Published by SET Publisher

Journal of Pharmacy and Nutrition Sciences

ISSN (online): 1927-5951

Participation of the TRPA1 Receptor in the Antinociceptive and Anti-Inflammatory Effect of the α, β-Amyrin Mixture in Adult Zebrafish (*Danio rerio*)

Marnielle Rodrigues Coutinho[1], Antonio Wlisses da Silva[2], Maria Kueirislene Amâncio Ferreira[2], Emanuela de Lima Rebouças[1], Jesyka Macedo Guedes[2], Amanda Maria Barros Alves[2], Marcia Machado Marinho[3], Emmanuel Silva Marinho[2], Jane Eire Silva Alencar de Menezes[2], Maria Izabel Florindo Guedes[1] and Helcio Silva dos Santosa[1,2,3,*]

[1]*State University of Ceara, Postgraduate Program in Biotechnology, Fortaleza, CE, Brazil*

[2]*State University of Ceara, Graduate Program in Natural Sciences, Fortaleza, CE, Brazil*

[3]*State University of Vale do Acaraú, Chemistry Course, Sobral, CE, Brazil*

Article Info:

Keywords:
Amyrin,
Inflammatory,
Antinociceptive,
Zebrafish,
TRPA1,
Camphor.

Timeline:
Received: April 16, 2025
Accepted: May 15, 2025
Published: June 19, 2025

Citation: Coutinho MR, da Silva AW, Ferreira MKA, de Lima Rebouças E, Guedes JM, Alves AMB, Marinho MM, Marinho ES, de Menezes JESA, Guedes MIF, dos Santosa HS. Participation of the TRPA1 receptor in the antinociceptive and anti-inflammatory effect of the α, β-amyrin mixture in adult zebrafish (*Danio rerio*). J Pharm Nutr Sci 2025; 15: 52-59.

DOI: https://doi.org/10.29169/1927-5951.2025.15.06

Abstract:

Inflammatory pain is a symptom associated with different human diseases, and they are treated with drugs that have several serious side effects. The triterpene mixture of α, β-amyrin (ABAM) has already been reported with antinociceptive and anti-inflammatory effects in mice. The antinociceptive potential of ABAM possibly involves the mechanisms of opioid and vanilloid receptors. This study aimed to determine the antinociceptive and anti-inflammatory potential of ABAM in adult zebrafish by modulating TRPA1. Thus, ABAM had its antinociceptive effect investigated by the nociception model in adult zebrafish induced by formalin. Furthermore, the possible neuromodulation by the TRPA1 channel was evaluated by pre-treatment with camphor. ABAM exhibited significant antinociceptive and anti-inflammatory effects in adult zebrafish at all tested doses. These effects, potentially mediated through TRPA1 modulation, were observed without signs of toxicity over a 96-hour period."

*Corresponding Author

E-mail: helciodossantos@gmail.com

1. INTRODUCTION

Inflammatory pain is a common and debilitating condition associated with functional and morphological changes in the spinal cord, whose underlying neurobiological mechanisms remain incompletely understood despite advances in recent decades [1]. Among the molecular components involved in nociceptive signaling, the transient receptor potential ankyrin 1 (TRPA1) stands out as a non-selective cation channel that helps define the nociceptive threshold. It is expressed in C-fiber nociceptors and various non-neuronal cells, contributing to the release and modulation of pro-inflammatory mediators [2]. TRPA1 is also known as a polymodal sensor, activated by thermal and chemical stimuli, and structurally distinguished from other TRP channels by its ankyrin repeats and specific glycosylation sites [3].

Chronic pain (also known as inflammatory pain) is characterized as unrelieved and persistent, lasting more than 3 months, and usually treated with non-steroidal anti-inflammatory drugs (NSAIDs), anticonvulsants, tricyclic antidepressants and opioids [3,4]. However, even having all these treatment alternatives, many patients still complain that their pain cannot be controlled sufficiently [5], and many of these drugs for the treatment of inflammatory pain cause serious side effects such as dependence, which makes it necessary to the search for new targets to develop analgesics with greater power of action in inflammatory pain and less adverse effects.

The triterpene mixture of α, β-amyrin derived from *Protium heptaphyllum* Aubl. has already been reported with several pronounceable pharmacological effects, from actions on the central and peripheral nervous system to the gastrointestinal tract and immune system [6]. ABAM also has reported analgesic and anti-inflammatory effects. ABAM caused peripheral and central analgesic effects without the involvement of opioid channels and anti-inflammatory activity. This activity was potentiated by both indomethacin and thalidomide, indicating the potential involvement of prostaglandins and inhibitions of tumor necrosis factor-alpha (TNF-α) [7]. The antinociceptive effect of ABAM was also investigated in the orofacial pain model induced by formalin or capsaicin, demonstrating a potential effect on inflammatory pain [8].

