1,25	0
BV (1.82) + KET (39.94)	112.6±3,86	25
TRA (6.63)	114.7±4,55	0
BV (1.82) + TRA (6.63)	108.4±7,43	25

DISCUSSION

The obtained results show that KET, TRA, and BV produced dose-dependent anti-nociceptive effects in the mouse HP test. BV was tested at doses up to 10 mg/kg, and the substances produced a clear-cut AE with the ED_{20} value of 3.64 mg/kg, confirming the AE of BV in the HP test. Likewise, KET was tested at doses up to 250 mg/kg, and showed a strong AE with the ED_{20} value of 79.88 mg/kg in the HP test. TRA tested at doses up to 30 mg/kg, produced AE with the ED_{20} value of 13.26 mg/kg. IA demonstrated that combination BV with both KET and TRA resulted in a supra-additive (synergistic) interaction in the mouse HP test. Consequently, their molecular mechanisms of action should be taken into account in order to comprehend the synergistic interaction between BV and these 2 analgesics observed in the current study.

With respect to BV, scientists have proposed several mechanisms for anti-nociceptive effect of the venom, which involve the activation of α2-adrenergic receptors in the central nervous system [21, 26, 27, 30, 48], as well as activation of the descending serotonergic pathway [25].

Roh et al. [26, 30] demonstrated the potential anti-hyperalgesic and anti-allodynic effects of BV, as well as the relevance of the α2-adrenoceptors in a rodent neuropathic pain model. Pre-treatment with the α2-adrenoceptor antagonist yohimbine totally prevented the anti-nociceptive action of BV; however, administration of the opioid antagonist naloxone or the serotonin antagonist methysergide had no effect [48]. Moreover, Baek et al. [21] showed that the anti-nociceptive action of BV in inflammatory pain, including in the collagen-induced arthritis rat model, involved the α2-adrenergic receptor but not the opioid receptor. BV can reduce cold allodynia in sciatic nerve chronic constriction injury rats by activating just the α2-adrenoceptor in the spinal cord, rather than the α1-adrenoceptor or β-adrenoceptor. Because the effect of high-dose BV was completely blocked by intrathecal pre-treatment of the α2-adrenoceptor antagonist idazoxan, but not prazosin (α1-adrenoceptor antagonist) or propranolol (non-selective β-adrenoceptor antagonist)

[49]. In contrast, intrathecal injections of idazoxan or methysergide significantly decreased BV-induced antinociception in the rat formalin test. These data confirm that both the α2 adrenergic and serotonergic receptors are activated to cause BV-induced antinociception [25].

Other mechanisms have also been suggested to explain the anti-inflammatory and anti-nociceptive effects induced by BV. In experimental rheumatoid arthritis, BV treatment significantly decreased the level of inflammation-related mediators, such as cyclooxygenase-2 (COX-2), phospholipase A_2, tumour necrosis factor alpha (TNF-α), interleukin (IL)-1, interleukin (IL)-6, nitric oxide (NO), and reactive oxygen species (ROS) [11]. BV inhibits pro-inflammatory cytokines by activating NF-κB [50]. BV also decreases the production of pro-inflammatory cytokines, prostaglandin E2, NO, and COX-2 in murine glial cell cultures induced by lipopolysaccharides [51].

KET, a non-steroidal anti-inflammatory drug belonging to the class of derivatives of arylcarboxylic acid, has analgesic, anti-pyretic, and anti-inflammatory qualities [52]. Its main modes of action involve inhibiting both forms of cyclooxygenase COX-1 and COX-2, causing a reduction in prostaglandin production in the peripheral and central nervous systems [53], as well as inhibition of lipoxygenase, leading to a reduction in leukotriene synthesis [54]. The drug is beneficial in the treatment of many diseases, such as: rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, acute bouts of gout, inflammation-related pain, post-operative pain, trauma, and also dental pain [55]. Moreover, KET exhibits efficient anti-nociception in different animal models when pain is provoked by tissue inflammation [56].

