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Analgesia induced by ibuprofen and extract of XL E MS in preclinical murine assays

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Abstract

Several medicinal plants are used empirically as analgesics because their mechanism of action is not known. The study presented is related to the antinociceptive and anti-inflammatory properties of a crude extract of XL E MS that is used in different types of pain. The efficacy of the extract was compared with that of ibuprofen based on its ED50's of dose-response curves of each substance in the murine models of acetic acid writhing (WT), tail flick (TF), formalin hind paw (FHP), hot plate (HP) and Complete Freund´s adjuvant (CFA), tests. The data exhibit he following order of potency for ibuprofen: TF > CFA 1 DAY > CFA 7 DAYS > FHP II > FHP I > HP > WT. In the case of the XL E MS extract, the order of potency obtained was: CFA 7 DAYS > CFA 1 DAY > WT > FHP I > FHP II > HP > TF tests. The results demonstrate that XL E MS extract is less effective than ibuprofen in all the murine assay, with the exception of CFA 1 DAY, CFA 7 DAYS and WT. This difference could be due to the fact that the extract does not activate the same mechanisms or mediators of antinociception and anti-inflammation that ibuprofen triggers and the characteristics of the pain induced by each test. The results obtained suggest that although the effect of XL E MS extract is relatively moderate, it is useful as an analgesic-anti-inflammatory agent when used in traditional herbal medicine.

Keywords: Ibuprofen; Herbal extract; Antinociception: Antiinflammation; Murine assays

1. Introduction

The mainstream pharmacotherapy for the different types of pain includes non-steroidal anti-inflammatory drugs (AINEs), opioids, and co-analgesics, but the side effects restrict their use. Then, medicinal plants are utilized as alternatives due to with low frequency of side effects. However, they lack for a certain mechanism of action, even though several of them are efficacy in animal pain models [1].

Pain it is an unpleasant experience, sensitive and emotional, associated with a current, potential tissue, or related to the same. It has a sensitive and affective component. The use of behavioral studies in rodents is used to evaluate preclinical models of pain. Several murine models include diverse stimuli as thermal (tail flick, hot plate tests), chemical (formalin hind paw, acetic acid writhing tests) or inflammatory (complete Freund´s adjuvant (CFA) assay [2,3].

The tail flick is a nocifensive test characterized by the simplicity and a non-tactile stimulus. The hot plate is a rapid and inexpensive test that can be repetitive without tissue injury. Chemical tests are used to assessment peripheral analgesia (acetic acid writhing test) and central analgesia (formalin test). CFA induces local inflammation and persistent pain. Furthermore, it is necessary to remember that there is no animal model that represents, at least, the main characteristics induced by nociceptive and inflammatory pain.

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The antinociceptive properties of the traditional crude extract of XL E MS used in different types of pain and inflammation are recognized, including gastric pain, toothache, renal colic, and some types of inflammation. The purpose of the present work was to evaluate, in vivo, in murine models of antinociception and anti-inflammation, the properties of the chemical crude extract XL E MS of a medicinal plant.

2. Materials and methods

2.1. Isolation of plant extract

The fresh roots of the plant were collected in the post-flowering summer period, washed, followed by natural drying in the shade and ground into powder. The plant powder was macerated in sterile distilled water and the lyophilizate was filtered using filter paper by double infusion in sterile distilled water, the extract was called XL E MS [4]. The reference drug (ibuprofen) was provided by local laboratory, CFA was purchased from Sigma-Aldrich Chemical Co, USA.

2.2. In vivo assays. Animals

Male CF-1 mice (25-30 g) from the central animal facility of the Faculty of Medicine of the University of Chile were used. Animals were kept under a 12-h light–dark cycle at 22± 2 °C with water and food provided ad libitum. All animal experiments were performed according to the ethical guidelines of the International Association for the Study of Pain and approved by the Animal Care and Use Committee of the Faculty of Medicine (CBA 0852/FMUCH/2019). Mice were acclimatized for 1 hour to the laboratory, utilized only once to the experimental work and euthanized intraperitoneally (i.p.) with 60 mg/kg of pentobarbital after the algesimeter assay. The number of animals was the minimum required for statistical interpretation.

