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Antinociceptive effects of hydroalcoholic extract from *Euterpe oleracea* Mart. (Açaí) in a rodent model of acute and neuropathic pain

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Abstract

Background: Plants rich in flavonoids, such as açai (*Euterpe oleracea* Mart.), can induce antinociception in experimental animals. Here, we tested an extract obtained from the stones of açai fruits (açai stone extract, ASE), a native plant from the Amazon region of Brazil, in models of acute/inflammatory and chronic pain.

Methods: Antinociceptive effects of ASE were evaluated in the hot plate, formalin, acetic acid writhing, carrageenan, and neuropathic pain models, as well as in thermal hyperalgesia and mechanical allodynia models induced by spinal nerve ligation. Antinociceptive activities were modulated by the administration of cholinergic, adrenergic, opioid, and L-arginine-NO antagonists.

Results: Oral administration of ASE (30, 100, or 300 mg·kg⁻¹) dose-dependently reduced nociceptive responses to acute/inflammatory pain in mice, including thermal hyperalgesia, acetic acid-induced writhing, and carrageenan-induced thermal hyperalgesia. Moreover, ASE reduced the neurogenic and inflammatory phases after intraplantar injection of formalin in mice. The antinociceptive effect of ASE (100 mg·kg⁻¹) in a hot plate protocol, was inhibited by pre-treatment with naloxone (1 mg·kg⁻¹), atropine (2 mg·kg⁻¹), yohimbine (5 mg·kg⁻¹), or L-NAME (30 mg·kg⁻¹). Furthermore, ASE prevented chronic pain in a rat spinal nerve ligation model, including thermal hyperalgesia and mechanical allodynia.

Conclusion: ASE showed significant antinociceptive effect via a multifactorial mechanism of action, indicating that the extract may be useful in the development of new analgesic drugs.

Keywords: *Euterpe oleracea* Mart, Arecaceae, Hyperalgesia, Allodynia, Acute and chronic pain

Background

Pain can reduce normal activities and negatively impact quality of life. Current options for the pharmacological treatment of pain include non-steroidal anti-inflammatory drugs and opioids, which unfortunately cause several side effects. The biodiversity present in countries like Brazil represents a potentially important source for development of new analgesic compounds [1].

Euterpe oleracea Mart., popularly known as “açai”, belongs to the family *Arecaceae* and is widely distributed in the Amazon region of Brazil. The fruit is an important source of food and is used as a medicinal plant for fever, pain, inflammation and anemia treatment [2].

Açaí fruits are rich in anthocyanic compounds (cyanidin 3-O-rutinoside) and other polyphenols, such as epicatechine, catechine homoorientin, orientin, isovitexin, and taxifolin deoxyhexose [3, 4], which have important biological effects.

Previously, we demonstrated that the hydro-alcoholic extract from açai stones (açai stone extract, ASE), which is rich in polymeric proanthocyanidins, have important vasodilatory [5], antihypertensive [6], antioxidant [7], and anti-inflammatory [8] activities. As extracts from plants

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51 rich in flavonoids can show antinociceptive effects [9, 10],
52 we tested the effects of ASE in acute and chronic models
53 of pain, as well as the mechanisms underlying these
54 effects.

55 **Methods**

56 **Preparation of ASE**

57 *E. oleracea* Mart. fruits (açai) were obtained from Ama-
58 zon Bay (Belém do Pará, Brazil; excicata number 29052,
59 Museu Goeldi-Belem do Pará). Hydro-alcoholic extracts
60 were obtained from a decoction of the seeds of the fruits
61 as previously described by Moura et al. [8]. Briefly, 200 g
62 of açai stone were boiled in 400 ml of distilled water for
63 5 min, mixed for 2 min, and then boiled again for 5 min.
64 The decoction was cooled to room temperature and
65 extracted by addition of 400 ml of ethanol with shaking
66 for 2 h. The extract was stored in dark bottles inside a re-
67 frigerator (4 °C) for 10 days. After this maceration period,
68 hydroalcoholic extracts of açai were filtered through
69 Whatman filter paper. Ethanol was evaporated by using a
70 rotary evaporator (Fisatom Equipamentos Científicos Ltda
71 São Paulo, São Paulo, Brazil) under low pressure at 55 °C.
72 The extract was lyophilized (LIOTOP model 202, Fisatom
73 Equipamentos Científicos Ltda São Paulo) at temperatures
74 from -30 to -40 °C and under a vacuum of 200 mmHg,
75 and frozen at -20 °C, until use. Typically, 100 g of stone
76 yielded approximately 5 g of lyophilized extract.

