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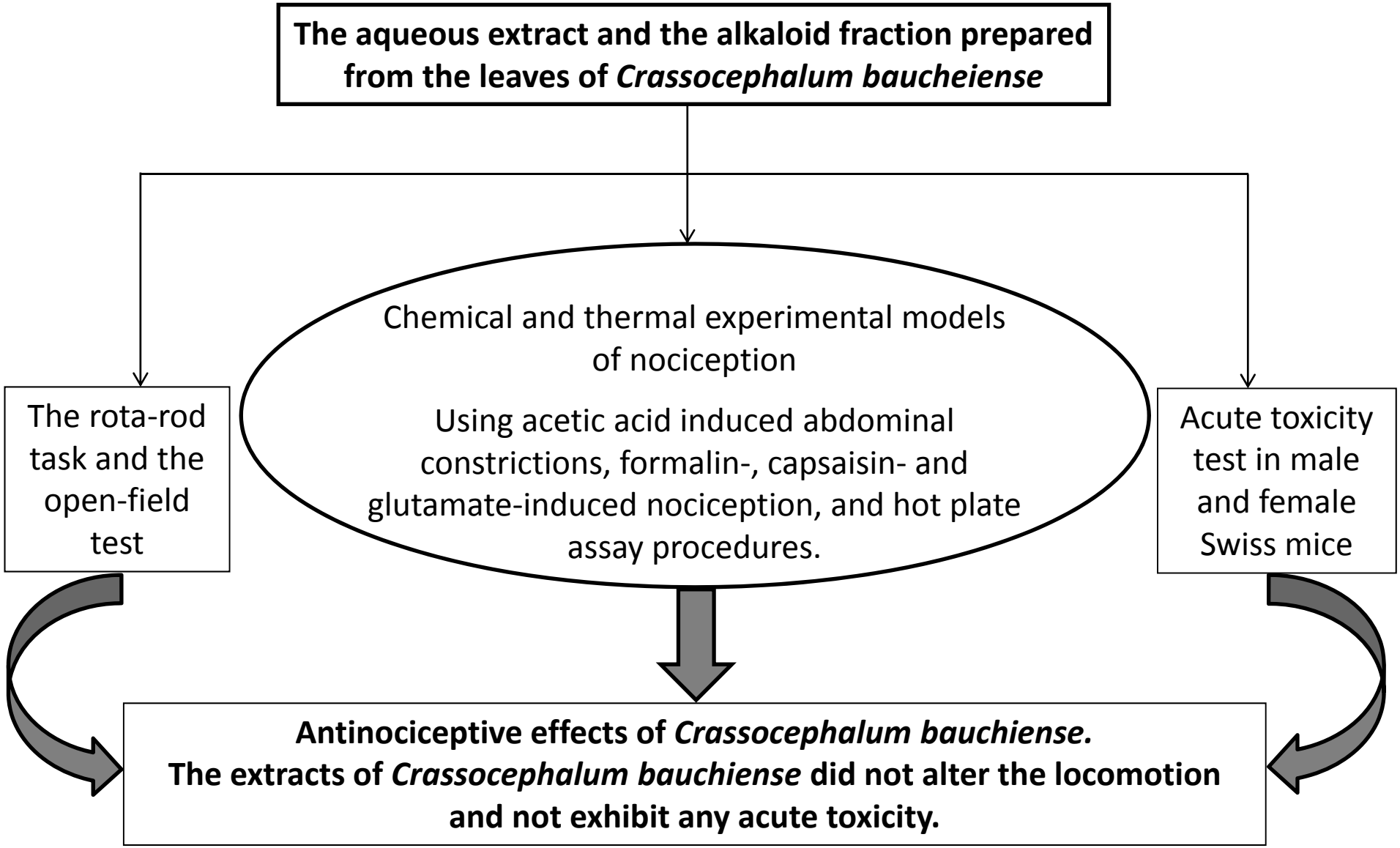
The aqueous extract and the alkaloid fraction prepared from the leaves of *Crassocephalum baucheiense*
Chemical and thermal experimental models of nociception
Using acetic acid induced abdominal constrictions, formalin-, capsaisin- and glutamate-induced nociception, and hot plate assay procedures.
The rota-rod task and the open-field test
Acute toxicity test in male and female Swiss mice
Antinociceptive effects of *Crassocephalum bauchiense*.
The extracts of *Crassocephalum bauchiense* did not alter the locomotion and not exhibit any acute toxicity.

Evaluation of antinociceptive effects of *Crassocephalum bauchiense* Hutch (Asteraceae) leaf extract in rodents

Germain Sotoing Taïwe[a,b,c,*], Elisabeth Ngo Bum[d], Emmanuel Talla[e], Théophile Dimo[b], Neteydji Sidiki[d], Amadou Dawe[f], Richard Marcel Nguimbou[g], Paul Désiré Djomeni Dzeufiet[b] and Michel De Waard[d,h]

[a]Department of Zoology and Animal Physiology, Faculty of Science, University of Buea, P.O. Box 63 Buea, Cameroon.

[b]Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, P.O. Box 812 Yaoundé, Cameroon.

[c]Unité Inserm U836, Grenoble Institute of Neuroscience, Chemin Fortuné Ferrini, Site santé de la Tronche, P.O. Box 170, 38042 Cedex 9, Université Joseph Fourier, Grenoble France.

[d]Department of Biological Sciences, Faculty of Science, University of Ngaoundéré, P.O. Box 454 Ngaoundéré, Cameroon.

[e]Department of Chemistry, Faculty of Science, University of Ngaoundéré, P.O. Box 454 Ngaoundéré, Cameroon.

[f]Ecole Normale Supérieure, University of Maroua, P.O. Box 55 Maroua, Cameroon.

[g]ENSAI, University of Ngaoundéré, P.O. Box 455 Ngaoundéré, Cameroon.

[h]Smartox Biotechnologies, Floralis, Biopolis, 5 Avenue du Grand Sablon, 38700 La Tronche, France.

***** Corresponding author. Tel.: 00237 77 71 86 70; Fax: 00237 22 15 73 70; E-mail address: taiwe_sotoing@yahoo.fr (G.S. Taïwe)

Abbreviations

AE, aqueous extract; AF, alkaloid fraction; Fr, fraction; ID_{50}, dose of extract necessary to reduce the response by 50% relative to the control value; LD_{50}, median lethal dose; NIH, National Institutes of Health; S.E.M, standard error of the means; v, volume; w, weight.

Abstract

Ethnopharmacological relevance: The leaves of *Crassocephalum bauchiense* have long been used in traditional Cameroonian medicine for the treatment of epilepsy, pain, inflammatory disorders, arthritis and intestinal pain.

Aim of the study: In this study, we attempted to identify the possible antinociceptive action of the aqueous extract and the alkaloid fraction prepared from the leaves of *Crassocephalum baucheiense*.

Materials and methods: Using acetic acid induced abdominal constrictions, formalin-, capsaisin- and glutamate-induced nociception, and hot plate assay procedures, the antinociceptive effects of the aqueous extract and the alkaloid fraction was assessed after oral administration in mice. Morphine sulphate was used as reference analgesic agent. Mice were submitted to the rota-rod task and open-field test in order to assess any non-specific muscle-relaxant or sedative effects of the extracts of *Crassocephalum bauchiense*. Male and female Swiss mice were used to assess acute toxicity of these extracts.