Zebrafish is frequently used as a model vertebrate organism for pre-clinical trials to evaluate the pharmacodynamics (absorption, distribution, metabolism and excretion) and pharmacokinetics of new bioactive compounds [9]. The genome encodes two TRPA1 genes in zebrafish: trpa1a and trpa1b [10]. Increased locomotor activity in zebrafish was previously observed by thermal and chemical activation of TRPA1 channels [11]. Harmful agents such as formalin and cinnamaldehyde have already been reported in the literature as responsible for causing motor impairment through neuromodulation of TRPA1 channels [12, 13].

In this context, the present study is the first to evaluate the antinociceptive and anti-inflammatory effects of ABAM in adult zebrafish through the modulation of TRPA1 channels. By using a formalin-induced nociception model combined with TRPA1 pharmacological manipulation, we aimed to clarify the mechanism of action of ABAM and highlight its biotechnological potential as a natural analgesic agent for inflammatory pain with fewer adverse effects.

2. MATERIALS AND METHODS

2.1. Drugs and Reagents

In this study, the following substances were used: Formaldehyde (Neo Química®), camphor (Can; Sigma-Aldrich) and carrageenan (Sigma-Aldrich).

2.2. Obtaining a Mixture of α- and β-Amyrin

To obtain the ABAM (Figure **1**), the *P. heptaphyllum* was isolated. Fractionation of the resin (20 g) was performed by column chromatography on silica gel with hexane, chloroform, ethyl acetate and methanol. Fractions extracted with chloroform (5.2 g) were repeatedly chromatographed on silica gel and eluted with increasing amounts of hexane-ethyl acetate. Fractions obtained from hexane: acetate (1:1) were analyzed by TLC and contained 450 mg of alpha and beta-amyrin [14].

2.3. Zebrafish

Adult wild zebrafish (*Danio rerio*) of both sexes (age 90 to 120 days; 0.4 ± 0.1 g, 3.5 ± 0.5 cm) was purchased at a local store (Fortaleza, CE). The fish were kept in a glass aquarium (30 × 15 × 20 cm) of 10 L (n = 3 / L), at a temperature of 25 ± 2 ° C, in light-dark cycles of 24 h with chlorinated water (ProtecPlus®) and air pump with submerged filters, under a temperature of 25 ° C and pH 7.0, Circadian cycle of 10 - 14 h (light/dark). The fish received food twice daily (Alcon BASIC™, Alcon, Brazil) up to 12 h before the experiments [15]. Before

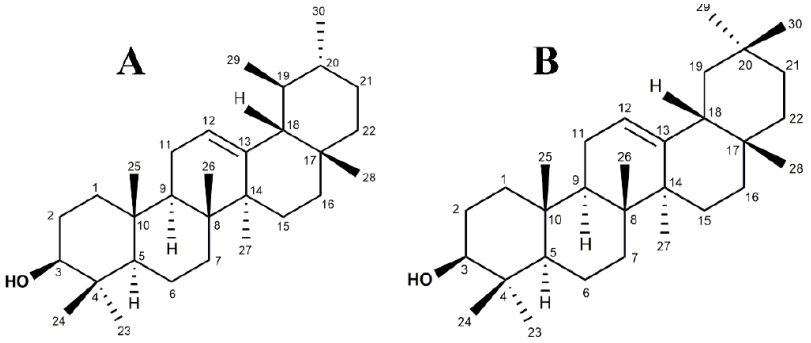

Figure 1: Structural formula of α, β-amyrin compounds: (**A**) α-amyrin (3α-hydroxyurs-12-ene) and (**B**) β-amyrin (3β-hydroxyolean-12-ene).

drug applications, the animals were anesthetized in ice water After the experiments, the animals were euthanized by immersion in ice water (2 and 4 °C) until loss of opercular movements [16]. The work was approved by the Ethics Committee for the Use of Animals of the State University of Ceará), following the Ethical Principles of Animal Experimentation (CEUA-UECE; nº 04983945/2021).

2.4. General Protocol

Adult fish of both sexes were chosen randomly in the experiments, transferred to a damp sponge, and treated with the test sample or controls orally (*p.o.*) or intramuscularly (*i.m.*), based on the methodologies proposed by Bezerra *et al*. [12]. Then, the animals were conditioned individually in a container (500 ml) containing 350 ml of aquarium water and kept at rest. For intraperitoneal (*i.p.*) and intramuscular (*i.m.*) treatments, insulin syringes (0.5 mL; UltraFine® BD) with a 30G needle were used. Next, groups of animals were treated with ABAM and submitted to toxicity, nociceptive and inflammation tests. In nociception tests, animal behavior was recorded by blind analyzers. After the treatments and application of the noxious agent, the animals were individually placed in a glass Petri dish containing four quadrants (10 × 15 cm). The nociceptive behavior response was quantified through the number of times crossed in the lines of the noxious agent's quadrants by fish for the formalin-induced nociception model described below.

