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Research Article
Analgesic Effect of Herbal Extract

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Abstract

Background and Objective: Regardless of the socioeconomic level and fear of dental care, most people let their dental diseases to progress until pain becomes unbearable, turning acute pain into chronic pain. This represents 40% of the causes for which people seek dental care. The objective of this study was to determine the analgesic effect of a Swedish herbal extract in an animal model. **Materials and Methods:** This study included 80 *Mus musculus* mice 4-6 weeks of age, randomly distributed into 4 groups of 20 mice. The experiment was carried out in three stages. The extract was administrated orally and the response on the hot plate at 15 and 30 min was evaluated. The Turkey HSD test and a variance of analysis test were used for comparisons between groups. **Results:** The results in the three days were 16.60±3.81, 17.44±5.79 and 16.10±1.84 sec for the Swedish herbal extract compared to 11.51±3.84, 15.47±3.17 and 17.84±3.02 sec for meloxicam, 15 min after compound administration. In the 30min tests, the results were 16.52±5.17, 12.34±2.55 and 13.97±6.33 sec for the Swedish herbal extract compared with meloxicam which was 17.72±6.05, 17.84±5.63 and 17.38±6.33. **Conclusion:** The analgesic effect of the Swedish herbal extract was effective and similar to meloxicam. It is an excellent option as an alternative or complementary drug treatment.

Key words: Pain, analgesia, medicinal plant, dental care, alternative medicine

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Regardless of socioeconomic level and fear of dental care, most people let their dental diseases to progress until pain becomes unbearable, turning acute pain into chronic pain which is partially or permanently incapacitating. This situation represents 40% of reasons for seeking dental care[1]. Different plants and chemical substances have been used to control pain. These analgesics have been grouped into two main categories: opioids and non steroidal anti-inflammatory drugs[2] however, an effective low cost alternative is needed to help patients control pain while seeking and receiving dental care. This alternative would be very useful in communities without immediate access to dental care.

The International Association for the Study of Pain (IASP) defines pain as "A unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage"[3]. This is an early alarm that forces an individual to obtain proper care to avoid or prevent future damage.

After many observations and investigations, it now know that pain is a response to stimuli on special receptors, nociceptors, that transform these stimuli into electrical signals that move through nerves and reach areas in the brain where they are interpreted[2].

The sensation of pain is a totally personal experience that cannot be shared, expressed or measured and it is one of the most common reasons why people seek help. Specifically, dental pain is considered one of the most disturbing pains a person may experience[4].

Pain is frequent in the orofacial region and can physical and psychological in nature. Physical pain can be generated by external or internal factors (acute pain) or should have a neuro-pathological nature (chronic pain). Tooth pain can be generated by the effect of different factors on nociceptors[5].

To treat pain in dental care, it must recognize all its characteristics. Advances in the study and treatment of pain provide a way to evaluate and measure it. Measuring pain should lead to better treatment and dosification[6]. Different pain evaluation scales have been developed to evaluate, reevaluate and compare it[7]. The Pain Relief Ladder of the World Health Organization is composed of three steps, which show different medications and their uses[7].

The causes of pain and its intensity obligate the use of different drugs and to find the appropriate dose to achieve complete relief. It is important to develop a standard measure of drugs and pain relief[8]. Low-cost alternatives are needed to help patients control pain while seeking and receiving dental care. These alternatives would be very useful in communities with limited access to dental care.

Countless products have been used in modern therapeutics. These have been tested in preclinical and clinical studies that support their efficacy. The Swedish herbal extract also known as "Bitter Swedish" is an original Swedish formula that has persisted to this day. Its ingredients are: 10 g Aloe, 5 g Myrrh, 0.2 g Saffron, 10 g Senna leaves, 10 g Camphor (Chinese and natural), 10 g Riubardo roots, 10 g Ceodaria roots, 10 g Manna, 10 g Theriac, 5 g Carlina roots, 10 g Angelica roots[1,9] (Fig. 1).