Results: The aqueous extract and the alkaloid fraction of *Crassocephalum bauchiense* produced a significant antinociceptive effects in the acetic acid, formalin, glutamate, capsaicin and hot plate tests. These antinociceptive effects of *Crassocephalum bauchiense* were significantly attenuated by pretreatment with naloxone. The extracts of *Crassocephalum bauchiense* did not alter the locomotion of animals in the open-field or rotarod tests, which

suggest a lack of a central depressant effect. The animals did not exhibit any acute toxicity to the aqueous extract and the alkaloid fraction, so it was not possible to calculate the LD_{50}.

Conclusion: The results confirm the popular use of *Crassocephalum bauchiense* as an antinociceptive, and contribute to the pharmacological knowledge of this species because it was shown that the aqueous extract and the alkaloid fraction of *Crassocephalum bauchiense* produced dose related antinociception in models of chemical and thermal nociception through mechanisms that involve an interaction with opioidergic pathway.

Keywords: *Crassocephalum bauchiense*, aqueous extract, alkaloid fraction, antinociceptive action, opioidergic pathway.

1. Introduction

The genus *Crassocephalum* belongs to the very large and widely distributed Asteraceae family in the tribe Senicioneae. It has been reported that *Crassocephalum* genus consists of nearly 24 species (Wagner et al., 1999). Many of these species are used widely as food additives or in traditional medicine, prompting phytochemical investigations that have in turn uncovered a variety of alkaloids, diterpenes and coumarins (Asada et al., 1985; Kongsaeree et al., 2003; Mohamed-Elamir et al., 2008). Well accepted that many plant-derived compounds possess analgesic and anti-inflammatory properties. Recent studies have shown that the labdane diterpene of the air-dried parts of *Crassocephalum mannii* has anti-inflammatory activity through its cyclooxygenase inhibitory activity (Liua et al., 2006; Heras et al., 2007; Mohamed-Elamir et al., 2008). Although the potent activity of non-steroidal anti-inflammatory drugs is noteworthy, they have also many severe adverse effects. The aim is therefore to identify medicinal plant agents with very little side effects as substitute therapeutics.

Crassocephalum bauchiense Huch (Asteraceae) is commonly used in traditional medicine in the north of Cameroon. The leaf extract has been used to treat several diseases, including epilepsy, pain, arthritis, intestinal pain and colics (Arbonnier, 2000). A decoction of the leaves has been reported to be useful in relieving bronchitis and the attendant fever. According to Cameroonian traditional healers, the plant is also effective in cases of cerebral deficit, behavioural disturbances in mentally-retarded children, inflammatory disorders and neuropathic pain. However, there is no detailed study on the alleged antinociceptives properties of this medicinal plant. To provide scientific evidence for its antinociceptive activities known in folk medicine, the main purpose of the present study was to evaluate the effects of the aqueous extract and the alkaloid fraction of *Crassocephalum bauchiense* leaves using different assays of the chemical nociception and the thermal model of nociception.

2. Materials and methods

2.1. Plant material

Fresh leaves of *Crassocephalum bauchiense* used in this study were harvested in the Mawi area of Ngaoundéré, Cameroon in july 2007. Botanical identification was performed at the National Herbarium, Yaoundé, Cameroun. Voucher specimen No. 7954/SRF/Cam was deposited at the Yaoundé herbarium.

2.2. Extraction and fractionation

For the preparation of the aqueous extract of *Crassocephalum bauchiense*, 100 g of dried and powdered leaves was soaked in 1000 ml of distilled water for 72 h and filtered. The filtrate was then dried in the oven (Gallenhamp®, England) at 60°C to give an extract with a 7.5% yield (w/w).

The dried and powdered leaves of *Crassocephalum bauchiense* (1000 g) were extracted with acetone/H_2O (7/3; 5 l) at room temperature. The combined extracts were evaporated *in vacuo* to afford a dark residue (652.45 g). The residue was suspended in warm water (1 l) and then extracted successively with ethyl acetate (0.5 l × 3) and n-butanol (0.5 l × 3), and concentrated to give residue A (207.13 g) and B (385.51 g), respectively. The latter was resolved in warm water (1 l), acidified with 1 mol/l HCl to pH between 4 and 5, and extracted with $CHCl_3$ (0.5 l × 3). The aqueous layer was neutralized with 1 mol/l NaOH to pH 9 to 10 and extracted with $CHCl_3$ (0.5 l × 3) once again and concentrated *in vacuo* to obtain the crude base (158.64 g; 41.15%). The crude base (158.64 g) was subjected to chromatography column on silica gel eluted using a gradient of $CHCl_3$:CH_3OH: 28% NH_4OH (from 50:1:0.1 to 3:1:0.1, v/v) to afford twelve fractions: Fr. I (16.54 g; 10.42%), Fr. II (16.34 g; 10.30%), Fr. III (12.68 g; 7.99%), Fr. IV (180.30 mg; 0.11%), Fr. V (2.44 g; 1.53%), Fr. VI (92.40 mg; 0.06%), Fr. VII (75.40 mg; 0.05%), Fr. VIII (41.27 mg; 0.03%), Fr. IX (39.20 mg; 0.02%), Fr. X (4.23 g; 2.66%), Fr. XI (135.20 mg; 0.08%) and Fr. XII (63.50 mg; 0.04%). The fraction Fr. II (16.30 g) was chromatographed on silica-gel chromatography column using a gradient of petroleum ether:Me_2CO:28%NH_4OH (from 30:1:0.2 to 2:1:0.1, v/v) to give Fr. XIII (47.14 mg; 0.28%), Fr. XIV (32.75 mg; 0.02%), Fr. XV (21.63 mg; 0.01%), Fr. XVI (18.71 mg; 0.01%), Fr. XVII (32.13 mg; 0.02%) and Fr. XVIII (29.34 mg; 0.18%). The aqueous extract and the alkaloid fraction of *Crassocephamum bauchiense* were dissolved in saline 0.90% containing dimethyl sulfoxyde 2% (vehicle) and subjected to the following pharmacological studies.

2. 3. Laboratory animals

Swiss albino mice (20 - 25 g) of either sex were used in this study. All animals were housed in a controlled environment, with free access to food and water and were maintained on a 12

h/12 h day/night cycle. Each animal was used only once. The investigation conforms to the Guide for the Care and Use of Laboratory Animal published by the US National Institutes of Health (NIH No. 85 - 23, revised 1996). In all the experimental studies each group consisted of six to eight animals and received approval of the local ethical committee for animal handling and experimental procedure.

2.4. Drugs and chemicals

Acetic acid, dipyrone, formalin, glutamate, morphine sulphate were purchased from Sigma chemical Co. (St. Louis, MO, USA). Naloxone was obtained from Arkopharma (Carros, France). Dipyrone, glutamate and morphine were prepared in saline (0.90% NaCl) and contained 2% dimethyl sulfoxyde. In all the pharmacological test naloxone was administered 15 min before the administration of the extracts of *Crassocephalum bauchiense*. Formalin stock solution was prepared in phosphate buffer solution (phosphate buffer solution concentration in mM: NaCl 137, KCl 2.70 and phosphate buffer, 10). Acetic acid was prepared in saline (0.90% NaCl). Capsaicin stock solution (10^{-2} M) was prepared by successively dissolving capsaicin in 10% ethanol, 10% tween 80 and 80% NaCl 0.90%. The stock solution was further diluted in saline upon administration to 80 mg/ml.