77 ASE was analyzed on an RP-18 column (250 mm ×
78 4 mm, 5 μm particles) according to a procedure re-
79 ported by Peng et al. [11]. Elution was conducted with
80 solvents A (0.2 % v/v phosphoric acid) and B (82 % v/v
81 acetonitrile, 0.04 % v/v phosphoric acid) at a flow rate
82 of 1 ml.min⁻¹. Ultraviolet-visible (UV-vis)-DAD ab-
83 sorption spectra were recorded on-line during High-
84 Performance Liquid Chromatography (HPLC) analysis.
85 The HPLC elution profile of ASE can indicate the
86 presence of proanthocyanidins [11]. The peak eluting at
87 37.2 min corresponded to catechin, as confirmed by
88 co-injection of a standard and by comparison of the
89 UV absorption spectra. The late elution (at 54.7 min)
90 and UV spectrum of the main peak are consistent with
91 the presence of polymeric proanthocyanidins, as previ-
92 ously described [8].

93 **Animals and housing conditions**

94 Male Swiss mice (18–25 g) and male Wistar rats (180–
95 220 g), obtained from Vital Brasil Institute and the
96 Federal University of Rio de Janeiro, respectively, were
97 housed under a 12 h light–dark cycle at 21 °C and 60 %
98 humidity, with food and water ad libitum. Protocols
99 were reviewed and approved by the institutional Animal
100 Care and Use Committee (CEUA, Ref. #DFBCICB061).

101 **Drugs**

102 Atropine, carrageenan, acetylsalicylic acid, indometh-
103 acin, formaldehyde and L-nitro arginine methyl ester
104 (L-NAME) were purchased from Sigma (St Louis, MO,
105 USA). Yohimbine hydrochloride was purchased from
106 Tocris (Ellisville, MO, USA). Tramadol, naloxone, ami-
107 triptyline hydrochloride and morphine sulfate were do-
108 nated by Cristália Produtos Químicos e Farmacêuticos
109 Ltda (Itapira, SP, Brazil). ASE was dissolved in distilled
110 water (10 mg.ml⁻¹, stock solution).

111 **Hot plate test**

112 The hot plate test in mice [12] was used to test the effect
113 of orally administered ASE (30, 100 or 300 mg.kg⁻¹) on
114 pain responses mediated by the central nervous system
115 (CNS). Oral tramadol (2 mg.kg⁻¹) was used as a positive
116 control. Withdrawal latency (reaction time of the animal
117 when placed on a surface heated to 52 °C) was measured
118 before and 30 min after oral administration of either saline
119 tramadol (2 mg.kg⁻¹) or ASE (30, 100 or 300 mg.kg⁻¹).
120 Additional measurements were performed every 15 min
121 up to 120 min to determine the maximum possible effect
122 (%MPE), which occurred 20–25 min after ASE adminis-
123 tration. Analgesic activity was calculated as the %MPE by
124 using the formula: %MPE = [(latency observed) – (latency
125 control) × 100] / [(cut-off) – (latency control)] (Fig. 1).

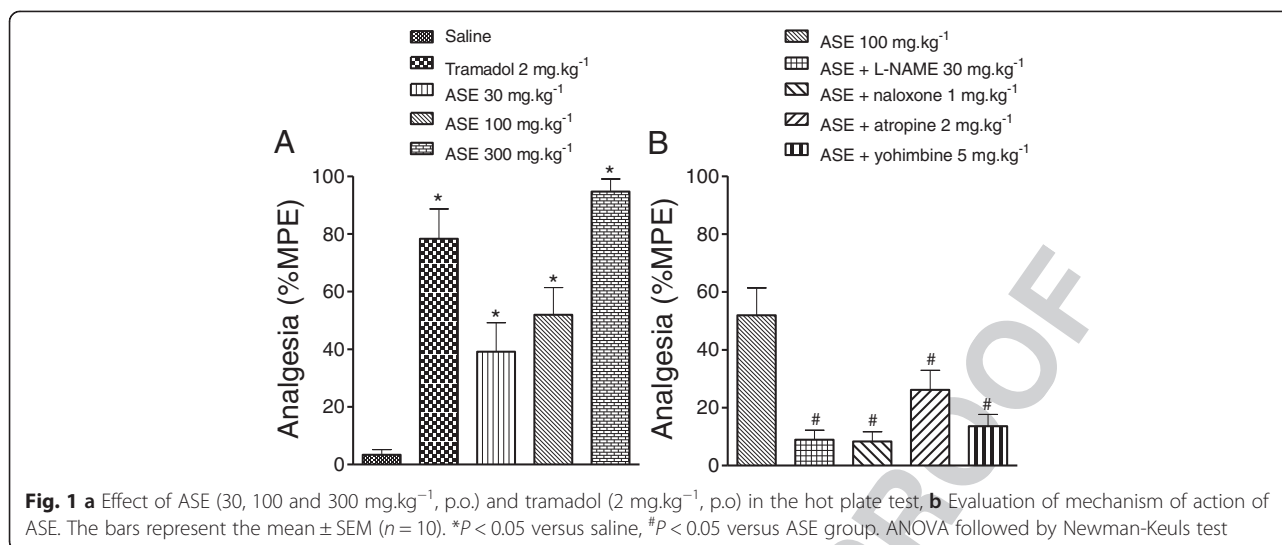
126 To investigate the possible mechanisms involved in
127 ASE activity animals received pre-administration of the
128 following antagonists: 30 mg.kg⁻¹ N^ω-nitro-L-arginine
129 methyl ester (L-NAME, selective NOS inhibitor),
130 1 mg.kg⁻¹ naloxone (opioid antagonist), 2 mg.kg⁻¹ atro-
131 pine (muscarinic antagonist), or 5 mg.kg⁻¹ yohimbine
132 (α₂ adrenoceptor antagonist). A 100 mg.kg⁻¹ dose of
133 ASE, produced a MPE of 50 % and was used for the
134 mechanism of action experiments.