In turn, TRA is a centrally acting analgesic, an atypical opioid, structurally similar to both morphine and codeine. TRA's two enantiomers increase pain inhibition by modulating neurotransmitters: the (+)-enantiomer inhibits serotonin reuptake, while the (-)-enantiomer inhibits norepinephrine reuptake. Furthermore, both the (+)-enantiomer and (+)-O-desmethyl-tramadol, the main metabolite of tramadol, function as μ-opioid receptor agonists [57]. Due to the synergistic analgesic effects of both tramadol enantiomers, tolerability is increased by a reduction in the adverse effects of other opioids, such as constipation, respiratory depression, and abuse potential [57, 58].

By considering the molecular mechanisms of action of both BV and the analgesic drugs, it can be shown that the investigated compounds' comparable mechanisms of action lead to the reduction of nociception in the nervous systems. As for BV and KET, inhibition of cyclooxygenase seems to be the main mechanism. In turn, TRA and BV combination inhibits serotonin reuptake which is common for these substances as they are likely to be responsible for the observed synergistic interaction in the HP test in mice. It is well acknowledged that drugs with similar modes of action result in an additive interaction when the partial effects of each individual drug in the combination are added up [59].

Kim et al., [60] showed more beneficial effects of BV and morphine combination on oxaliplatin-induced neuropathic pain in mice, compared to the BV or morphine alone. The analgesic effect of this combination was significantly blocked by intrathecally administered naloxone and MDL-72222 (5-HT_3 receptor antagonist), but not by idazoxan, demonstrating that opioid and 5-HT_3 receptors, but not α2-adrenergic receptors, are responsible for this effect. Therefore, as in the case of morphine and also in the case of TRA, it cannot be assumed that the synergistic effect of this drug with BV results from the activation of serotonin receptors by both substances. In turn, BV enhances the analgesic effect of clonidine, which belongs to the α2-adrenoceptor agonists group, in chronic constriction injury-induced neuropathic pain model [61]. In this case, the synergistic effect is probably due to the activation of α2-adrenergic receptors.

However, it is possible that the observed synergistic interaction in the HP combination test of BV with the selected analgesic drugs results from the activation of different but complementary mechanisms of anti-nociception. Consequently, the systemic administration of the combination of BV and selected analgesic drugs would produce synergistic interactions. According to Miranda and Pinardi [62], the supra-additivity effect of clonidine with NSAIDs may be explained by the fact that they work through distinct mechanisms: NSAIDs acts through spinal COX-2 inhibition while clonidine acts directly on dorsal horn neurons and via activation of descending inhibitory mechanisms.

Comparing the doses of BV and KE or TRA in the mixture at a fixed ratio of 1:1, a considerable reduction of drug doses can be observed when both drugs were used in combination. Consequently, reducing drugs' doses may contribute to limiting the acute adverse effects caused by these chemicals when used alone at high effective doses [63]. Taking this into consideration, the authors conducted behavioural tests for both KET and TRA given separately and their combination with BV at doses corresponding to their ED_{20} values. The results showed no adverse effects for the tested combinations in impairing motor coordination and skeletal muscle strength in the tested animals.

To summarise, if the findings of the current research could be extrapolated to clinical settings and additionally confirmed in different experimental models of pain, the combination of BV with KET or TRA may be effective for the alleviation of pain in patients. However, the findings show that BV, when combined with KET or TRA, has a synergistic effect in the HP test, and it should be borne in mind that injecting BV may cause anaphylaxis or systemic reactions in those who are allergic. Furthermore, the cellular and molecular mechanisms of BV as an anti-nociceptive substance remain unclear and require further substantial research to limit possible negative effects [12].

CONCLUSIONS

BV and the selected analgesics – KET and TRA – showed an anti-nociceptive effect, and when combined at a constant ratio of 1:1, demonstrated a supra-additive (synergistic) interaction in the mouse HP test. Additionally, in behavioural tests, neither combination significantly affected skeletal muscle strength or motor coordination in the tested animals. Nevertheless, the results obtained regarding the synergistic interaction of BV with these two drugs should be confirmed in other models of acute and/or chronic pain. Furthermore, additional research is needed to fully understand the molecular mechanism of the action of BV, and evaluate the long-term safety of its therapeutic use.

Results are presented as: (1) mean grip-strengths (in gfs ± S.E.M.) from the grip-strength test, assessing muscular

strength in mice; and (2) percentage of animals showing motor coordination impairment in the chimney test in mice.

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