2.5. Pharmacological analysis

2.5.1. Abdominal constrictions induced by acetic acid

The aqueous extract of *Crassocephalum bauchiense* (20, 40, 80 and 160 mg/kg, p.o.), the alkaloids fractions from *Crassocephalum bauchiense* (Fr. I, Fr. II, Fr. III, Fr. IV, Fr. V, Fr. VI, Fr. VII, Fr. VIII, Fr. IX, Fr. X, Fr. XI, Fr. XII, Fr. XII, Fr. XIV, Fr. XV, Fr. XVI, Fr. XVII and Fr. XVIII; 40 mg/kg, p.o.), the aqueous extract + naloxone (160 mg/kg, p.o. + 1 mg/kg, i.p.), the alkaloid fraction + naloxone (Fr. XVI, 40 mg/kg, p.o. + 1 mg/kg, i.p.), morphine

sulphate (positive control, 5 mg/kg, s.c.) or vehicle (10 ml/kg, p.o.) were administered 1 h prior to acetic acid treatment. Acetic acid (0.60%, 10 ml/kg) was injected i.p. and the number of abdominal constrictions (writhings) during the following 30 min period was observed (Taïwe et al., 2011). A significant reduction in the number of abdominal constrictions by any treatment compared with vehicle treated animals was considered as an antinociceptive response.

2.5.2. Formalin-induced nociception

The formalin test was carried out as described by Tjolsen et al (1992). Mice were given the aqueous extract of *Crassocephalum bauchiense* (20, 40, 80 and 160 mg/kg, p.o.), the alkaloid fraction from *Crassocephalum bauchiense* (Fr. XVI; 5, 10, 20 and 40 mg/kg, p.o.), the aqueous extract + naloxone (160 mg/kg, p.o. + 1 mg/kg, i.p.), the alkaloid fraction + naloxone (Fr. XVI; 40 mg/kg, p.o. + 1 mg/kg, i.p.), morphine sulphate (positive control, 5 mg/kg, s.c.) or vehicle (10 ml/kg, p.o.) 1 h before injecting formalin. Pain was induced by injecting subcutaneously in the right hind paw 20 µl of 2.50% formalin (0.90% formaldehyde). The amount of time spent licking the injected paw was measured and considered as an indication of pain. The first phase of the nociceptive response normally peaks 0 – 5 min after injection and the second phase 15 – 30 min after formalin injection. These two phases correspond to the neurogenic and inflammatory pain responses, respectively (Hunskaar and Hole, 1987).

2.5.3. Capsaicin-induced nociception

The animals were individually placed in a transparent plexiglas observation chamber (15 × 15 × 15 cm) for an adjustment period of 20 min. After this period, mice were given the aqueous extract of *Crassocephalum bauchiense* (20, 40, 80 and 160 mg/kg, p.o.), the alkaloid fraction from *Crassocephalum bauchiense* (Fr. XVI; 5, 10, 20 and 40 mg/kg, p.o.), the aqueous extract

+ naloxone (160 mg/kg, p.o. + 1 mg/kg, i.p.) and the alkaloid fraction + naloxone (Fr. XVI; 40 mg/kg, p.o. + 1 mg/kg, i.p.). Control animals received vehicle (10 ml/kg, p.o.) or morphine sulphate (positive control, 5 mg/kg, s.c.). One h after these treatments, the right hind paw was injected with 20 µl of capsaicin (1.60 µg/paw). Nociception was assessed immediately after injection and quantified by paw licking time during a 5 min period (Santos and Calixto, 1997).

2.5.4. Glutamate-induced nociception

Animals were treated with the aqueous extract of *Crassocephalum bauchiense* (20, 40, 80 and 160 mg/kg; p.o.), the alkaloid fraction from *Crassocephalum bauchiense* (Fr. XVI; 5, 10, 20 and 40 mg/kg; p.o.), the aqueous extract + naloxone (160 mg/kg, p.o. + 1 mg/kg, i.p.), the alkaloid fraction + naloxone (Fr. XVI; 40 mg/kg, p.o. + 1 mg/kg, i.p.) and dipyrone (positive control, 60 mg/kg, p.o.) or vehicle (10 ml/kg; p.o.) 1 h before test. A volume of 20 µl of glutamate (30 µmol/paw) was injected intraplantarly in the ventral surface of the right hindpaw. Animals were observed individually for 15 min following glutamate injection. The amount of time they spent licking the injected paw was recorded with a chronometer and was considered as indicative of nociception (Beirith et al. 2002).

2.5.5. Hot plate test

The method is an adaptation of that described by Eddy and Leimbach (1953). The hot plate was maintained at 55 ± 0.50°C. Animals were placed on the hot plate and the time between placement and licking of the hind paws or jumping was recorded as the index of response latency. The reaction time was recorded at 0, 30, 60, 120 and 180 min after administration of various doses of the aqueous extract of *Crassocephalum bauchiense* (20, 40, 80 and 160 mg/kg; p.o.), the alkaloid fraction from *Crassocephalum bauchiense* (Fr. XVI; 5, 10, 20 and

40 mg/kg; p.o.), morphine sulphate (positive control, 5 mg/kg, s.c.), or vehicle (10 ml/kg, p.o.). A cut-off time of 30 sec was maintained to minimize tissue damage.

2.6. Measurement of locomotor activity and motor performance

The study of ambulatory behavior was carried out on mice according to a slightly modified method (Ngo Bum et al., 2009). The open field used was a wooden square box 40 × 40 × 45 cm; the floor was divided into 16 smaller squares of equal dimensions (10 × 10 cm). During all the experiments, the laboratory room was dark. The mouse was placed individually into the centre of the arena and allowed to explore freely. The ambulations (the number of crossing sector lines with all four paws) were recorded, over 2 consecutive 30 min periods, 1 h after administration of the aqueous extract of *Crassocephalum bauchiense* (20, 40, 80 and 160 mg/kg; p.o.), the alkaloid fraction from *Crassocephalum bauchiense* (Fr. XVI, 5, 10, 20 and 40 mg/kg; p.o.), morphine sulphate (5 mg/kg, s.c.), or vehicle (10 ml/kg, p.o.).

The motor coordination test was performed to determine side effects of the *Crassocephalum bauchiense* extract using the rotating rod method (Duham and Miya, 1957). A preliminary selection of mice was made on the previous day of experiment excluding those that did not remain on the rotarod bar during a 1 min session each. The bar (diameter 2.50 cm) rotated at a constant speed of 12 revolutions per min. Selected animals were tested 1 h after administration of the aqueous extract of *Crassocephalum bauchiense* (20, 40, 80 and 160 mg/kg; p.o.), the alkaloid fraction from *Crassocephalum bauchiense* (Fr. XVI, 5, 10, 20 and 40 mg/kg; p.o.), morphine sulphate (5 mg/kg, s.c.), or vehicle (10 ml/kg, p.o.). The integrity of motor coordination was assessed on the basis of the number of falls from the rota-rod in 1 min. During the test session itself, i.e. after oral treatment, both the latency to fall from the revolving bar and the number of falls were recorded.