2.5. Treatments

To perform the nociception tests, the animals (n = 6/group) were orally treated (20 µL) with ABAM (4; 20 and 40 mg/kg), morphine (8 mg/kg - positive control) or vehicle (3% DMSO solution - negative control). In the inflammation test, the animals (n = 6/group) were treated orally (20 µl) with ABAM (4; 20 and 40 mg/kg, *p.o.*); ibuprofen (100 mg/kg, *p.o.* – Positive control); and group treated with 3% DMSO (negative control) was included. For oral treatments, an automatic 20 µl pipette was used.

2.6. Formalin-Induced Nociceptive Behavior

Groups of adult fish (n = 6/group) were pretreated (20 µl; *p.o.*) with ABAM (4 or 20 or 40 mg/kg) or morphine (8 mg/kg; positive control) or vehicle (DMSO solution to 3%; negative control), 30 min before i.m. of formalin (0.1%; TRPA1 agonist) applied to the tail of the animals. Analgesia was evaluated through locomotor activity by the number of crossings performed in the open field test in a petri dish analyzed over 30 minutes, calculated both in the neurogenic phase (0–5 min) and the inflammatory phase (15–30 min) [12].

To evaluate the neuromodulation of the antinociceptive effect of ABAM on nociception induced by formalin through TRPA1 channels, the mechanism of action with camphor (TRPA1 antagonist) was performed. A new group of adult animals (n = 6/group) was pre-treated (20 µL *i.p.*) with camphor (30.4 mg/kg), and after 30 min, they received the lowest effective dose (which induced antinociceptive behavior) of ABAM (4 mg/kg; *p.o.*). A vehicle group (3% DMSO solution; negative control) and a camphor-treated group (30.4 mg/kg; i.p.) were included. After 30 min, the groups received the *i.m.* of formalin (0.1%; TRPA1 agonist) applied to the animals' tails and then taken to the open field. The type of analgesia was evaluated as previously described [3].

2.7. Induction of Abdominal Edema by Carrageenan

The animals were initially identified and weighed, and then 3 groups (n=6, each) were treated with ABAM (4;

20 and 40 mg/kg, *p.o.*), another group with ibuprofen (100 mg/kg, *p.o.* – Positive control) and a group treated with 3% DMSO was included (negative control). After 1 hour at rest, 1.5% carrageenan was applied intraperitoneally in all groups. After this period, the fish were weighed every hour for 4 consecutive hours. These data were plotted, and the difference between the initial weight of the fish (without treatment) and the final weight after each hour was calculated to assess the growth of abdominal edema until the final time of 4h [17].

2.8. Assessment of Locomotor Activity (Open Field Test - OFT)

The open field test was performed to assess the presence or absence of changes in animal motor coordination [18], whether due to sedation and/or muscle relaxation. Initially, fish (n = 6/group) were treated orally (p.o.) with ABAM (4, 20 and 40 mg/kg) or vehicle (Negative control; 3% DMSO). After 30 min of treatments, the animals were added to glass Petri dishes (10 x 15 cm) containing the same aquarium water, marked with four quadrants, and analyzed for locomotor activity by counting the number of crossed lines (CL) by the animals during five minutes of analysis [12].

2.9. Acute Toxicity 96 Hours

After the open field test, the fish were left to rest for analysis of the mortality rate for a period of 96 h, recording every 24 h the number of dead fish in each group [19], the lethal dose being capable of killing 50% of the animals (DL50) determined by the mathematical probit method with a 95% confidence interval. During this period, the fish were fed twice daily with commercial flakes (Alcon).

2.10. Statistical Analysis

Results were expressed as mean values ± standard error of the mean for each group of 6 animals. After confirming the normal distribution and homogeneity of the data, the differences between the groups were submitted to analysis of variance one-way unidirectional ANOVA and two-way ANOVA for the mechanisms of action and inflammation test, followed by the Tukey test. All analyzes were performed using GraphPad Prism v. 8.0. The level of statistical significance was set at 5% (p<0.05).

3. RESULTS AND DISCUSSION

3.1. Formalin-Induced Nociceptive Behavior

The effect of ABAM on formalin-induced nociception was investigated. Doses of 20 (109.9%) and 40 (140.1%) mg/kg; 20µL; p.o.) * p < 0.05; ** p < 0.01 vs. control] significantly inhibited neurogenic nociception. In the inflammatory phase, all doses inhibited nociception [(4 (85.54%); 20 (127.33%) and 40 (168.11%) mg/kg; 20 µL; p.o.) * p < 0.05; ** p < 0.01 vs. control], restoring locomotion of animals in the open field test compared to control. Morphine also significantly inhibited formalin neurogenic and inflammatory nociception (* p < 0.05 vs. control) (Figure **2**).