135 **Formalin-induced hind paw-licking**

136 The antinociceptive effect of ASE on neurogenic and
137 inflammatory pain was tested by using the formalin test
138 in mice [13]. Formalin (2.5 %, 20 μl) was administered
139 by intraplantar injection into the right hind paw 15 min
140 after oral administration of saline, acetylsalicylic acid
141 (150 mg.kg⁻¹), morphine sulfate (30 mg.kg⁻¹) or ASE
142 (30, 100, or 300 mg.kg⁻¹). The duration of licking and
143 biting of the injected paw was monitored over 0–5 min
144 (early phase, neurogenic pain response) and 15–30 min
145 (late phase, inflammatory pain response).

146 **Carrageenan-induced pain**

147 Carrageenan-induced thermal hyperalgesia was evalu-
148 ated in mice as described [12, 14]. Animals were placed
149 in transparent boxes on a glass surface, and a radiant
150 heat stimulus was applied through the glass onto the
151 hind paws until withdrawal. Latency was defined as the



152 time between heat application and hind paw withdrawal.
 153 Peripheral inflammation was induced by intraplantar injection of carrageenan (1 %, 20 μ l) into the right hind
 154 paw at time zero. The latency of each animal to react to the thermal stimulus was measured at different time
 155 points before (control measure) and after carrageenan injection. Saline, acetylsalicylic acid (150 mg.kg⁻¹), or
 156 ASE (30, 100, or 300 mg.kg⁻¹) was administered orally 15 min before carrageenan. A cut-off time of 15 s was
 157 used to avoid tissue damage.

162 Acetic acid-induced writhing

163 Mice received intraperitoneally (i.p.) administered acetic
 164 acid (0.6 %, 10 μ l.g⁻¹ v/v), as previously reported [15],
 165 and were placed in a box (40 x 30 x 25 cm) in a quiet, il-
 166 luminated room. The resulting abdominal contortions
 167 (writhes) were counted for 20 min, beginning 10 min
 168 after acetic acid administration, as previously described
 169 [16]. Saline, the reference drug indomethacin (2 mg.kg
 170 ⁻¹) or ASE (30, 100, or 300 mg.kg⁻¹) was administered
 171 orally 15 min before acetic acid.

172 Spinal nerve ligation (SNL)

173 Neuropathic pain signs (thermal hyperalgesia and mech-
 174 anical allodynia) were induced by SNL as described [17].
 175 Briefly, after anesthesia with ketamine (100 mg.kg⁻¹ i.p.)
 176 and xylazine (5 mg.kg⁻¹ i.p.), Wistar rats (180–220 g),
 177 were placed in the prone position. The right L5 spinal
 178 nerve was isolated and tightly ligated with 6.0 silk
 179 threads. After the procedure, the wound was sutured.
 180 Animals were individually housed after surgery for the
 181 remainder of the study.

Antinociceptive effect on SNL-induced thermal hyperalgesia and mechanical allodynia

182 Thermal hyperalgesia was assessed using latency of paw
 183 withdraw [14, 18] from a radiant heat source applied to
 184 the plantar surface of the hind paws. Animals were
 185 placed in transparent acrylic boxes for 20–30 min to
 186 acclimatize before application of radiant heat through
 187 the glass flooring. Latency from stimulus onset to paw
 188 withdrawal was measured across three trials with a cut-
 189 off of 30 s.