The hot plate test is one of the oldest[10-12] and most widely used in experimental methods to assess nociception in rats and mice[13]. The test consists of placing a rodent on an enclosed hot plate and measuring the latency to lick a hind paw or jump out of the enclosure[13]. The advantages of this test are that it is objective, quantifyable can be administered repeatedly without causing inflammation and assesses supra spinal-organized responses to a noxious stimulus[14].

This study was performed to determine the analgesic effect of this Swedish herbal extract based on a previous study that demonstrated its anti-inflammatory[1], antibiotic and anticancer[8] effects.

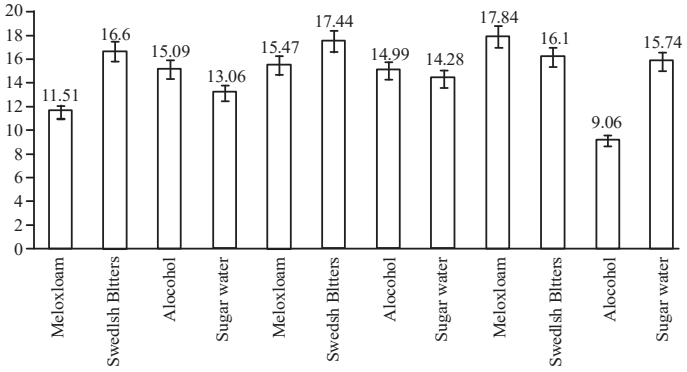

Fig. 1: Swedish herbal extract

Table 1: Statistical variables used for evaluation of the compounds

Independent	Dependent
Weight: 7.4-16.74 g	Swedish herbal extract (Milligrams according to weight)
Gender: Female	Swedish herbal extract vehicle, 40% alcohol (Milligrams according to weight)
Age: 4-6 weeks	Meloxicam (Milligrams according to weight)
Strain: *Mus Musculus*	Meloxicam vehicle, sugar water (Milligrams according to weight)

Table 2: Mean dose of study compounds administered according to body weight of mice

Group	Component	Mean weight(g)	Dose (μLg^{-1})
One	Swedish herbal extract	10.686	83
Two	Meloxicam	11.77	130
Three	Medicinal Plant Compound vehicle	10.291	106
Four	Meloxicam vehicle	10.622	106

MATERIALS AND METHODS

This was a comparative, experimental *in vivo* study performed with female *Mus musculus* mice (Harlan Mexico, S.A.de C.V, Mexico city). Animals were cared according to the University of California, San Francisco Institutional Animal Care and Use Committee/Laboratory Animal Resource Center (UCSF IACUC/LARC) standard procedures (http://iacuc.ucsf.edu/Policies/aw Standard Procedures. asp) and the Mexican Official Norm NOM-062-ZOO-1999 on the production, care and use of experimental animals,

The study was conducted in three stages divided into three days; 20 *Mus musculus* mice, 4-6 weeks of age and weighing 7.4-16.74 g were randomly distributed into 4 groups of 5 mice. The mice were exposed to each element, the Swedish herbal extract, the vehicle of the Swedish herbal extract (alcohol), meloxicam and the vehicle of meloxicam (sugar water). Colored tail marks were used to distinguish the mice in the groups (Table 1).

One week before the experiment, all mice were placed on the hot plate in order to obtain a mean of the time they could remain on the hot plate before suffering burns or other problems while testing the elements. The reaction was observed and the time registered. The mice could lick their front or back paws, jump or squeal; licking their paws was the most common reaction. The animals were left to rest this day and were then left to rest a week before beginning with the administration of the study elements. Substances were prepared and administered to the mice during the following week using this formula: The compound was calculated at 2 mg kg^{-1}, this was multiplied by mean weight (x). The concentration of the compound was 0.5 mg mL^{-1} (y); thus $x \times y$ = Microliters per mouse (Table 2).