To investigate the mechanism of action of ABAM via the TRPA1 receptor, the acute nociception test with camphor (TRPA1 antagonist) was performed. As a

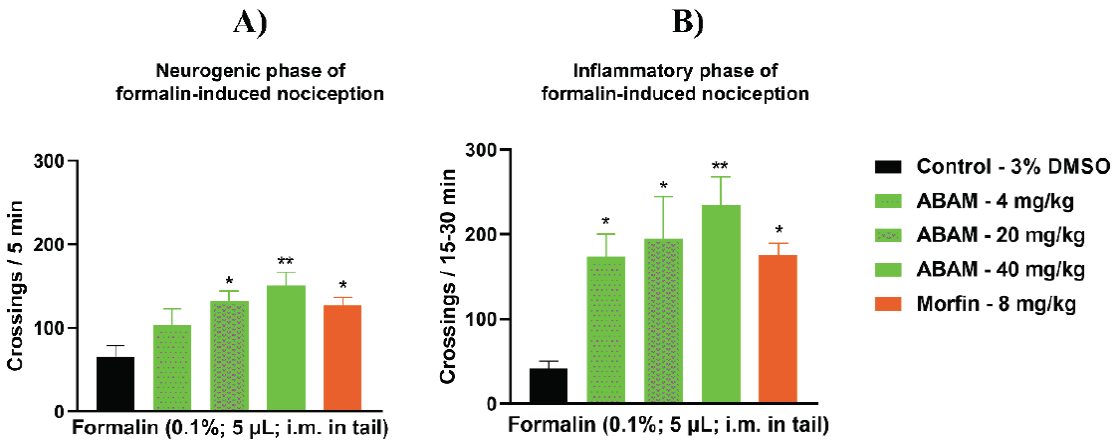

Figure 2: Effect of ABAM on nociception induced by formalin (0.1%) in adult zebrafish in the neurogenic (0-5 min) – (**A**) and inflammatory (15-30 min) – (**B**) phase. Each column represents the mean ± standard error of the mean (n = 6/group). Control: vehicle (3% DMSO; 20 µl; *i.p.*), ABAM - triterpene mixture of α, β-amyrin (4, 20 or 40 mg/kg; 20 µl; *p.o.*). One-way ANOVA followed by Tukey's test. (*p<0.05; **p<0.01 *vs*. control).

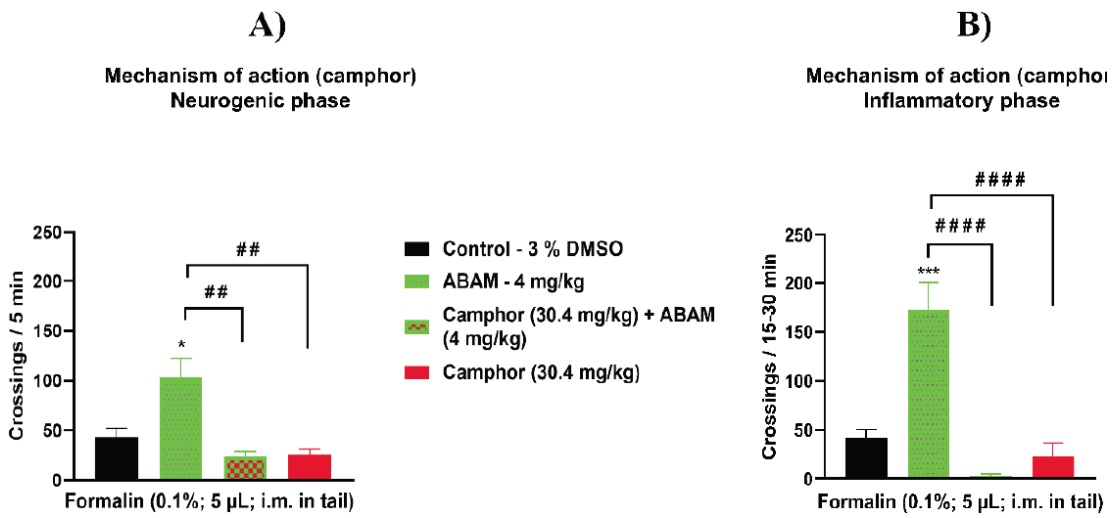

Figure 3: Effect of camphor on the antinociceptive activity of ABAM (4 mg/kg) in adult zebrafish in the neurogenic (0-5 min) - **A** and inflammatory (15-30 min) - **B** phase. Each column represents the mean ± standard error of the mean (n = 6/group). Control: vehicle (3% DMSO; 20 µl; *p.o.*), Can: camphor (30.4 mg/kg). Two-way ANOVA followed by Tukey's test. (*p<0.05; ***p<0.001 *vs*. control; $^{\#\#}$p <0.01; $^{\#\#\#\#}$p <0.0001 vs. ABAM).

result, the antinociceptive effect of ABAM (4 mg/kg) was completely prevented ($^{\#\#\#}$ *p* <0.001, $^{\#\#\#\#}$ *p* <0.0001 vs. ABAM) by camphor in the neurogenic and inflammatory phase of formalin-induced nociception, indicating that the antinociceptive effect of the ABAM triterpene mixture may occur through TRPA1 receptor neuromodulation (Figure **3**).