190 Mechanical allodynia was assessed by using a digital
 191 version of the Von Frey filaments [18]. Rats were placed
 192 in individual acrylic boxes for 30 min to acclimatize.
 193 Stimuli were applied to the plantar region of the hind
 194 paw, and the withdrawal threshold was assessed across
 195 five trials with a cutoff of 120 g. Control measurements
 196 were taken before and 7 days after SNL. Rats were sub-
 197 jected to thermal hyperalgesia and mechanical allodynia
 198 tests to confirm the success of SNL surgery and the on-
 199 set of neuropathic pain. After daily treatment with ASE
 200 for 7 days, pain tests were repeated.
 201
 202

203 Statistical analysis

204 Data are reported as the mean \pm standard error of the mean
 205 (S.E.M.). One-way ANOVA followed by the Newman-Keuls
 206 test was used to analyze the effects of ASE on the hot
 207 plate test, formalin-induced pain, and acetic acid-
 208 induced writhing. Two-way ANOVA followed by the
 209 Bonferroni post-hoc test was used to analyze the effects
 210 of ASE on the carragenin-induced pain and on the SNL
 211 experiments. Data were graphed and statistically ana-
 212 lyzed by using GraphPad Prism 5.0. Differences were
 213 considered significant when the *p* value < 0.05.

214 Results and discussion

215 Hot plate test

216 Treatment with 30, 100, or 300 mg.kg⁻¹ ASE dose-
217 dependently increased the %MPE to 39.1 ± 10.0, 51.9 ± 9.5,
218 or 94.7 ± 4.4 %, respectively (Fig. 1a, n = 10 per group,
219 *p* < 0.05). The %MPE was also increased by tramadol
220 (2 mg.kg⁻¹) to 78.3 ± 10.3 % (n = 10, *p* < 0.05). Pre-
221 treatment with i.p. administration of L-NAME (30 mg.kg
222 ⁻¹), naloxone (1 mg.kg⁻¹), yohimbine (5 mg.kg⁻¹) or atro-
223 pine (2 mg.kg⁻¹) reduced the antinociceptive effect of ASE
224 (100 mg.kg⁻¹) from 51.9 ± 9.5 to 8.9 ± 3.3, 8.3 ± 3.3, 13.6 ±
225 4.1, or 26.2 ± 6.8 %, respectively (Fig. 1b, n = 10 per group,
226 *p* < 0.05).

227 Nociception induced by thermal stimulation (hot plate
228 test) is used to evaluate antinociceptive agents that act
229 centrally but not peripherally [19]. This test involves vari-
230 ous physiological systems, including cholinergic, adrener-
231 gic, opioid, and L-arginine/NO, which may be targets for
232 antinociceptive compounds.

233 The importance of the sympathetic nervous system in
234 pain modulation has been known since 1904, when Weber
235 [20] demonstrated the antinociceptive effect of epineph-
236 rine injected in the spinal cord of a cat. Intrathecal or
237 intraperitoneal administration of α2-adrenoceptor agonists
238 induces significant antinociceptive effects in the hot plate
239 test in rodents [21]. Here, the α2-adrenoceptor antagonist
240 yohimbine inhibited the antinociceptive effect of ASE,
241 supporting involvement of the adrenergic system on pain
242 modulation, consistent with others flavonoids [22]. The
243 antinociceptive effect of ASE is probably dependent on
244 flavonoids content, because flavones [23], and quercetin
245 [24] have similar effect in animals. Specifically, polymeric
246 proanthocyanidins, which are common compounds in
247 our extract, may underlie the antinociceptive effects, as
248 seem with proanthocyanidins obtained from *Croton*
249 *celtidifolius* bark [9].

250 Morphine is considered to be the gold standard drug
251 for systemic pain treatment. However, prolonged use of
252 morphine induces tolerance and hyperalgesia. In the
253 present study naloxone, an opioid antagonist blocked
254 the anti-nociceptive effects of ASE. Opioid mechanisms
255 also modulate the antinociceptive effects of flavones
256 compounds [23] and quercetin [22]. Muscarinic cholin-
257 ergic receptors are present along the pain pathway
258 from the dorsal root ganglia to somatosensory cortex
259 [25], and muscarinic agonists have antinociceptive
260 effects in rodents [18]. Inhibition of muscarinic recep-
261 tors by atropine reduced, but did not abolish the anti-
262 nociceptive effect of ASE. This finding suggests that
263 cholinergic mechanisms may mediate these activities.