2.3. Measurement of analgesia

Analgesia was evaluated by the following murine assays:

- The tail flick test (TF), The tail flick test (TF) as described previously [5]. A radiant heat with and automatic tail wagging (Ugo Basile, Comerio, Italy) was used to measure response latencies. Baselines were obtained before the protocol and after administration of medications. A time limit of 8 seconds to avoid tissue damage. TF latencies were converted to percentages of the maximum possible effect (% MPE) observed in ibuprofen control animals (n = 8).
- The hot plate test (HP). The assay describe previously was used (5). A commercial device (Ugo Basile, Comerio, Italy) was calibrated at 50° C ± 0.2 and the cut-off time was set to 30 sec to, prevent tissue damage. The time in sec between placement licking or shaking the hind paw or jumping was recorded as the response latency and is a signal of thermal nociception. Each mice was tested twice, before and after the extract XL E MS. HP latency was expressed as % MPE.
- The acetic acid writhing test (WT) as described previously [6]. Animals were injected i.p. with 10 ml/kg of 0.6 % of acetic acid solution and the writhing response was counted for the next 5 minutes and the drugs were administered 30, min prior to the acetic acid solution. Antinociception was expressed as % MPE.
- The formalin hind paw (FHP) test as described previously [6]. To achieve the test, 20 µl of 2% formalin solution was injected into the dorsal surface of the right hind paw. The pain was assessed as the time spent licking or biting the injected paw, expressed in seconds, and converted to % MPE. This test shows two phases, each associated to a different type of pain. The phase I occur withing 5 min post-formalin injection and reflects tonic acute pain. Phase II spans 10 min, starting 20 min after formalin injection and reflects inflammatory pain.
- Complete Freund´s adjuvant (CFA) assay. This inflammatory pain model was induced as previously reported [7]. Mice were intraplantar injected with 50 µl of CFA in the left hind paw. Control mice were injected with 50 µl of saline. CFA induce a severe inflammatory pain measured after 30 min of drugs administration at 1 day (acute assay) and 7 days (chronic assay) using the paw latency in the hot plate test and expressed in % MPE.

2.4. Experimental design

The analgesic or the angi-inflammatory activity of ibuprofen and the extract XL E MS was assessed from the dose response curves, administered 30 min before the CFA-1 DAY and 7 DAYS, TF, FHP-I and II, WT and HP tests. The different curves were obtained using 8 mice for each at least 4 doses. The ED_{50} values were calculated from a linear regression of the corresponding dose-response curves

2.5. Statistical data analysis

The test results are tabulated as the mean ± S.E.M. Significance of the results results was assessed through one-way ANOVA, followed by Tukey's post-test analysis, where values of $P < 0.05$ were deemed significant.

3. Results

The extract XL E MS and ibuprofen did not induce at all doses used changes in the behavioral or in the motor dysfunction of the mice.

3.1. Analgesic efficacy induced by ibuprofen and the extract XL E MS

To evaluate whether the analgesic activity of ibuprofen and XL E MS extract are similar in all tests, complete dose-dependent curves were obtained in each test for the compounds. The results demonstrate a significant antinociceptive correlation between ibuprofen and the XL E MS extract in the different tests of this work. The data exhibit he following order of potency for ibuprofen: TF >CF A 1 DAY >CFA 7 DAYS> FHP II > FHP I > HP >WT tests. In the case of the XL E MS extract, the order of potency obtained was: CFA 7 DAYS > CFA 1 DAY > WT > FHP I > FHP II > HP > tests. Furthermore, the relationship between the ED_{50} values, denoted as the AR relationship, varied between 5.1 and 1.2 in the following order: CFA 7 DAYS > CFA 1 DAY > WT > FHP I > FHP II > HP > TF tests. All data are in shown in Table 1 and Figure 1.