Recent studies show that noxious stimuli (such as exposure to chemicals) activate nociceptive channels and elicit a behavioral response in zebrafish characterized by reduced locomotor activity [12, 20, 21]. Evidence indicates that TRPA1-mediated hyperlocomotion in Zebrafish larvae and adults has the potential to be used as a screening tool for antinociceptive drugs [4, 12, 22], as noxious agents such as formalin and glutamate were able to cause nociceptive behavior effect by compromising Zebrafish locomotion [12, 17]. Nociception through the activation of the TRPA1 receptor by formalin action has already been investigated in adult zebrafish by applying this noxious agent in the tail (intramuscular) [12, 17, 21]. This same method was used to evaluate the antinociceptive effect of ABAM in which all doses prevented the animals from neurogenic and inflammatory nociception caused by formalin.

After identifying TRPA1 as a cation channel expressed in nociceptive neurons, it was proposed as a relevant target for treating pain and inflammation [23]. An intriguing feature of this receptor is that several modulators exert a bimodal effect, acting as agonists at low (micromolar) concentrations and as antagonists at higher concentrations [23]. Camphor has been reported as an inhibitor of TRPA1 basal currents in HEK-293 cells from transfected mice, with an IC50 of 0,66 mM [24]. The study of the bimodal effect of cinnamaldehyde and camphor on nociception in mice confirmed that the extracellular application of 1 mM camphor induced a significant inhibition of basal TRPA1 currents at negative potentials [23]. ABAM analgesia was demonstrated in the model of orofacial pain induced by formalin or capsaicin.

The animals were treated with ABAM (10, 30 and 100 mg/kg, *i.p.*), which caused significant inhibition of nociception in both models. However, in the formalin model, there was inhibition of nociception only in the second phase, that is, significant action only in inflammatory pain with a dose of 30 mg/kg [8]. In that study, pretreatment with a dose of 30.4 mg/kg of camphor abolished the antinociceptive effect of ABAM on neuropathic and inflammatory algesia induced by formalin in adult zebrafish, indicating an effect on both nociceptive phases. In another study, Silva *et al*. (2011) [22] reported the effect of α,β-amyrin with long-lasting antinociceptive and anti-inflammatory properties in 2 models of persistent nociception via activation of cannabinoid receptors and inhibition of cytokine production and NF-κB expression. Finally, another study indicated that the antinociceptive potential of ABAM possibly involves the mechanisms of opioid and vanilloid receptors (TRPV1). Here, the findings indicate the antinociceptive effect of ABAM through modulation of the TRPA1 receptor, confirming the antinociceptive potential of ABAM through several neurotransmission

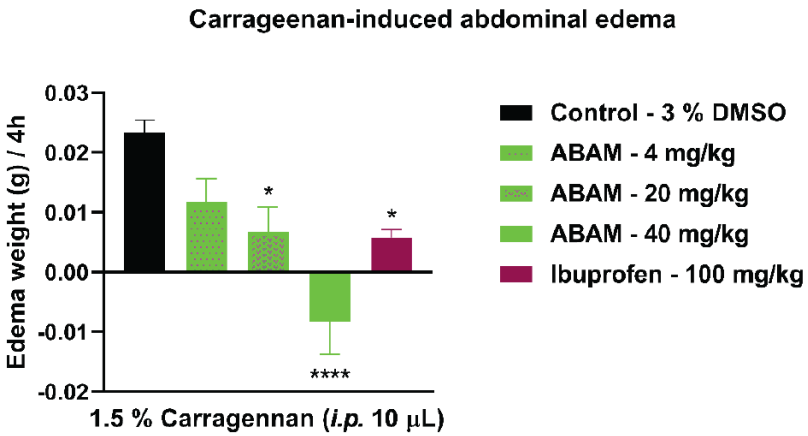

Figure 4: Effect of ABAM on carrageenan-induced abdominal edema in adult zebrafish, analyzed after 4 h. Each column represents a mean ± standard error of the mean (n=6/fish). One- way ANOVA followed by Tukey's test (*p < 0.05; ****p < 0.0001 *vs*. control).

pathways.

3.2. Induction of Abdominal Edema by Carrageenan

The highest doses of ABAM [(20 and 40 mg/kg; 20 µl; *p.o.*) * $p < 0.05$, **** $p < 0.0001$ vs. control] and ibuprofen [(100 mg/kg; 20 µl; *p.o.*) * $p < 0.05$ vs. control] significantly reduced carrageenan-induced abdominal edema after 4 h of analysis, indicating the anti-inflammatory effect of ABAM and ibuprofen in adult Zebrafish (Figure **4**).

The use of carrageenan to induce edema is a classic model used to screen drugs to treat and control inflammatory pain [2]. An inflammatory model based on carrageenan-induced paw edema in rats was developed in 1962 to study inflammatory mechanisms [25]. The evaluation of the action of carrageenan via TRPA1 in rats indicated the pharmacological blockade of this receptor through its selective antagonist (HC 030031), preventing and reversing hyperalgesia induced by carrageenan, detected by mechanical or chemical stimulus (low dose of capsaicin), but not affected the expression of carrageenan-induced cytokines or the migration of neutrophils [2]. Thus, it was concluded that TRPA1 plays an important role in developing and maintaining carrageenan-induced inflammatory hyperalgesia, directly contributing to nociceptor excitability.