The medicinal plant compound and all the other elements were administrated orally with a special probe. The hot plate was turned on before administration and was used after reaching a temperature of 55±1.5°C, which is the variation needed to maintain the temperature at 55°C. While the hot plate reached the determined temperature, mice were placed in a different area so they could relax before the experiment. After administration of the compounds, 15 min were measured, then the mice were placed on the hot plate one by one; the time they remained was measured and their reaction was observed. After all mice were placed on the hot plate and readings were taken, a further 15 min were measured and the mice were placed on the hot plate again, observing their reactions and taking the corresponding readings from the second test.

Experiments were carried out on 3 different days, with two replicates per day per group, one at 15 min and another at 30 min after administration of the elixir, meloxicam and their vehicles.

Statistical analysis: ANOVA one factor analysis of variance was conducted from the data obtained from the experimental groups. Tukey's HSD test[15] was used for multiple comparisons between groups.

RESULTS

To determine the effectiveness of the Swedish herbal extract, 3 different days of tests were performed. Mean values for day 1 were 16.60±3.81 sec. The minimum reaction time was 12.09 sec and the maximum 20.79 sec. On day 2, using the same procedure, the mean reaction time was 17.44±5.79 sec, ranging from 11.49-24.27 for the Swedish extract. On day 3, using the same procedure, mean values were 16.10±1.84 sec for the Swedish herbal extract ranging from 14.70-19.18 sec (Fig. 2).

Tests performed at 30 min with the same procedure on day 1 showed a mean reaction time of 16.52±5.17 for the Swedish herbal extract, ranging from 10.67-26.81 sec. On day 2 the mean reaction time was 12.34±2.55 for the Swedish herbal extract, ranging from 9.37-16.38 sec. On day three the mean reaction time for the Swedish herbal extract was 13.97±6.33 sec with a confidence interval of 6.10-21.83 sec.

ANOVA was used to compare the reaction times at 15 min in the four groups. No significant difference was found between the groups on the first or second day ($p = 0.306$ and $p = 0.596$, respectively). On the 3rd day, a significant difference in reaction time was found ($p = 0.001$) (Table 3).

The ANOVA comparing the reaction times of the four groups at 30 min did not show a statistical significance during any of the 3 days ($p = 0.134$, $p = 0.115$ and $p = 0.059$, respectively (Table 4).

Using Tukey's HSD test, it was possible to conclude that there was a significant difference in the reaction times of some groups in comparison with alcohol, mainly meloxicam ($p = 0.001$) and the Swedish herbal extract ($p = 0.001$) in the third test performed at 15 min. No other significant differences were found with the Tukey HSD test with regard to the reaction time of any of the study groups ($p>0.05$).

DISCUSSION

Experiments were done to demonstrate the analgesic effect of the Swedish herbal extract using *Mus musculus* mice on the hot plate test at 15 and 30 min in comparison with meloxicam, a well-known analgesic. No significant difference was found between the two compounds ($p>0.05$), verifying that the Swedish herbal extract can be useful for pain control.

The results showed that the Swedish herbal extract has a faster analgesic response than meloxicam at 15 min. However, meloxicam at 30 min was slightly more effective than the Swedish herbal extract.

It was expected that smaller mice would have an immediate reaction when placed on the hot plate but after all the tests were done, it was found that they responded the same as those with greater weight. It was also expected that the first reaction would be a jump or a shriek; however, their first reaction was that they licked their front and back paws.

Complementary and/or alternative treatments, change continuously and once their effectiveness is proven, they should be incorporated into conventional health treatments. This study defined the analgesic effects of a Swedish herbal extract observing latency results similar to the analgesic effect of meloxicam and their vehicles.