2.7. Acute toxicity test

The acute toxicity test for the aqueous extract and the alkaloid fraction of *Crassocephalum bauchiense* (Fr. XVI,) was carried out to evaluate any possible toxicity. Mice of either sex were divided into control and test groups. The first group served as a normal control treated with vehicle (10 ml/kg; saline 0.90% containing 2% DMSO, p.o.). The aqueous extract of *Crassocephalum bauchiense* (40, 80, 160, 320, 640, 1280, 2560 and 5120 mg/kg) and the alkaloid from *Crassocephalum bauchiense* (Fr. XVI, 40, 80, 160, 320, 1280, 2560 and 5120 mg/kg) were administered orally to different groups of mice. After administration of these extracts, mice were allowed access to food and water *ad libitum* and behaviour parameters including convulsion, hyperactivity, sedation, grooming, loss of righting reflex, increased or decreased respiration, food and water intake and mortality were observed for a period of 14 days. The median lethal dose (LD_{50}) was estimated according to the method described by Litchfield and Wilcoxon (1949).

2.8. Data analysis and statistics

Data were expressed as mean ± standard error of the means (S.E.M.) per group. Statistical differences between control and treated groups were tested by two-way repeated measures analysis of variance (ANOVA), followed by Newman-Keuls post hoc test. The differences were considered significant at $P<0.05$. The ID_{50} (dose of extract necessary to reduce the response by 50% relative to the control value) and 95% confidence intervals values were determined by using linear regression. The statistical package used for the analysis was Graphpad Prism 5.01 for Window (Graphpad Prism Software, San Diego, CA, USA).

3. Results

3.1. Effects of Crassocephalum bauchiense on abdominal constriction induced by acetic acid

Oral administration of the aqueous extract of *Crassocephalum bauchiense* (80 and 160 mg/kg) did not produce any irritation action "*per se*", but caused a dose-related and significant inhibition [$F(25, 97) = 109.58$; $p<0.001$] of acetic acid-induced abdominal constriction in mice (Figure 1). The percentage of inhibition of constrictions was calculated as 36.12% (80 mg/kg) and 57.81% (160 mg/kg). The calculated mean ID_{50} for oral administration of the aqueous extract of *Crassocephalum bauchiense* was 133.82 (112.86 – 154.58) mg/kg. The alkaloids fractions from *Crassocephalum bauchiense* (Fr. II, Fr. VII, Fr. VIII and Fr. XVI) exhibited significant reduction [$F(25, 97) = 173.19$; $p<0.001$] in abdominal writhes induced by acetic acid compared to the control group. Such effects were observed in mice pre-treated by the narcotic analgesic, morphine sulphate (5 mg/kg) used as a reference drug. Naloxone antagonized antinociceptive effect of the extracts of *Crassocephalum bauchiense* in acetic acid-induced abdominal constriction in mice

3.2. Effects of Crassocephalum bauchiense on the formalin test

In vehicle treated control animals the mean paw licking response time was 65.81 ± 1.23 s in the early phase (0 to 5 min) and 71.58 ± 1.27 s in the late phase (15 to 30 min). Morphine sulphate treatment resulted in a marked reduction of response time to 24.35 ± 2.73 s and 23.82 ± 2.41 s in the early and late phases, respectively. The results of Table 1 show that the aqueous extract of *Crassocephalum bauchiense* (80 – 160 mg/kg) caused a significant dose-related inhibition of the neurogenic (0 to 5 min) [$F(11, 56) = 163.15$; $p<0.001$] and the inflammatory (15 to 30 min) [$F(11, 85) = 131.49$; $p<0.001$] of the formalin-induced licking. The calculated mean ID_{50} value for these effects were 111.05 (98.54 – 148.21) and 114.25 (99.73 – 157.87) mg/kg, and the maximal inhibitions were 61.03 and 54.84%, respectively. Pre-treatment of animals with the alkaloid fraction from *Crassocephalum bauchiense* has significant antinociceptive effects on both early (0 to 5 min) [$F(11, 76) = 152.48$; $p<0.001$]

and late phase (15 to 30 min) [F(11, 37) = 152.46; p<0.001] of formalin test as shown on Table 1. Its neurogenic-induced pain blockade, occurred at 40 mg/kg (60.20%), whereas beginning from 10 mg/kg (32.14%), the alkaloid fraction significantly blocked pain emanating from inflammation. The calculated mean ID_{50} values for these effects were 27.82 (17.41 – 38.87) and 23.47 (19.21 – 36.23) mg/kg, against the early and the late phase of the formalin response, respectively. The maximal inhibitions of the early and the late phase were 60.24 and 65.60%, respectively. Pre-treatment of mice with naloxone (1 mg/kg, i.p.), a non selective opioid receptor antagonist, completely and significantly reversed the antinociceptive effects of the extracts of *Crassocephalum bauchiense* in both phases of formalin test.

3.3. Effects of Crassocephalum bauchiense on the capsaicin test

The result of Figure 2 show that the aqueous extract of *Crassocephalum bauchiense* (20 – 160 mg/kg) and the alkaloid fraction (5 – 40 mg/kg) given systematically produced dose-dependant and equipotent inhibition of capsaicin-induced licking. The calculated mean ID_{50} values and the maximal inhibition were 81.25 (68.14 – 98.41) and 13.66 (8.11 – 19.30) mg/kg, and 82.82 [F(6, 71) = 179.12; p<0.001] and 83.70% [F(6, 42) = 106.22; p<0.001], respectively. Morphine sulphate used as positive control, also reduced the licking behavior induced by capsaicin in mice, producing significant inhibition of 80.75% [F(6, 71) = 179.12; p<0.001]. Pretreatment of mice with naloxone (1 mg/kg; i.p.) 15 min before administration of the extracts of *Crassocephalum bauchiense* reversed the reduction in nociception inhibition.

3.4. Effects of Crassocephalum bauchiense on the glutamate test

Interestingly in the glutamate induced nociception in mice, the aqueous extract and the alkaloid fraction of *Crassocephalum bauchiense*, caused marked and the dose-related antinociception (Figure 3). The calculated mean ID_{50} values and the maximal inhibition were

95.62 (64.84 – 97.21) and 13.25 (8.32 – 18.70) mg/kg, and 72.45 [F(6, 35) = 134.51; p<0.001] and 76.01% [F(6, 42) = 115.17; p<0.001], respectively. Given orally dipyrone produced significant inhibition of 74.83% [F(6, 71) = 179.12; p<0.001] of the glutamate-induced nociception in mice (Figure 3). Pre-treatment of mice with naloxone (1 mg/kg, p.o.), significantly prevented the antinociceptive effect induced by the oral administration the extracts of *Crassocephalum bauchiense*.

3.5. Effects of Crassocephalum bauchiense on the hot plate test

All the extracts of *Crassocephalum bauchiense* exhibited varying degree of antinociceptive activity in the thermal nociception model in mice. Figure 4 shows that the aqueous extract of *Crassocephalum bauchiense* tested marked increase [F(6, 79) = 115.25; p<0.001] in the latency response in the hot plate algesiometer model of nociception, with the higher dose administered (160 mg/kg) and the maximal effect was observed in later times after oral administration (120 - 180 min). Besides, the best result was obtained with the alkaloid fraction from *Crassocephalum bauchiense* (40 mg/kg). In this regard, since 60 min after its oral administration it could be observed a significant increase [F(6, 35) = 134.51; p<0.0001] in baseline that reached maximal level (100% increase in baseline) at 180 min. Naloxone antagonized antinociceptive effect of the extracts of *Crassocephalum bauchiense* in hot plate assay procedures.