Table 1 ED_{50} values (means ± SEM) in mg/kg for the antinociceptive and anti-inflammatory activity of ibuprofen and the extract XLEMS in different algisimeter assays of mice

ASSAY	ED50 IBUPROFEN	ED50 XL E MS	AR
TF	15.5 ± 1.1	125.4 ± 5.4	8.09
CFA-1 DAY	21.3 ± 0.8	17.8 ± 0.8	0.83
CFA-7 DAYS	22.5 ± 0.9	15.7 ± 0.9	0.70
FHP II	36.9 ± 3.6	89.5 ± 2.1	2.42
FHP I	37.4 ± 3.2	74.1 ± 8.8	1.98
HP	42.1 ± 7.3	101.2 ± 6.3	2.40
WT	56. 9 ± 3.4	39.3 ± 3.0	0.69

Note: AR: ratio between ED_{50} XLEMS / IBUPROFEN treatment. All results are statistically significant compared both group ($P < 0.005$).
Abbreviations: TF: tail flick, CFA-1 DAY: complete Freund´s adjuvant, 1 day, CFA-7 DAYs: complete Freund´s adjuvant, 7 days, FHP II: formalin hind paw, phase II; FHP I: formalin hind paw, phase I; HP: hot plate and WT, acetic acid writhing tests.

4. Discussion

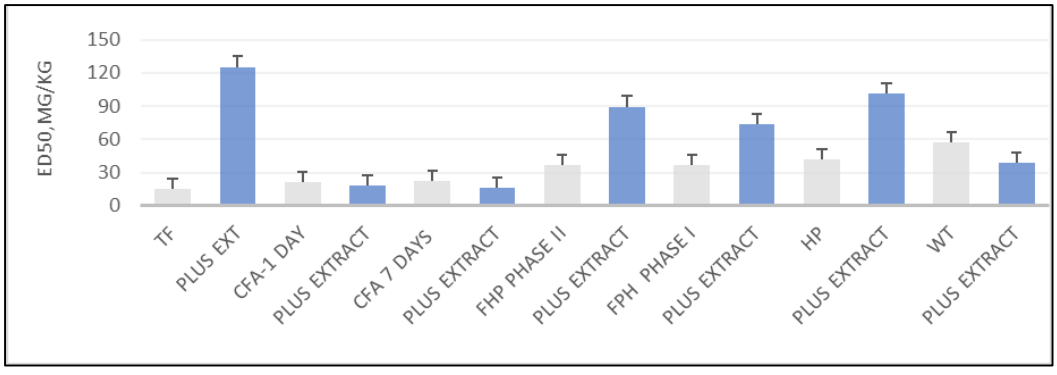

Figure 1 Effect antinociceptive and anti-inflammatory activity of the ED_{50} of ibuprofen and the ED_{50} of extract XLEMS in diverse assays of mice. Abbreviations: TF: tail flick, CFA-1 DAY: complete Freund´s adjuvant, 1 day, CFA-7 DAYs: complete Freund´s adjuvant, 7 days, FHP II: formalin hind paw, phase II; FHP I: formalin hind paw, phase I; HP: hot plate and WT, acetic acid writhing tests. The ED_{50}'s of ibuprofen and the extract XL R MS are shown in white and blue columns, respectively. Columns represent the mean ± SEM of 8 mice. All the results are statically significant, $P < 0.05$, versus ibuprofen

Biomedical research has used various animal models to study the pathogenesis of different diseases, as well as their therapeutic profiles. There are several studies on pain behavior in animals, especially murine animals, that are frequently used and focus on their mechanisms of action. Nevertheless, it is necessary to establish that there is no animal model that captures the complete profile of nociception. Analysis of plants used for medicinal purposes demonstrates the existence of a wide variety of different phytochemical constituents such as amino acids, flavonoids, steroids, alkaloids, triterpenoids, glycosides, and others, all of which contribute to their multiple effects [7].

For the evaluation of the antinociceptive activity of a phasic pain test, nociceptive stimuli of chemical nature (acetic acid, formalin) or thermal nature (tail flick, hot plate) were used. On the other hand, to cause inflammatory pain, nociception induced by CFA (Complete Freund's Adjuvant) or formalin is usually used. The antinociceptive activity obtained by the acetic acid-induced writhing model is essential due to the activation of prostaglandin synthesis induced by cyclooxygenases (COXs) that produce an increase in pain perception in sensory nerve endings. Furthermore, it has been described that in the peripheral analgesia produced by ibuprofen, other mediators such as serotonin, nitridergic, GABAergic, and opioidergic receptors are likely to be involved [8]. Therefore, it can be deduced that there are central and peripheral processes involved in abdominal contortions. The results demonstrate that XL E MS extract is less effective than ibuprofen in the acetic acid-induced writhing test. This difference could be due to the fact that the extract does not activate the same mechanisms or mediators of nociception that ibuprofen activates.