264 The L-arginine–nitric oxide (NO)/cyclic guanosine
265 monophosphate (cGMP) pathway also modulate pain
266 responses [26]. NO activates soluble guanylyl cyclase,
267 leading to the production of cGMPn which activates

268 cGMP-dependent protein kinase to open ATP-sensitive
269 K⁺ channels, leading to neuronal hyperpolarization and
270 spinal and peripheral antinociception [27]. In this study,
271 the NO synthesis inhibitor L-NAME inhibited the anti-
272 nociceptive effect of ASE. This inhibition demonstrates
273 the involvement of the L-arginine-NO-pathway to the
274 antinociceptive activities of ASE. Inhibition of NO syn-
275 thesis antagonizes the activities of several antinociceptive
276 compounds [28].

277 Taken together, these results indicate that ASE has an
278 antinociceptive effect that is modulated by the choliner-
279 gic, adrenergic, opioid, and L-arginine-NO pathways. In
280 addition, reactive oxygen species can enhance nocicep-
281 tive responses [29], and ASE may block these responses
282 via antioxidant activities and increasing NO-synthase to
283 release NO [30].

Formalin-induced hind paw-licking test

284 The total amounts of time spent licking, scratching, or
285 biting during the neurogenic and inflammatory phases
286 after intraplantar injection of formalin were 73.1 ± 6.1 s
287 and 207.8 ± 19.0 s, respectively (Fig. 2). Reactivity in the
288 neurogenic phase was not affected by oral administra-
289 tion of the lowest doses of ASE (30 mg.kg⁻¹) or acetyl-
290 salicylic acid (150 mg.kg⁻¹), but was reduced by higher
291 doses (100 and 300 mg.kg⁻¹ ASE) to 45.6 ± 5.0 s and
292

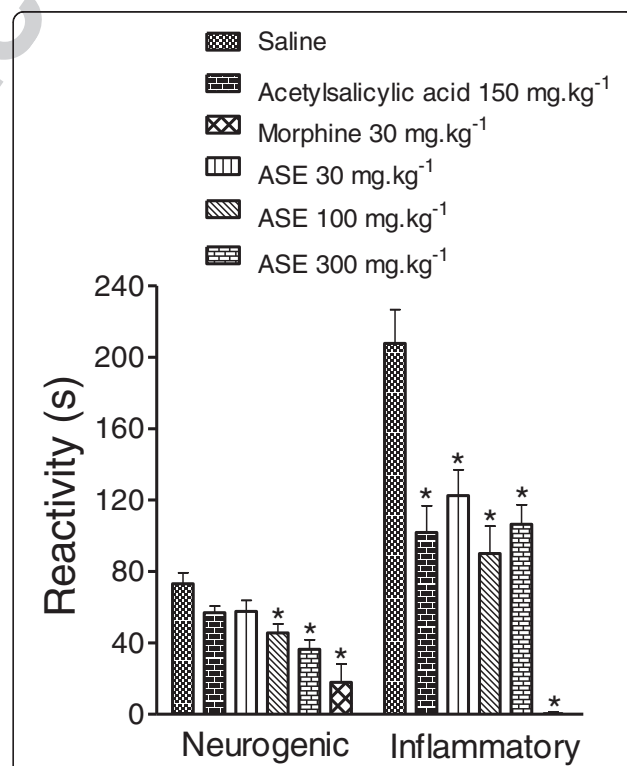


Fig. 2 Effect of ASE and acetylsalicylic acid on the formalin test. The bars represent mean ± SEM (n = 10). **P* < 0.05 versus saline. ANOVA followed by Newman-Keuls test

293 36.4 ± 5.3 s, respectively ($p < 0.05$). Reactivity in the
 294 inflammatory phase was reduced by acetylsalicylic acid to
 295 101.9 ± 14.9 s and by (30, 100, or 300 mg.kg^{-1} ASE) to
 296 122.5 ± 14.5 s, 90.1 ± 15.2 s and 106.4 ± 11.0 s, respectively
 297 ($p < 0.05$).

298 Intraplantar injection of formalin in rodents induces
 299 nociceptive-related behavior when assessed over two
 300 temporally distinct phases [13]. The first phase is induced
 301 by a direct activation of peripheral afferent C-fibers. The
 302 second phase is mediated by ongoing stimulation of noci-
 303 ceptors by inflammatory mediators (serotonin, histamine,
 304 bradykinin, NO, and prostaglandins) released from injured
 305 tissue, leading to activity-dependent sensitization of CNS
 306 neurons within the dorsal horn [31]. Local anesthetics and
 307 morphine inhibit the first phase whereas NSAIDs in-
 308 hibit the second inflammatory phase. In this study, we
 309 found that ASE inhibited the first phase, probably due to
 310 interaction with CNS targets. ASE reduced reactivity in
 311 the second phase; this finding suggests that ASE has anti-
 312 inflammatory activities, perhaps via inhibition of cyclooxy-
 313 genase 1 and 2 [4].