3.6. Effects of Crassocephalum bauchiense on locomotor activity and motor performance

Mice treated with the aqueous extract and the alkaloid fraction from *Crassocephalum bauchiense*, failed to display any detectable alteration in the locomotor activity, when compared to the cumulative value recorded from control vehicle-treated animals over 0 [F(9,

47) = 171.36; p>0.05], 30 [F(9, 47) = 171.36; p>0.07] and 60 [F(9, 47) = 171.36; p>0.06] minutes (Table 2).

The aqueous extract and the alkaloid fraction of *Crassocephalum bauchiense* 1 h after oral administration did not significantly affect the motor response of the animals. The control response in the rotarod test was 58.24 ± 1.31 vs. 60 s in the presence of the aqueous extract [F(9, 47) = 171.36; p>0.06] and the alkaloid fraction of *Crassocephalum bauchiense* [F(9, 47) = 171.36; p>0.06], respectively. Likewise, as shown in Table 2, none of the aqueous extract [F(9, 82) = 193.41; p>0.07] or the alkaloid fraction of *Crassocephalum bauchiense* [F(9, 82) = 193.41; p>0.07], altered the number of falls from the revolving bar in the rotarod test.

3.7. Acute toxicity test

The aqueous extract (40 - 5120 mg/kg, p.o.) and the alkaloid fraction (40 - 5120 mg/kg, p.o.) prepared from the leaves of *Crassocephalum bauchiense* given to mice, had no effect on their behavioral and no mortality during the observation period of 14 days after administration, so it was not possible to calculate the LD_{50}. Therefore, it can be indicated that the *Crassocephalum bauchiense* extracts has low toxicity profile.

4- Discussion

The result of the current study show that the aqueous extract and the alkaloid fraction prepared from the leaves of *Crassocephalum bauchiense*, produced dose-related and marked antinociceptive effect when assessed in different assays of the chemical nociception and the thermal model of nociception. In general, acetic acid-induced abdominal constriction is used to evaluate the compounds for peripheral antinociceptive activities (Gene et al., 1998; Le Bars et al., 2001; Sanchez-Mateo et al., 2006). Acetic acid injection produces peritoneal inflammation, which triggers a response characterized by writhing (Koster et al., 1959).

Related studies have demonstrated that acetic acid indirectly induces the release of endogenous mediators of pain (such as prostaglandins, kinins, histamin, etc.) that stimulate the nociceptive neurons, which are sensitive to non steroidal anti-inflammatory drugs and opioids (Derardt et al., 1980; Sanchez-Mateo et al., 2006). Our results indicated that the extracts of *Crassocephalum bauchiense* could reduce the number of writhings in animal models, implying that it had a powerful antinociceptive effect. However, the results of this writhing test alone were unable to ascertain whether the antinociception was central or peripheral effect.

The advantage of the formalin model of nociception is that it can discriminate pain in its central and/or peripheral components. It has been reported that formalin-induced persistent pain in mice paws produced a distinct biphasic nociception (Hunskaar and Hole, 1987; Tjolsen et al., 1992). The nociceptive behavior after formalin injection was distinctly recorded in two phases. The first phase of paw licking/biting response starts immediately after injection and is considered probably due to direct stimulation of nociceptors (Dubuisson and Dennis, 1977). The second phase which appears a little later is considered to be due to a combination of an inflammatory reaction in the peripheral tissue and changes in central processing (Tjolsen et al., 1992). Central analgesics, such as narcotics, inhibit both phases, while peripherally acting drugs, such as steroids (hydrocortisone, dexamethasone) and non-steroidal anti-inflammatory drugs suppress mainly the late phase (Hunskaar and Hole, 1987). A significant and dose related antinociceptive effect was clearly evident for all the tested extracts of *Crassocephalum bauchiense* against both neurogenic (early phase) and inflammatory (late phase) pain behavior caused by formalin injection in mice.

In the present study, the possible involvement of the transient receptor potential vanilloid 1 receptor inhibition in the *Crassocephalum bauchiense* induced antinociception was investigated. Our findings showed that *Crassocephalum bauchiense* significantly

inhibited capsaicin-induced nociceptive behavior in a dose-dependent manner. Capsaicin, when injected into the mice paw, is capable of activating in a distinctive subpopulation of primary afferent fibers, which transmit the nociceptive information to central nervous system for the releasing of pro-inflammatory neuropeptides (Holzer, 1991). The intraplantar injection of capsaicin in mice hindpaw induced deep pain-like behaviour related to neurogenic pain, characterized by biting and licking the injected paw (Sakurada, 1992; Santos and Calixto, 1997). Furthermore, the effect of capsaicin was antagonized with a transient receptor potential vanilloid 1 receptor antagonist (Calixto et al., 2005). It is known that there is a release of several chemical mediators, such as excitatory amino acid, substance P, kinins, calcitonin gene-related peptide, prostaglandin E_2 and nitric oxide that contribute for the increase of pain process (Wu et al., 1998). Our results, allow us to suggest the influence of the *Crassocephalum bauchiense* extracts on the actions of neuropeptides.

Currently, it is well-known that between different neurotransmitters that are involved on the pain models cited previously, the excitatory amino acids (glutamate and aspartate) presents a relevant role. It was reported that this nociceptive response caused by glutamate involves peripheral, spinal and supraspinal sites of action with glutamate receptors play an important role modulating this nociceptive response (Beirith et al., 2002). The data presented in our study showed that the *Crassocephalum bauchiense* was capable of interfering with the nociceptive response induced by glutamate, demonstrating, once more, the involvement of neurogenic pathways. Thus, such results suggest that the antinociception induced by *Crassocephalum bauchiense* is associated with its interaction with the glutamatergic system.

To corroborate that the aqueous extract and the alkaloid fraction from the leaves of *Crassocephalum bauchiense* have antinociceptive activity, hot plate test was conducted. In the hot plate test, a central model that has a selectivity for opioid-derived analgesics (Abbott and Melzack, 1982), oral administration of the aqueous extract and the alkaloid fraction from

Crassocephalum bauchiense exerts a potent antinociceptive action confirming the central activity of this extract.

The present results reveal that naloxone was able to significantly attenuate the antinociceptive activity of the investigated *Crassocephalum bauchiense*. This observation suggests a role for opioid mechanism in the antinociceptive action of the aqueous extract and the alkaloid fraction. However, other mechanisms responsible for the analgesic effect of *Crassocephalum bauchiense* have to be.

The present study further demonstrates that, systemic administration of investigated *Crassocephalum bauchiense* did not produce any motor dysfunction, sedation or alteration in locomotor activity of animals. In the present study, we did not observe any mortality case up to the dose of 5120 mg/kg of the aqueous extract and the alkaloid fraction prepared from the leaves of *Crassocephalum bauchiense*. Therefore, we may suggest that the extract has no lethal toxicity in mice even in a dose of 5120 mg/kg they may be considered to be relatively safe.

In conclusion, the present study demonstrated the central and peripheral antinociceptive activity of *Crassocephalum bauchiense* in the test models of chemical nociception induced by acetic acid, formalin, capsaicin and glutamate, as well as in the test model of nociception induced by thermal stimuli, and further suggested that antinociceptive activity of *Crassocephalum bauchiense* might be related to the involvement of the opioïdergic system, which merited further studies regarding the precise site and the mechanism of action. In further investigations, the different fractions of *Crassocephalum bauchiense* will be evaluated and the structural characterization of responsible components will be clarified.