To evaluate antinociceptive activity, with another chemical stimulus model, the formalin test of the hind paw was used, in which ibuprofen was able to induce analgesia in both phases of the test. It has been proposed that this antinociceptive effect is due to the upregulation of substance P, serotonin, nitric oxide, prostaglandins, opioids, cannabinoids, and histamine [9,10]. This trial is a model of tonic and visceral pain in which the extract used as an antinociceptive agent demonstrated a less affinity in both phases of the test by increasing the ED_{50} two-fold compared to that of ibuprofen.

Preclinical studies have shown that the tail movement induced in the tail flick depends on the activation of peripheral nociceptors located at the spinal level that are modulated supraspinal. In this work, the NSAID ibuprofen was able to provoke an antinociceptive response in the tail flick test, in contrast to previously reported, the lack of response produced by NSAIDs in this preclinical model [11,12]. It is suggested that ibuprofen response is due to the activation of histaminergic, cannabinoid, purinergic, and transient receptor potential channels [8]. In this thermal test, the XL E MS extract used as an analgesic agent produced an ED_{50} that was 8.09 times lower in efficacy than the ED_{50} of ibuprofen. The deficient antinociceptive role of the extract, both in the formalin hind paw and in the tail flick, tests, could be due to its limiting effect on the mediators of which nociception depends.

The hot plate test was used to assess central thermal nociception. The analgesic activity induced by ibuprofen in this trial is believed to be due to the opening of K+ channels in the central and peripheral nervous system as an important mechanism. Furthermore, this NSAID is capable of inducing analgesia by interacting directly with other neuronal channels, such as Na, Ca, and Cl. On the other hand, it has been proposed that the antinociceptive activity in the hot plate is due to the activation of COXs and the following receptors nicotinergic, adrenergic, serotonergic, opioidergic, and cannabinoidergic [8,12]. The results demonstrate that XL E MS extract is less effective than ibuprofen in the hot plate test. This 2.40 times decrease in the ED_{50} of the extract compared to the ED_{50} of ibuprofen could be due to the lower potency of the extract to activate antinociception mechanisms.

In this study, the CFA (Complete Freund's Adjuvant) test was used as a preclinical model of inflammatory pain, a dose-dependent agent that produces local inflammation on day 1 (acute phase) and persistent pain on day 7 (chronic phase). Ibuprofen produced effective antinociception in both phases of inflammatory pain due to CFA. It has been postulated that CFA is capable of stimulating an immune response by promoting the release of cytokines, eicosanoids, and neurotrophins [8,13]. Also, it has been proposed changes in certain amino acid level. Additional mechanisms involved in CFA-induced pain include the production and release of prostaglandin E_2, NO, leukotriene B_2, TNF, IL-2, and IL-17 [13]. The potency of XL E MS extract as an analgesic agent in the CFA trial produced an ED50 that was 0.83 times and 0.70 times lower in efficacy at day 1 and 7 days, respectively, than the ED50 of ibuprofen. This significant decrease in the potency of the extract could be due to the lack of activation of the same antinociceptive mediators described for ibuprofen.

5. Conclusions

The present study demonstrated that ibuprofen has a higher antinociceptive and anti-inflammatory efficacy than that induced by the XL E MS extract in the murine models of acetic acid writhing (WT), tail flick (TF), hind paw formalin (FHP), plate hot. (HP) and complete Freund's adjuvant (CFA). The differences in effectiveness between the tests could be due to differences in the mechanism of action of the agents and the characteristics of the pain induced by each test.

The results obtained suggest that although the effect of XL E MS extract is relatively moderate, it is useful as an analgesic when used in traditional herbal medicine.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest.

Authors contribution

All authors contributed equally in preparing all parts of the work and approved the version submitted for revision.