The study coincides with Gunn *et al.*[14], Onasanwo *et al.*[16] and Toro-Vega[17] ,regarding the effectiveness of the hot plate test for measuring an analgesic effect although with different variables. Among these, weight, analgesic dose, response time and habituation time, correlated with latency. Gunn *et al.*[14] stated that her test with the hot plate is reliable and easy to use to evaluate nociceptive and adrenal responses. The advantages of this test are that it is objective, quantifyable, could be repeatedly done without causing any damage and evaluates organized supra spinal responses through a harmful response. They describe and perform a model of classic somatic pain, a heat-induced nociceptive test. Toro-Vega[17] used a modified method previously described by Menendez *et al.*[18], where animals were placed on a hot plate at 45±1°C. Gunn *et al.*[14] placed the rats at 52.5±1°C and Onasanwo *et al.*[16] placed them between 45°C and 52.5±1°C. The latency of pain sign was licking both front and/or hind legs or leaping off the hot surface. This was recorded with a timer, previously determining the mean time that the animal remains on the hot plate without suffering damage. For all the experiments, the reactions obtained were that of jumping, licking legs and paws and on some occasions, a squeal. Therefore, this previously used hot plate method has proven effectiveness.

In the study by Rodgers *et al.*[19], it was found that obese mice lose sensitivity. It was demonstrated using the hot plate that sensation decreases considerably in obese mice. In this study, they confirmed that the weight of the mouse influences in the impression received by induced heat. They did not use any type of analgesic; however, they did use the hot plate test. In the present study, the use of obese mice was not important

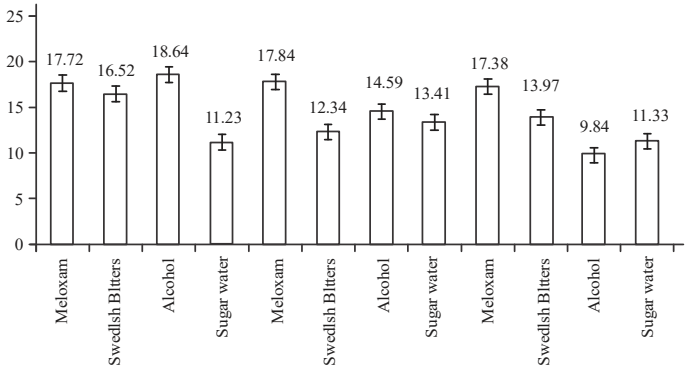

Fig. 2: Mean reaction time in sec. of each study group in the fifteen min test according to the compound used

Table 3: Analysis of variance, 15 min test, comparing mean reaction time between groups

Days	Square sums	df	Mean- square	F-value	Sig.
1					
Between groups	74.18	3	24.73	1.32	0.306
In the groups	282.06	15	18.80		
Total	356.24	18			
2					
Between groups	27.46	3	9.15	0.65	0.596
In the groups	226.45	16	14.15		
Total	253.91	19			
3					
Between groups	190.27	3	63.42	10.26	0.001
In the groups	92.73	15	6.18		
Total	283.00	18			

Table 4: Analysis of variance, thirty-minute test, comparing mean reaction time between groups

Days	Square sums	df	Mean-square	F-value	Sig.
1					
Between groups	157.37	3	52.46	2.17	0.134
In the groups	362.90	15	24.19		
Total	520.27	18			
2					
Between groups	84.98	3	28.33	2.31	0.115
In the groups	196.36	16	12.27		
Total	281.34	19			
3					
Between groups	152.52	3	50.84	3.09	0.059
In the groups	246.66	15	16.44		
Total	399.18	18			

since the real importance was to measure the analgesic effect produced by the Swedish herbal extract in comparison with meloxicam, with a low-weight model (all mouse weights were between 7.4 and 16.74 g) used to do the tests, thus weight, as a condition in this case, was ruled out.

Menendez *et al.*[18] conducted tests based on comparative studies using morphine in conjunction with the hot plate test. The result was that morphine influences the thermal response and produces analgesia in the mouse. Also, Kuhar *et al.*[20] performed mediated desensitization, where JNK2 was required to verify the analgesic tolerance mediated with morphine. They used the hot plate test to verify this induced analgesia. Both of these studies are comparative with this one because they used an analgesic product and did the hot plate test; however, the difference was that we used meloxicam and compared the analgesic effect with a complementary product, the Swedish herbal extract proving with this comparison, that both had a similar analgesic activity demonstrated with the hot plate test.