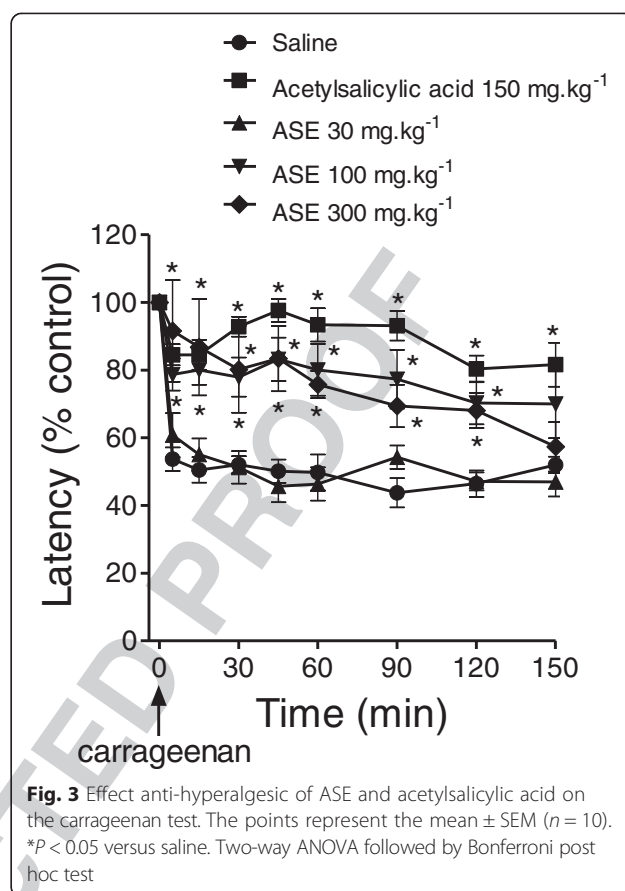
314 Carrageenan-induced pain test

315 Intraplantar administration of carrageenan reduced paw
 316 withdrawal latency to heat stimulation to 55.4 ± 5.7 % of
 F3 317 control (Fig. 3). The effect of carrageenan was noted
 318 5 min after administration, sustained for 150 min, and not
 319 affected by oral administration of ASE (30 mg.kg^{-1}).
 320 However, higher doses of ASE (100 and 300 mg.kg^{-1}) or
 321 acetylsalicylic acid (150 mg.kg^{-1}) reduced the effect of car-
 322 rageenan on paw withdrawal latency.

323 After carrageenan-induced inflammation, noxious stimuli
 324 elicit an enhanced pain response (hyperalgesia) [14].
 325 This enhanced synaptic transmission is essential for central
 326 sensitization. ASE prevented the appearance of this
 327 sensitization, supporting its antinociceptive effects in
 328 inflammatory pain. Some flavonoids in açai are modu-
 329 late proinflammatory cytokine production [32]. Carra-
 330 geenan stimulates the release of tissue necrosis factor
 331 (TNF)- α , interleukin (IL)-1 β and IL-6, with subsequent
 332 increases in COX products and IL-8, to stimulate local
 333 production of sympathetic amines [33]. Therefore, ASE
 334 may block the cascade of cytokine release induced by
 335 carrageenan-induced sensitization to produce analgesia
 336 in inflammatory pain.