Statement of ethical approval

Hereby, Hugo F. Miranda, I assure that the manuscript: "**Analgesia induced by ibuprofen and extract of XL E MS in preclinical murine assays** " complies with being an original research work, that has not been sent for publication elsewhere, that it has been prepared by both authors and are responsible for their content. All animal procedures were performed in accordance with the ethical guidelines of the International Association for the study of pain and approved by the animal care and use committee of the Faculty of Medicine, University of Chile, (CBA 0852/FMUCH/2018).

References

[1] Dragos D, Gilca M, Gaman L, Vlad A, Iosif L, Stoian I, Lupescu O. Phytomedicine in Joint Disorders. Nutrients. 2017 ;9(1):70-88.

[2] Gregory NS, Harris AL, Robinson CR, Dougherty PM, Fuchs PN, Sluka KA. An overview of animal models of pain: disease models and outcome measures. J Pain. 2013;14(11):1255-1269.

[3] Larson CM, Wilcox GL, Fairbanks CA. The Study of Pain in Rats and Mice. Comp Med. 2019;69(6):555-570.

[4] Mahnashi, M. H., Alqahtani, Y. S., Alyami, B. A., Alqarni, A. O., Ahmed Alshrahili, M., Abou-Salim, M. A., Mohammed N. Alqahtani., Sadaf Mushtaq., Abdul Sadiq., and Muhammad Saeed Jan 7. GC-MS analysis and various in vitro and in vivo pharmacological potential of habenaria plantaginea lindl. Evid. Based Complement. Altern. Med. 2022, 7921408. doi:10.1155/2022/7921408

[5] Mahrous A. Abou-Salim,3 Mohammed N. Alqahtani,4 Sadaf Mushtaq,5 Abdul Sadiq,6 and Muhammad Saeed Jan 7 1 Department of Pharmaceutical Chemistry, College of Pharmacy, N

[6] Miranda HF, Noriega V, Sierralta F, Sotomayor-Zarate R, Prieto JC. Risperidone in analgesia induced by paracetamol and meloxicam in experimental pain. Fundam Clin Pharmacol. 2022;36(3):494-500. doi:10.1111/fcp.1275

[7] Nuro GB, Tolossa K, Giday M. Medicinal Plants Used by Oromo Community in Kofale District, West-Arsi Zone, Oromia Regional State, Ethiopia. *J Exp Pharmacol*. 2024; 16:81-109

[8] Yam MF, Loh YC, Oo CW, Basir R. Overview of Neurological Mechanism of Pain Profile Used for Animal "Pain-Like" Behavioral Study with Proposed Analgesic Pathways. Int J Mol Sci. 2020 ;21(12):4355-4380.

[9] Arrau S, Rodríguez-Díaz M, Cassels BK, Valenzuela-Barra G, Delporte C, Barriga A, Miranda HF. Antihyperalgesic Activity of Quillaic Acid Obtained from Quillaja Saponaria Mol. Curr Top Med Chem. 2019;19(11):927-930.

[10] Miranda Hugo F., Noriega V., Aguilera V., Sierralta F., Prieto JC. Pharmacodynamic differences between racemic ibuprofen and dexibuprofen in murine poreclinical study. Intertnational Journal of Frontiers in Chemistry and Pharmacy Research 2023; 03(02), 001-007.

[11] Dogrul A, Gülmez SE, Deveci MS, Gul H, Ossipov MH, Porreca F, Tulunay FC. The local antinociceptive actions of nonsteroidal antiinflammatory drugs in the mouse radiant heat tail-flick test. Anesth Analg. 2007 ;104(4):927-935.

[12] Connor J, Makonnen E, Rostom A. Comparison of analgesic effects of khat (Catha edulis Forsk) extract, D-amphetamine and ibuprofen in mice. J Pharm Pharmacol. 2000 ;52(1):107-110.

[13] Lazarević M, Stanisavljević S, Nikolovski N, Dimitrijević M, Miljković Đ. Complete Freund's adjuvant as a confou nding factor in multiple sclerosis research. Front Immunol. 2024; 15:1353865. doi: 10.3389/fimmu.2024.1353865. PMID: 38426111