The proliferation of pro-inflammatory cytokines and iNOS, as an inflammatory mediator, has been reported in carrageenan-induced abdominal edema in adult Zebrafish [26]. Therefore, the effect of ABAM on carrageenan-induced abdominal edema was investigated, and a significant reduction in edema was observed, an effect superior to that of the ibuprofen control group. Furthermore, the effect of ABAM on acute cerulein pancreatitis in mice was investigated [27]. As a result, α,β-amyrin greatly suppressed pancreatic edema, inflammatory cell infiltration, acinar cell necrosis, and TNFα and inducible nitric oxide synthase expressions. However, ABAM, in addition to attenuating inflammatory pain via TRPA1, supposedly reduced abdominal edema by reducing pro-inflammatory cytokines and iNOS.

3.3. Assessment of Locomotor Activity (Open Field Test) and 96h Acute Toxicity

The ABAM triterpene mixture did not alter the locomotion of the adult Zebrafish in the open field test ($p > 0.05$), as the treated animals did not show locomotor activity different from the control group (Figure **5A**). Furthermore, there were no animal deaths during the 96 h of analysis and no apparent anatomical changes (Figure **5B**), confirming the preclinical safety of ABAM for use in experiments with adult Zebrafish.

In addition, ABAM showed no motor impairment or toxicity, confirming its safety in zebrafish over 96 hours, as there was no change or evidence of toxicity caused by ABAM in adult Zebrafish, ensuring its preclinical safety for studies in animal models corroborating with several studies carried out [6].

This study has some limitations that should be considered. First, although behavioral tests indicate an antinociceptive effect of ABAM, the underlying molecular mechanisms were not investigated. Furthermore, the assays were performed in an acute animal model, which may not fully reflect clinical situations of chronic pain. Future investigations involving biochemical analysis, specific signaling

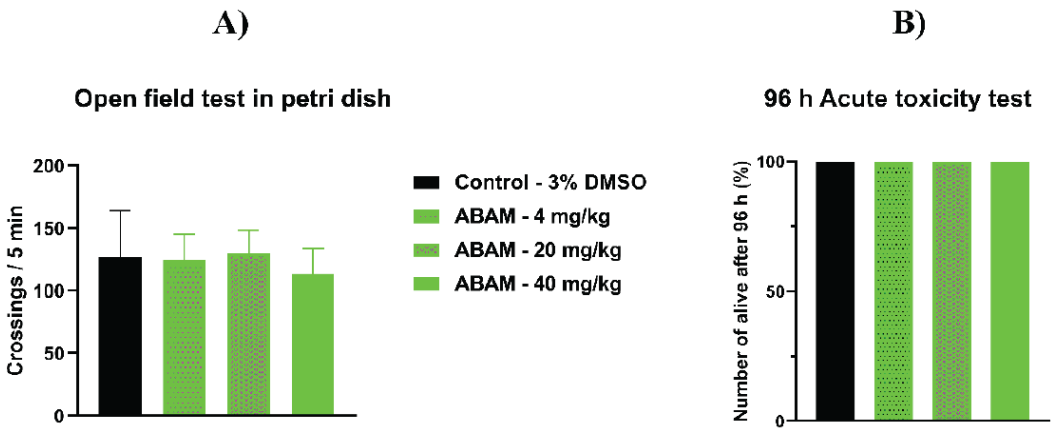

Figure 5: Effect of ABAM on the locomotor activity of adult zebrafish in the Open Field Test (0-5 min) – **A** and number of animals alive after 96 h of analysis (n=6) – **B**. Each column represents the mean ± standard error of the mean (n = 6/group). Control: vehicle (3% DMSO; 20 µl; *p.o.*). One- way ANOVA followed by Tukey's test.

pathways, and other pain models are necessary to deepen the understanding of the observed effect. Nevertheless, the results obtained provide promising initial evidence regarding the analgesic potential of the compound.

4. CONCLUSION

This study confirms the antinociceptive and anti-inflammatory potential of ABAM via TRPA1 neuromodulation in adult zebrafish, highlighting its promise as a biotechnological candidate for novel analgesic formulations.

ACKNOWLEDGEMENTS

Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support and scholarship. Helcio Silva dos Santos acknowledges financial support from CNPq (Grant 306008/2022-0) and FUNCAP (Grants#: ITR-0214-00060.01.00/23).

CONFLICTS OF INTEREST

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

AVAILABILITY OF DATA AND MATERIAL

Data supporting the findings of this study are available from the corresponding author [H.S. dos Santos] on request.