Chatterjea *et al.*[21] used the basic secretagogue compound C48/80 with a neutralizing TNF-α treatment. They discovered that intravenous administration of an anti-TNF-α antibody produces analgesia. When they placed treated male ND4 Swiss mice on the hot plate for thermal evaluation, they found that pain and swelling were effectively reduced. In comparison, in this study, the compound was administered orally by probe and its effect was to use the hot plate test to verify the analgesic effect produced by Swedish herbal extract in comparison with meloxicam. Gao *et al.*[22] used the traditional Chinese medicinal drug, norisoboldine (a benzylisoquinoline alkaloid isolated from *Radix linderae*) to verify analgesia. This substance reduces acetic acid and formalin-induced pain responses but its effectiveness was not what was expected when the hot plate test was used. This study was done with a natural alternative and/or complementary medicine, a Swedish herbal extract, which gave a good result as was expected. When mice were placed on the hot plate for corroboration, the analgesic effect was observed. Jackson *et al.*[23] used the kappa opioid receptor antagonist, LY2456302, as a relief for nicotine-induced withdrawal symptoms. In addition to this improvement, when the hot plate test was used, the analgesia produced by LY2456302 was verified. The present test were done only for analgesia and not for withdrawal to any other substance, mice were completely healthy and just received by probe the Swedish herbal extract when placed on the hot plate.

Venkatesh and Fatima[24] used the plant *T. plukenetii* in powder form, administering it parenterally. To increase its effect, a combination with chloroform was used. In this study, no combination of analgesic medicine was used since meloxicam is a well-known analgesic and the Swedish herbal extract is a well-known complementary and/or alternative analgesic herbal medicine. The hot plate test was used to determine their analgesic activity and with this comparison it can say that both had similar analgesic activity. Tasleem *et al.*[25] used the pure compound piperine along with hexane and ethanol extracts of *Piper nigrum* L. fruit. They discovered that piperidine had the most potent analgesic and anti-inflammatory effect. On the hot plate test, *Piper nigrum* L. possessed a significant analgesic activity similar to our test with the Swedish herbal extract; the analgesic effect was proven and nothing needed to be added to revise this effect. Saleh *et al.*[26] used the hot plate test to evaluate the analgesic effect of *Gleditsia triacanthos* L. methanolic fruit extract (MEGT) and its saponin-containing fraction (SFGT). They recorded the central and peripheral analgesic activity of these substances and concluded that this is why it is used as "folk medicine". The Swedish herbal extract, Swedish Bitters is also known as a "folk medicine" and as a complementary and/or alternative drug. But it was found that thermal sensitivity is considerably reduced with the administration of the Swedish herbal extract, with an effect that is similar to

commonly used medication, as already mentioned, to meloxicam with a final result of having no significant difference in its analgesic activity.

Barua *et al.*[27] used rats and mice to conduct experiments of the analgesic and anti/nociceptive activity of *Drymaria cordata* hydroethanolic extract (DCHE). They used different doses to perform tests on the hot plate and also the tail flick model. They showed that this compound could be a potent analgesic and anti-nociceptive agent. In comparison with this study, instead of doing the tail flick test, the only test done was with the hot plate.

This study focused on the analgesic activity of a Swedish herbal extract used for centuries. The only drugs used in this study were the comparator, meloxicam, which is already known for its analgesic effectiveness and the alternative compound Swedish Bitter, whose analgesic efficacy was proven.

CONCLUSION

The vehicles of meloxicam (sugar water) and Swedish herbal extract (40% alcohol) had no analgesic effect. Meloxicam and the Swedish herbal extract demonstrated a similar analgesic response during the three tests with a mean of 16.3. Therefore, the analgesic efficacy of the Swedish herbal extract, Swedish Bitter, is verified.

SIGNIFICANCE STATEMENT

This study establishes the analgesic effect of a Swedish herbal extract as a possible therapeutic alternative for the management of dental pain. This study provides the basis for further studies to define its effectiveness in clinical situations.

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