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Figure legends

Figure 1. Influence of the oral treatment with the aqueous extract and the alkaloids fractions prepared from the leaves of *Crassocephalum bauchiense* or morphine sulphate on acetic acid-induced writhing. Results are expressed as mean ± S.E.M., for 6 animals. The extracts at all doses used began manifesting its assuaging effect on the writing reflex 1 h following the administration. Data were analysis by two-way ANOVA, followed by Newman-Keuls post hoc test, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, significantly different compared to the vehicle.

Figure 2. Influence of the oral treatment with the leaves extracts of *Crassocephalum bauchiense* (panel A: aqueous extract, panel B: alkaloid fraction) or morphine sulphate on capsaicin-induced nociception. Results are expressed as mean ± S.E.M., for 6 animals. The amount of time spent licking and biting the injected paw was indicative of neurogenic pain and was recorded in 0 – 5 min. Data were analysis by two-way ANOVA, followed by Newman-Keuls post hoc test, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, significantly different compared to the vehicle.

Figure 3. Influence of the oral treatment with the leaves extracts of *Crassocephalum bauchiense* (panel A: aqueous extract, panel B: alkaloid fraction) or dipyrone on glutamate-induced nociception. Results are expressed as mean ± S.E.M., for 6 animals. The amount of time spent licking and biting the injected paw was indicative of neurogenic pain and was recorded in 0 – 15 min. Data were analysis by two-way ANOVA, followed by Newman-Keuls post hoc test, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, significantly different compared to the vehicle.

Figure 4. Influence of the oral treatment with the leaves extracts of *Crassocephalum bauchiense* (panel A: aqueous extract, panel B: alkaloid fraction) or morphine sulphate on hot plate-induced nociception in mice. Results are expressed as mean ± S.E.M., for 6 animals. Data were analysis by two-way ANOVA, followed by Newman-Keuls post hoc test. $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$, significantly different compared to the vehicle.

Table 1: Influence of the oral treatment of the aqueous extract and the alkaloid fraction prepared from the leaves of *Crassocephalum bauchiense* on formalin-induced pain.

Treatments	**Dose (mg/kg)**	**Licking time (s)**		**Inhibition (%)**	
		Early phase (0-5 min)	**Late phase (15-30 min)**	**Early phase**	**Late phase**
Vehicle	–	65.81 ± 1.23	71.58 ± 1.23	–	–
AE	20	57.75 ± 3.22	56.73 ± 2.31	12.24	20.74
AE	40	51.71 ± 2.37	42.34 ± 1.32*	21.42	40.84
AE	80	29.82 ± 2.91**	34.81 ± 2.54***	54.68	51.36
AE	160	25.73 ± 1.24***	32.33 ± 2.41***	61.90	54.83
AE + Naloxone	160 + 1	53.71 ± 4.15	63.82 ± 2.52	18.38	10.84
AF	5	58.52 ± 3.54	56.24 ± 3.21*	10.78	21.43
AF	10	44.73 ± 2.72*	46.22 ± 3.45**	32.03	35.42
AF	20	32.51 ± 2.32**	32.31 ± 3.72***	50.60	54.86
AF	40	26.24 ± 3.14***	24.72 ± 2.34***	60.12	65.46
AF + Naloxone	40 + 1	60.21± 5.23	67.75 ± 5.32	08.50	05.35
Morphine	5	24.35± 2.73***	23.82 ± 2.41***	62.99	66.72

Results are expressed as mean ± S.E.M., for 6 animals. The amount of time spent licking and biting the injected paw was indicative of pain and was recorded in 0 - 5 min (first phase) and 15 - 30 min (second phase). Data were analysis by two-way ANOVA, followed by Newman-Keuls post hoc test. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, significantly different compared to the vehicle.

Table 2: Influence of the oral treatment of the aqueous extract and the alkaloid fraction prepared from the leaves of *Crassocephalum bauchiense* on locomotor activity in the open field test and on motor performance in the rotarod test

Treatments	**Dose (mg/kg)**	**Locomotor activity**			**Motor coordination**	
		0 min	**30 min**	**60 min**	**Number of falls**	**Time in the rotating bar (s)**
Vehicle	–	18.45 ± 2.27	47.62 ± 8.61	74.82 ± 8.61	0.10 ± 0.10	58.24 ± 1.31
AE	20	18.24 ± 3.52	39.28 ± 6.43	78.53 ± 9.41	0.10 ± 0.10	58.87 ± 1.73
AE	40	17.86 ± 2.34	41.47 ± 7.59	79.63 ± 8.72	0.10 ± 0.00	59.70 ± 1.91
AE	80	18.43 ± 3.62	44.48 ± 8.08	82.11 ± 9.21	0.00 ± 0.00	60.00 ± 0.00
AE	160	18.62 ± 2.44	47.32 ± 9.21	81.82 ± 7.91	0.00 ± 0.00	60.00 ± 0.00
AF	5	18.07 ± 2.32	41.94 ± 9.47	76.09 ± 4.08	0.10 ± 0.10	58.82 ± 5.11
AF	10	17.86 ± 1.91	49.51 ± 8.22	81.58 ± 5.89	0.00 ± 0.00	60.00 ± 0.00
AF	20	17.72 ± 1.68	51.53 ± 7.54	83.39 ± 4.67	0.00 ± 0.00	60.00 ± 0.00
AF	40	18.22 ± 2.08	54.72 ± 6.48	86.47 ± 3.77	0.00 ± 0.00	60.00 ± 0.00
Morphine	5	18.07 ± 1.86	31.63 ± 5.39*	44.29 ± 4.69**	0.80 ± 0.20**	36.93 ± 3.93*

Results are expressed as mean ± S.E.M. of the number of crossing in a cumulative way or of the number of falls and the total time spent on therotating bar during a 1 min test, n = 6 animals. Data were analysis by two-way ANOVA, followed by Newman-Keuls post hoc test.*P<0.05, **P<0.01, significantly different compared to the vehicle.

Table 1: Influence of the oral treatment of the aqueous extract and the alkaloid fraction prepared from the leaves of *Crassocephalum bauchiense* on formalin-induced pain.