REFERENCES

[1] Chen Q, Dai J, Nan F, Xu J, Chen S. P66shc in the spinal cord is an important contributor in complete Freund's adjuvant induced inflammatory pain in mice. Biochem Biophys Res Commun 2023; 656: 63-69. https://doi.org/10.1016/j.bbrc.2023.03.028

[2] Bonet IJM, Fischer L, Parada CA, Tambeli CH. The role of transient receptor potential A 1 (TRPA1) in the development and maintenance of carrageenan-induced hyperalgesia. Neuropharmacology 2013; 65: 206-212. https://doi.org/10.1016/j.neuropharm.2012.09.020

[3] Alves AW da S, Sousa BL, Moura LFWG, Rebouças EL, Coutinho MR, Silva AW, Chaves RP, Carneiro RF, Bezerra EHS, Guedes MIF, Florean EOPT, Nagano CS, Sampaio AH, Rocha BAM. Codium isthmocladum lectin 1 (CiL-1): Interaction with N-glycans explains antinociceptive and anti-inflammatory activities in adult zebrafish (*Danio rerio*). Int J Biol Macromol 2022; 208: 1082-1089. https://doi.org/10.1016/j.ijbiomac.2022.03.209

[4] Ko MJ, Ganzen LC, Coskun E, Mukadam AA, Leung YF, van Rijn RM. A critical evaluation of TRPA1-mediated locomotor behavior in zebrafish as a screening tool for novel anti-nociceptive drug discovery. Sci Rep 2019; 9: 1-11. https://doi.org/10.1038/s41598-019-38852-9

[5] Dowell D, Haegerich TM, Chou R. CDC guideline for prescribing opioids for chronic pain-United States, 2016. JAMA - J Am Med Assoc 2016; 315: 1624-1645. https://doi.org/10.1001/jama.2016.1464

[6] Nogueira AO, Oliveira YIS, Adjafre BL, Moraes MEA, Aragao GF. Pharmacological effects of the isomeric mixture of alpha and beta amyrin from Protium heptaphyllum: a literature review. Fundam Clin Pharmacol 2019; 33: 4-12. https://doi.org/10.1111/fcp.12402

[7] Aragão GF, Pinheiro MCC, Bandeira PN, Lemos TLG, Viana GSB. Analgesic and anti-inflammatory activities of the isomeric mixture of alpha- and beta-amyrin from *Protium heptaphyllum* (Aubl.) March. J Herb Pharmacother 2007; 7: 31-47. https://doi.org/10.1300/J157v07n02_03

[8] Holanda Pinto SA, Pinto LMS, Cunha GMA, Chaves MH, Santos FA, Rao VS. Anti-inflammatory effect of α, β-Amyrin, a pentacyclic triterpene from *Protium heptaphyllum* in rat model of acute periodontitis. Inflammopharmacology 2008; 16: 48-52. https://doi.org/10.1007/s10787-007-1609-x

[9] Curtright A, Rosser M, Goh S, Keown B, Wagner E, Sharifi J, Raible DW, Dhaka A. Modeling nociception in zebrafish: A

way forward for unbiased analgesic discovery. PLoS One 2015; 10: 1-18. https://doi.org/10.1371/journal.pone.0116766

[10] Prober DA, Zimmerman S, Myers BR, McDermott Jr BM, Kim SH, Caron S, Rihel J, Krezel LS, Julius D, Hudspeth AJ, Schier AF. Zebrafish TRPA1 channels are required for chemosensation but not for thermosensation or mechanosensory hair cell function. J Neurosci 2008; 28: 10102-10110. https://doi.org/10.1523/JNEUROSCI.2740-08.2008

[11] Stevens JS, Padilla S, DeMarini DM, Hunter DL, Martin WK, Thompson LC, Gilmour MI, Hazari HS, Farraj AK. Zebrafish locomotor responses reveal irritant effects of fine particulate matter extracts and a role for TRPA1. Toxicol Sci 2018; 161: 290-299. https://doi.org/10.1093/toxsci/kfx217

[12] Bezerra AJN, Silva FCO, da Silva AW, Ferreira MKA, Marinho EM, Marinho MM, Magalhães FEA, Marinho ES, Menezes JESA, Santos HS. Antinociceptive effect of triterpene acetyl aleuritolic acid isolated from Croton zehntneri in adult zebrafish (*Danio rerio*). Biochem Biophys Res Commun 2021; 534: 478-484. https://doi.org/10.1016/j.bbrc.2020.11.056

[13] Silva FCO, de Menezes JESA, Ferreira MKA, Silva AW, Holanda CLA, Lima JR, Campos AR, Evaristo FFV, Teixeira EH, Magalhães FEA, Bandeira PN, Santos HS. Antinociceptive activity of 3β-6β-16β-trihydroxylup-20 (29)-ene triterpene isolated from Combretum leprosum leaves in adult zebrafish (*Danio rerio*). Biochem Biophys Res Commun 2020; 533: 362-367. https://doi.org/10.1016/j.bbrc.2020.07.107