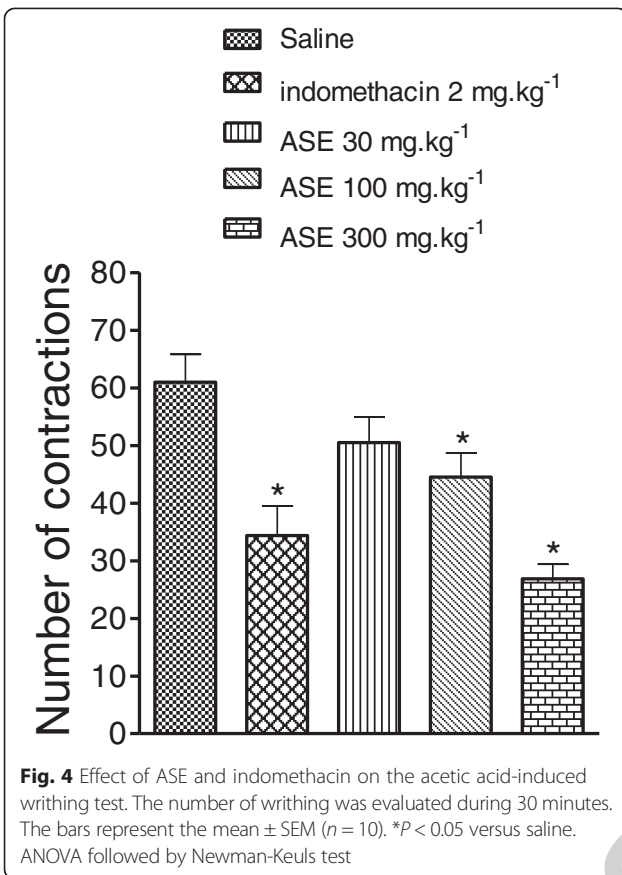
337 Acetic acid-induced writhing test

338 ASE at 100 and 300 mg.kg^{-1} dose-dependently reduced
 339 the number of abdominal contractions in response to
 340 acetic acid from 61.0 ± 4.8 (saline) to 44.5 ± 4.2 and $26.9 \pm$
 341 2.5 , respectively ($p < 0.05$). This effect was not significant
 342 at the lowest dose of ASE (30 mg.kg^{-1}), which slightly



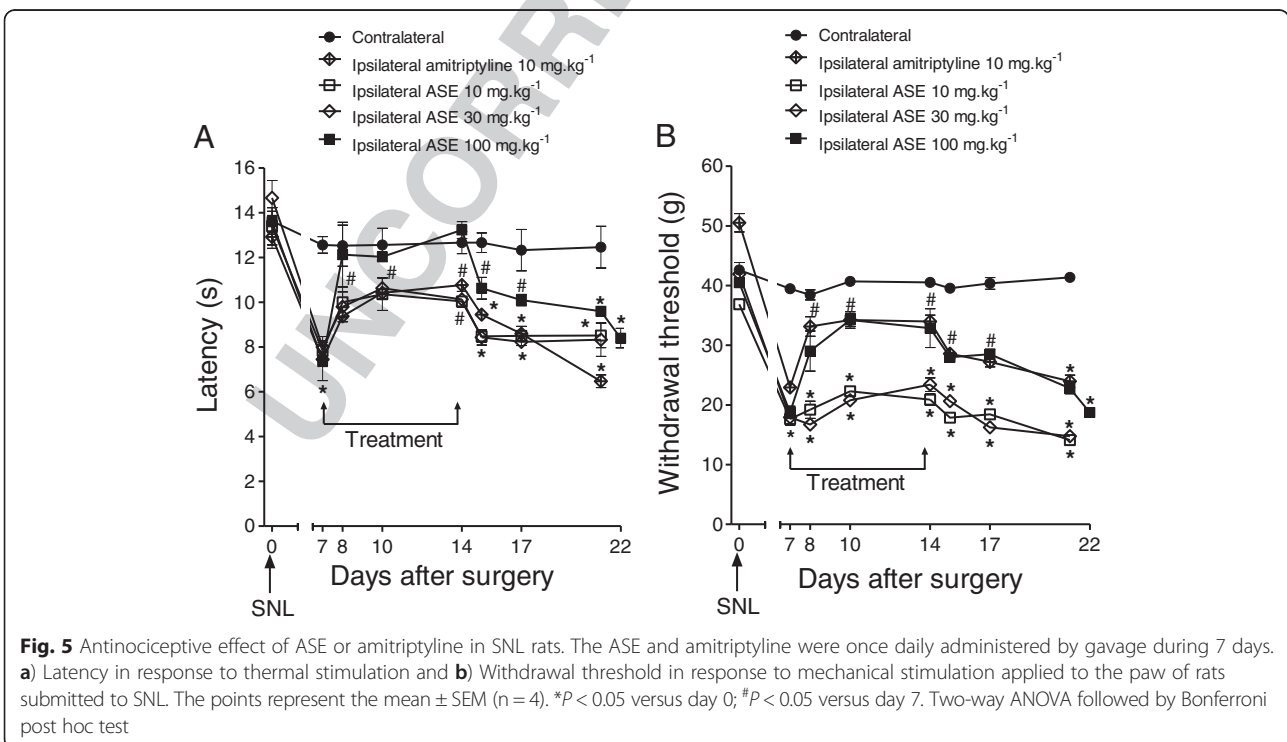
343 reduced contractions to 50.5 ± 4.4 . The reference drug
 344 indomethacin (2 mg.kg^{-1}) reduced contractions to $34.4 \pm$
 345 5.1 (Fig. 4).