Treatments	**Dose (mg/kg)**	**Licking time (s)**		**Inhibition (%)**	
		Early phase (0-5 min)	**Late phase (15-30 min)**	**Early phase**	**Late phase**
Vehicle	–	65.81 ± 1.23	71.58 ± 1.23	–	–
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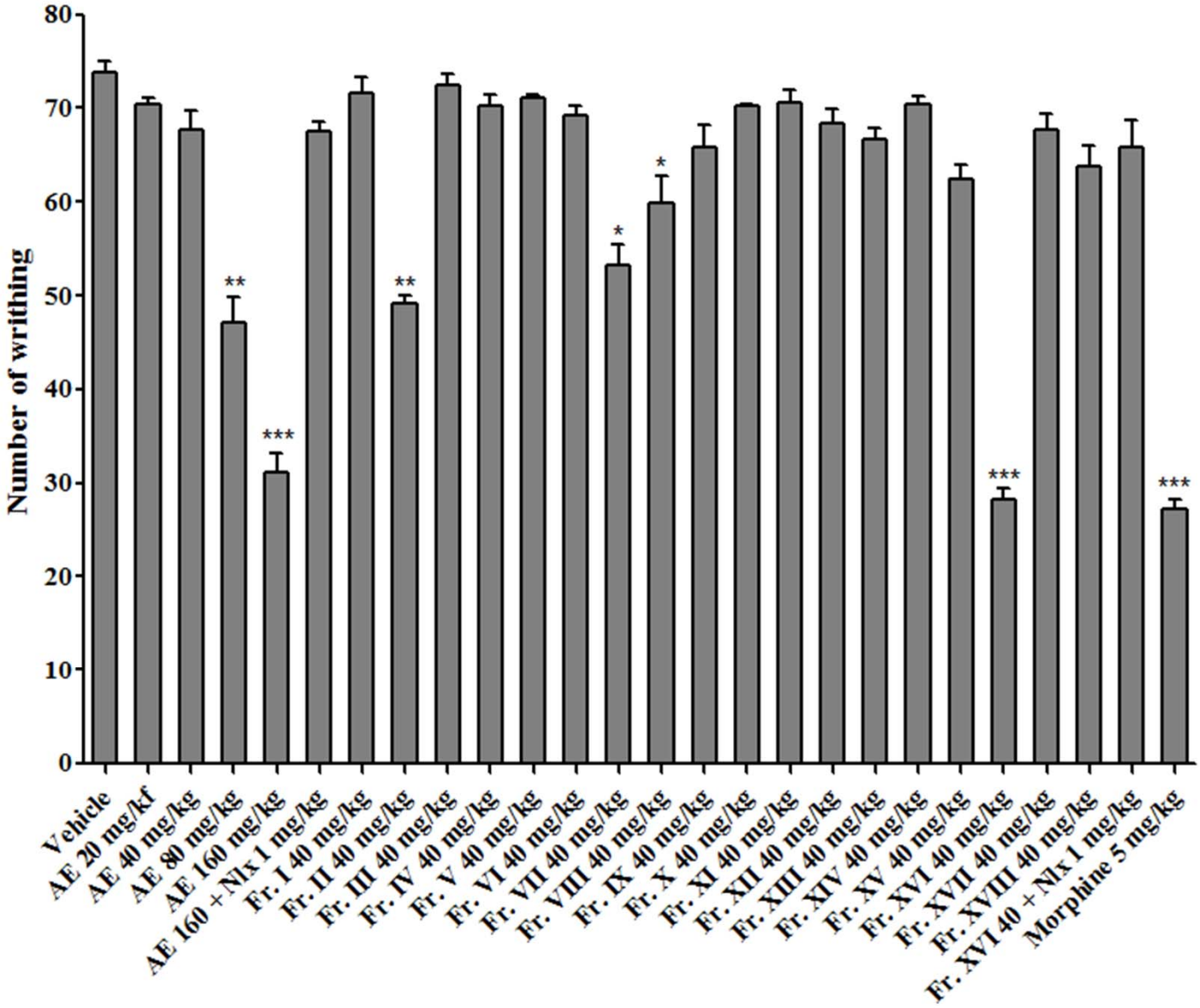
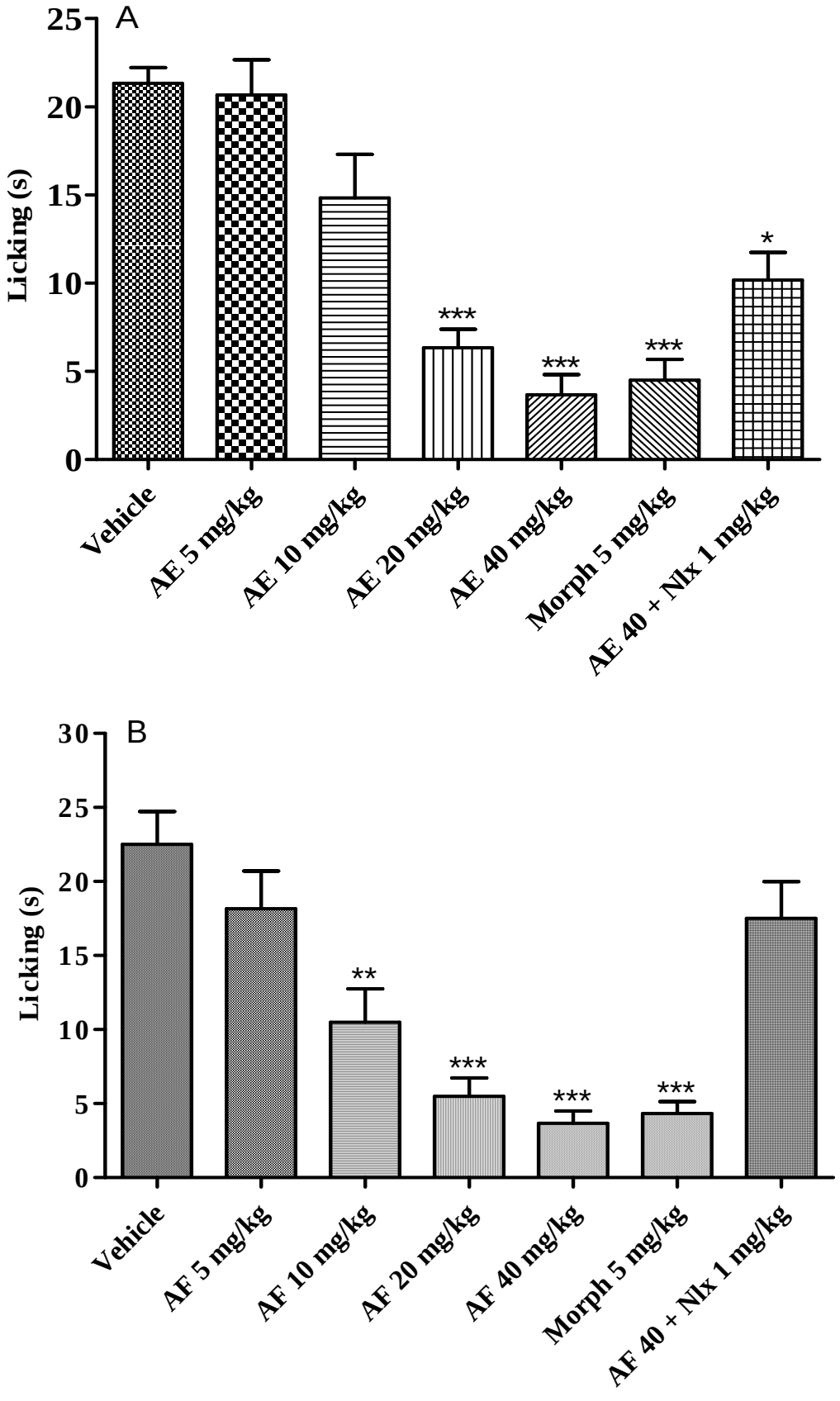


Figure 1:
Germain Sotoing Taïwe et al.



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Germain Sotoing Taïwe et al.