[14] Bandeira PN, Lemos TLG, Costa SMO, Santos HS dos. Obtention of derivatives from the alpha- and beta-amyrin triterpenoid mixture: 13C NMR data. Rev Bras Farmacogn 2007; 17: 204-208

[15] Avdesh A, Chen M, Martin-Iverson MT, Mondal A, Ong D, Rainey-Smith S, Taddei K, Lardelli M, Groth DM, Verdile G, Martins RN. Regular care and maintenance of a Zebrafish (*Danio rerio*) laboratory: An introduction. J Vis Exp 2012. https://doi.org/10.3791/4196

[16] CONCEA, Resolução Norm, Conselho Nacional de Controle de Experimentação Animal, 2018 No 37, 15 Fevereiro 2018, 22 de Abril,em www.mctic.gov.br/…/concea/…normativa/RN-37-%0AEutanasia-secao-1-22_02_18.pdf.%0A

[17] Silva LMR, Lima JDSS, Magalhães FEA, Campos AR, Araújo JIF, Batista FLA, Araújo SMB, Sousa PHM, Lima GC, Holanda DKR, Rolim RC, Figueiredo RW, Figueiredo EAT, Duarte ASG, Ricardo NMP. Graviola Fruit Bar Added Acerola By-Product Extract Protects Against Inflammation and Nociception in Adult Zebrafish (*Danio rerio*). J Med Food 2020; 23: 173-180. https://doi.org/10.1089/jmf.2019.0078

[18] Ferreira MKA, Da Silva AW, dos Santos Moura AL, Sales KVB, Marinho EM, Banderia PN, Magalhães FEA, Marinho ES, Menezes JESA, Santos HS. Chalcones reverse the anxiety and convulsive behavior of adult zebrafish. Epilepsy Behav 2021; 117: 107881. https://doi.org/10.1016/j.yebeh.2021.107881

[19] Oecd. Fish, acute toxicity test. Guidel Test Chem 1992; 1-9.

[20] Ohnesorge N, Heinl C, Lewejohann L. Current Methods to Investigate Nociception and Pain in Zebrafish. Front Neurosci 2021; 15: 1-13. https://doi.org/10.3389/fnins.2021.632634

[21] Batista FLA, Lima LMG, Abrante IA, Araujo JIF, Batista FLA, Abrante IA, Magalhães EA, Lima DR, Lima MCL, Prado BS, Moura LFWG, Guedes MIF, Ferreira MKA, Menezes JESA, Santos SAAR, Mendes FRS, Moreira RA, Moreira ACOM, Campos AR, Magalhães FEA. Antinociceptive activity of ethanolic extract of Azadirachta indica A. Juss (Neem, Meliaceae) fruit through opioid, glutamatergic and acid-sensitive ion pathways in adult zebrafish (*Danio rerio*). Biomed Pharmacother 2018; 108: 408-416. https://doi.org/10.1016/j.biopha.2018.08.160

[22] Simão Da Silva KAB, Paszcuk AF, Passos GF, Silva ES, Bento AF, Meotti FC, Calixto JB. Activation of cannabinoid receptors by the pentacyclic triterpene α,β-amyrin inhibits inflammatory and neuropathic persistent pain in mice. Pain 2011; 152: 1872-1887. https://doi.org/10.1016/j.pain.2011.04.005

[23] Alpizar YA, Gees M, Sanchez A, Apetrei A, Voets T, Nilius B, Talavera K. Bimodal effects of cinnamaldehyde and camphor on mouse TRPA1. Pflugers Arch Eur J Physiol 2013; 465: 853-864. https://doi.org/10.1007/s00424-012-1204-x

[24] Xu H, Blair NT, Clapham DE. Camphor activates and strongly desensitizes the transient receptor potential vanilloid subtype 1 channel in a vanilloid-independent mechanism. J Neurosci 2005; 25: 8924-8937. https://doi.org/10.1523/JNEUROSCI.2574-05.2005

[25] Ekambaram SP, Perumal SS, Pavadai S. Anti-inflammatory effect of Naravelia zeylanica DC via suppression of inflammatory mediators in carrageenan-induced abdominal oedema in zebrafish model. Inflammopharmacology 2017. https://doi.org/10.1007/s10787-016-0303-2

[26] Huang SY, Feng CW, Hung HC, Chakraborty C, Chen CH, Chen WF, Jean YH, Wang HMD, Sung CS, Sun YM, Wu CY, Liu W, Hsiao CD, Wen ZH. A novel zebrafish model to provide mechanistic insights into the inflammatory events in carrageenan-induced abdominal edema. PLoS One 2014; 9. https://doi.org/10.1371/journal.pone.0104414

[27] Melo CM, Morais TC, Tomé AR, Brito GAC, Chaves MH, Rao VS, Santos FA. Anti-inflammatory effect of α,β-amyrin, a triterpene from Protium heptaphyllum, on cerulein-induced acute pancreatitis in mice. Inflamm Res 2011; 60: 673-681. https://doi.org/10.1007/s00011-011-0321-x