F4 346 The acetic acid-induced writhing test is a screening tool
 347 for assessment of antinociceptive and anti-inflammatory
 348 agents [34]. Intraperitoneal injection of acetic acid in-
 349 creases pain mediators, such as prostaglandins, lipoxygen-
 350 ase, cyclooxygenase, histamine, serotonin, bradykinin,
 351 substance P, IL-1 β , IL-8, and TNF- α [34, 35], which
 352 increase vascular permeability and reduce the nociceptive
 353 threshold, causing stimulation of nociceptive terminals to
 354 induce abdominal writhing. The writhing response starts a
 355 few minutes after acetic acid injection. Reduction of this
 356 behavior is used to test the efficacy of drugs with visceral
 357 antinociceptive activity [36]. We measured the writhing
 358 response for 20 min starting 10 min after acetic acid in-
 359 jection to avoid counting stress reaction of the animal due to
 360 manipulation. We found similar writhing levels to other
 361 studies that measured the reaction for 30 min starting
 362 5 min after acetic acid administration [16, 37]. Pre-
 363 treatment with ASE reduced the acetic acid-induced writh-
 364 ing response, suggesting reduced synthesis or release of
 365 pain modulators.



SNL-induced thermal hyperalgesia and mechanical allodynia 366
 ASE (10, 30, or 100 mg.kg⁻¹) dose-dependently prevented 367
 development of thermal hyperalgesia and mechanical al- 368
 lodynia in SNL rats on the ipsilateral side (Fig 5a and b), but 369 **F5**
 no effect was observed on the contralateral side. At 7 days 370
 after surgery, the thermal withdrawal duration was re- 371
 duced from 13.6 \pm 0.5 s to 7.4 \pm 0.9 s ($n = 4$). ASE had 372
 significant effects from day 1 to 7 of treatment, reaching 373
 13.2 \pm 0.4 s. Treatment with 10 or 30 mg.kg⁻¹ ASE was as 374
 effective as 10 mg.kg⁻¹ amitriptyline. The mechanical with- 375
 drawal threshold was reduced 7 days after surgery from 376
 40.5 \pm 0.6 g to 18.8 \pm 1.0 g. After 7 days of treatment, ASE 377
 (100 mg.kg⁻¹, $n = 4$) increased this threshold to 32.9 \pm 378
 3.2 g, similar to amitriptyline (10 mg.kg⁻¹, $n = 4$). ASE had 379
 no effect on withdrawal duration or withdrawal threshold 380
 in the contralateral paw (Fig 5). 381

Chronic pain with neuropathic features affects 7-8 % 382
 of the general population [38]. Unfortunately, current 383
 pharmacotherapies used to treat the main symptoms of 384
 this disorder, hyperalgesia and allodynia, are not com- 385
 pletely effective. Oral administration of ASE over 7 days 386
 prevented the development of thermal hyperalgesia and 387
 mechanical allodynia in rats with SNL. Analgesic effects 388
 of ASE in this model were observed from 1 to 7 days 389
 after treatment with no signs of tolerance, which is a 390
 drawback of morphine [39]. Furthermore, side effects 391
 such as sedation were not observed after prolonged ASE 392
 treatment, providing an advantage over amitriptyline, 393
 which is sedative in humans [40]. A combination of the 394



395 CNS and anti-inflammatory effects of ASE may underlie
396 the antinociceptive effects in rats subjected to SNL.

397 Flavonoids such as the polyphenolic compounds rutin
398 and quercetin have anti-inflammatory [41], analgesic
399 [42], and antioxidant [43] effects. SNL is a neuropathic
400 pain model used in rats that mimics the pain sensations
401 experienced by human patients [44]. ASE had compar-
402 able efficacy to the clinical drug amitriptyline, in treating
403 SNL-induced neuropathic pain. Others flavonoids can
404 impact animal models of neuropathic pain. For example,
405 Azevedo et al. [24] showed that rutin and quercetin pre-
406 vented thermal and mechanical nociceptive responses in
407 oxaliplatin-induced neuropathic pain in mice by medi-
408 ating oxidative stress-induced damage.

409 Conclusions

410 The present study demonstrates a significant and potent
411 antinociceptive effect of oral ASE. The mechanism of this
412 antinociceptive effect is not completely understood, but
413 probably involves various pathophysiological systems.
414 These findings indicate the possibility for development of
415 a new analgesic drug.

416 Competing interests

417 Roberto Soares de Moura is inventor of a patent that may support the
418 development of a product. The others authors state no competing interests.

419 Authors' contributions

420 RTS, GZS and RSM idealized the study, designed the experiments and helped
421 to write the article. CESM, RVA, MLN and ACR were responsible to perform the
422 experiments. PJCS and RSM carry out the preparation of extract from *Euterpe*
423 *oleracea*. All authors read and approved the final version of the manuscript.

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