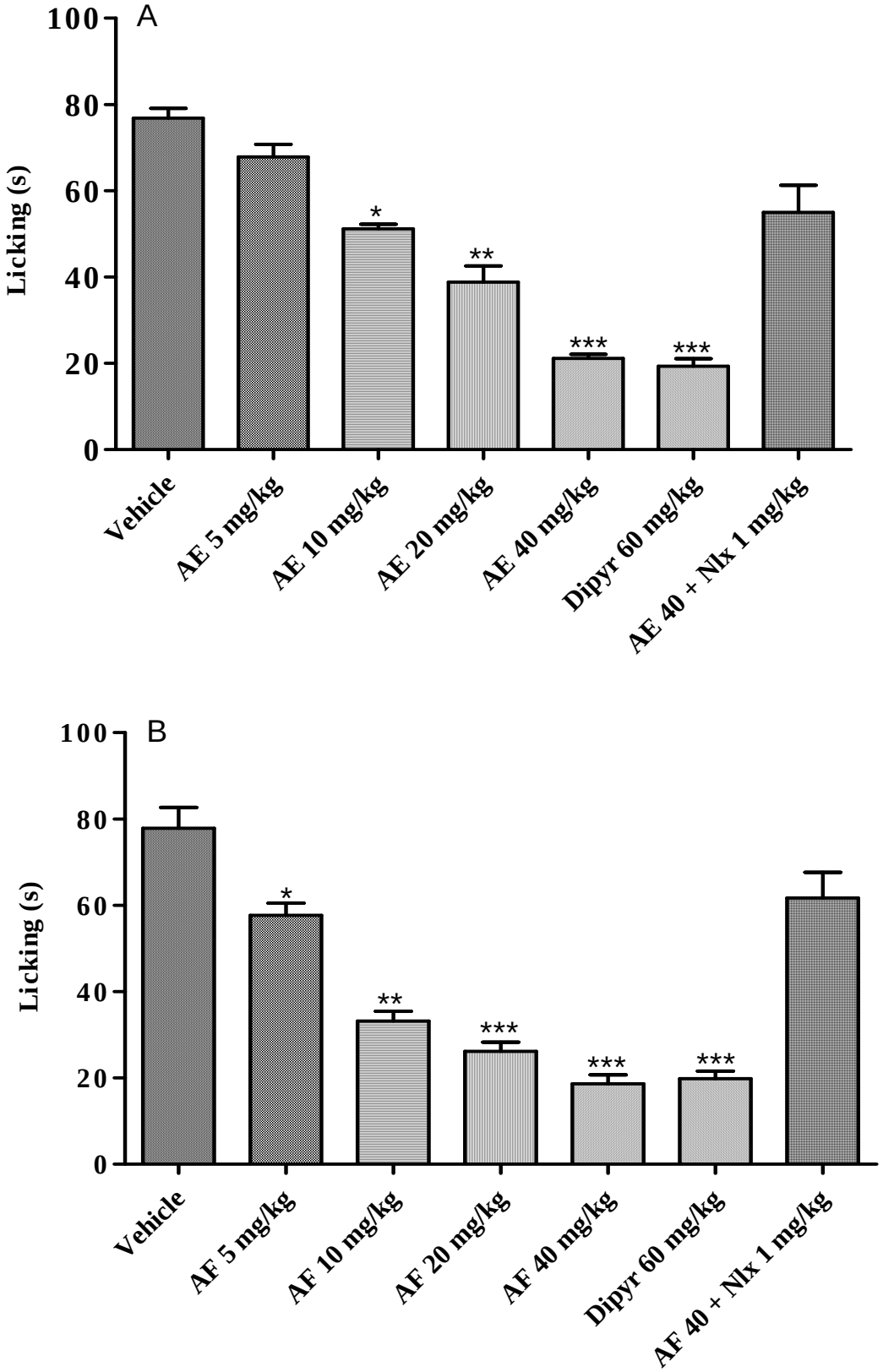

Figure 3:
Germain Sotoing Taïwe et al.

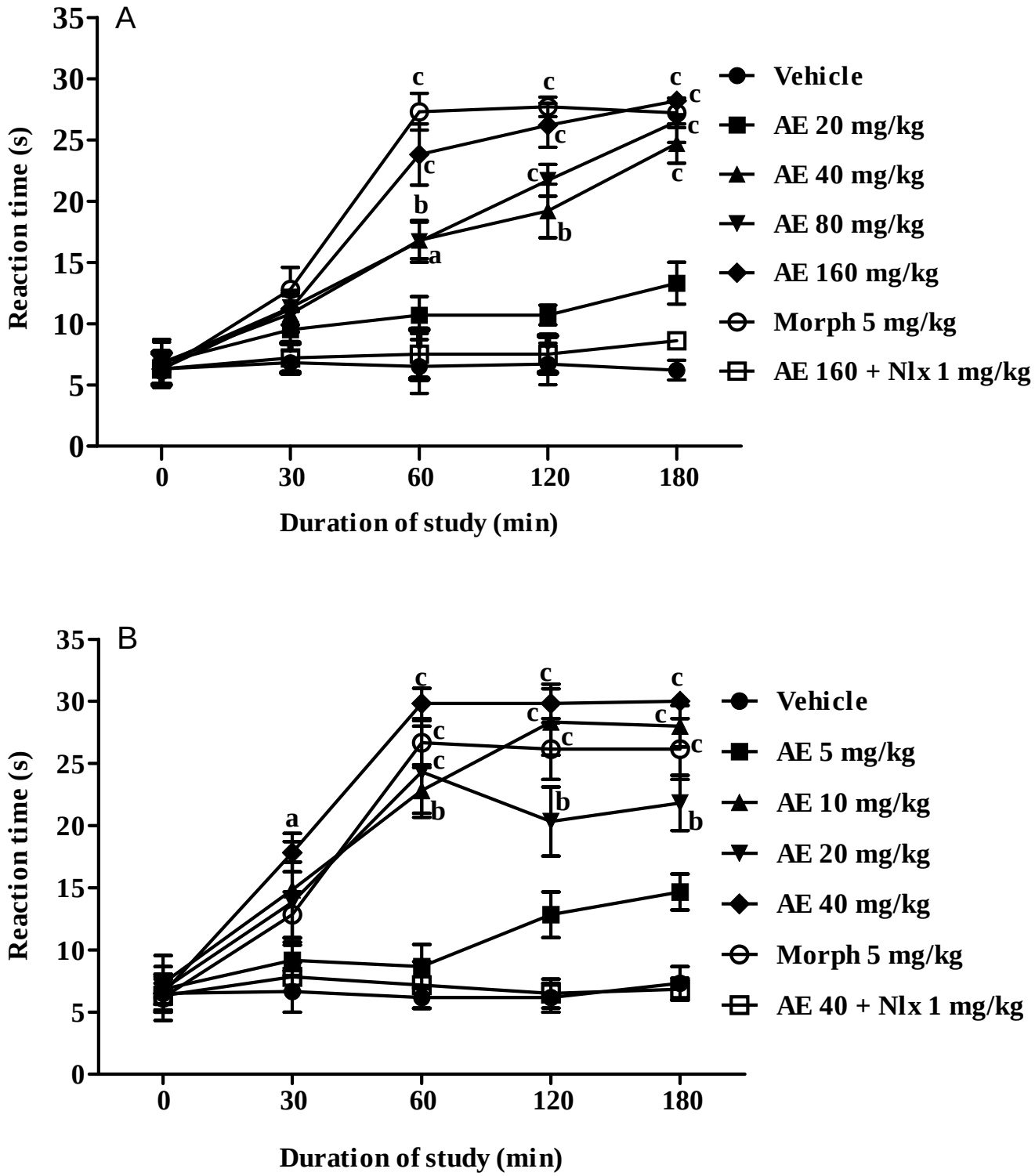

Figure 4:
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