investigation Article

The use of Turmeric as an analgesic using the formalin test in murine model

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Abstract:

Turmeric is a plant with multiple medicinal attributes. One of them being its analgesic effect. In this project the analgesic effect of the plant was evaluated with an inflammatory pain model: the 5% formalin model. Turmeric was administered to 250 g male Wistar rats (6 ± 2) provided by the Health Science department's vivarium. The dose-response curve of the plant was performed observing the analgesic effect. Once the dose was selected, they were administrated within different turmeric protocols. 400 mg orally every 24 h for two weeks, another group with turmeric *ad libitum* for two weeks, the control group with 0.9% saline solution for two weeks, and a group with administration of metamizole prior to evaluation with 5% formalin. Subsequently, each rat was evaluated by 5% formalin intraplantarly and the number of rat paw twitches per 1 h was observed. The analysis of the data generated through the obtained results was carried out. The dose of 400 mg of oral turmeric was administered for two weeks without observing any collateral effects. An excellent analgesic effect was found in this protocol, as well as, in the *ad libitum* administration for two weeks compared to the control group. Likewise, turmeric presents a milder effect than metamizole, a well-known analgesic.

Keywords: turmeric longa1, analgesia2, 5% formalin3.

1. Introduction

Turmeric is an herbaceous perennial plant, that reaches a height of up to 1 meter. With highly branched yellow to orange, cylindrical and aromatic rhizomes. The leaves are arranged in two rows. They are divided into leaf sheath, petiole and leaf lamina [1].

Kingdom: Plantae
Family: Zingiberaceae
Class: Liliopsida
Species: *Curcuma longa.*

It is a plant species of the ginger family cultivated in India, China, India, Indonesia, Jamaica, and Peru [2]. Its rhizomes (growing roots) are used to obtain a spice called turmeric. A rhizome under a thin light brown film, is an underground stem that grows horizontally from which the sprouts emerge towards the surface and the roots towards the ground. It is known worldwide as an aromatic spice, used in Asian cuisine to give a touch of color and spicy flavor to the typical dishes of that region. The phytochemical compounds that it contains in its rhizome is of a characteristic orange color, curcuminoids, confer to this plant important medicinal properties, from which curcumin has been isolated and thus attributed with an analgesic effect.

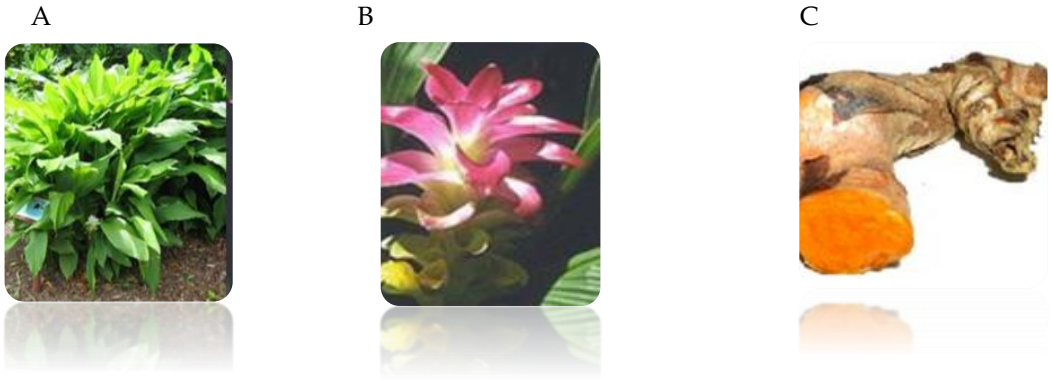


Fig 1. Images of the curcuma longa plant, A. plant, B. Flower, C. rhizome, source: Curcuma I. (Curcuma longa L.) [3].

The turmeric rhizome has been the subject of much research in India, an attempt has been made to find its active principles to optimize its activity and to explain its mechanism of action; Numerous extracts, ethanolic, methanolic and with different solvents have been prepared to analyze their biological activities [4-6]. Some uses of C. longa in popular medicine, in phytotherapy an infusion of rhizome is prepared against liver and gallbladder diseases, the ground rhizomes are used as a poultice in contusions on the back, it is also used for the treatment of amenorrhea, chronic constipation , diabetes, liver disorders, arterial and cardiotonic hypotension, uterine bleeding and varicose veins, among others [7,8].

Analgesic effect:

Turmeric, through one of its components, curcumin, refer [9]., explain the possible mechanisms of production of the analgesic effect and mention that one of the possible mechanisms of the analgesic effect at the level of the central and peripheral nervous system due to the inhibition of certain transcription factors involved in inflammation and alteration of pain signaling pathways through ion channels. curcumin inhibits the inducible nitric oxide synthase (iNOS) enzyme, involved in the regulation of cyclooxygenase-2 (COX-2) and the consequent inhibition of prostaglandin production producing the analgesic effect, curcumin can also act through antagonism of the ion channels of the «transient potential receptor vanilloid type 1 (TRPV1) ». TRPV1 channels are ion channels permeable to calcium ligands, involved in nociceptive Human Medicine signaling and located in the periphery of nociceptors; These contain neurotransmitters such as the calcitonin gene-related peptide (CGRP) and substance P. The molecular ring that curcumin presents is the structure that regulates the TRPV1 channels. It was found that, in addition to reducing thermal hyperalgesia in a dose-dependent manner, curcumin blocks capsaicin-induced currents in trigeminal ganglion neurons and reduces TRPV1 expression in HEK-293 cells (human embryonic kidney cells). It has also been suggested that, due to its solubility, it may adhere to the TRPV1 binding site across the membrane without restarting intracellular signaling pathways. These antagonistic effects on TRPV1 channels have been found in curcuminoid compounds synthesized in the laboratory that, in addition to demonstrating antinociception against noxious and inflammatory stimuli in neuropathic pain models in

mice, also decrease the expression of CGRP (peptide related to the gene of calcitonin) in chronic constriction lesions of peripheral nerves. [9] Among the effects of curcumin at the central nervous system level, its intrathecal administration has been shown to attenuate inflammatory pain with a cumulative effect, especially in hyperalgesia to heat. It has been suggested that this antinociceptive effect is carried out through its action on the dorsal root ganglia by inhibiting the activation of glial cells (astrocytes and microglia) and suppressing the expression of fibrillar acid protein, as well as the suspension of the secretion of NO, proinflammatory cytokines (IL-6 and IL-8), chemokines (MCP-1 and MIP-a) and TNF-α.77,78 TNF-α activates the nuclear factor kappa- B (Nfκ-β) and induces the transcription of genes for inflammation and pain mediators. Blocking the activation of microglia and astrocytes in the spinal cord prevents or delays painful hypersensitivity. It is suggested that curcumin inhibits the expression of inflammation mediators in the central nervous system and that it probably occurs through modulating effects of the adrenergic systems of the brainstem [9].

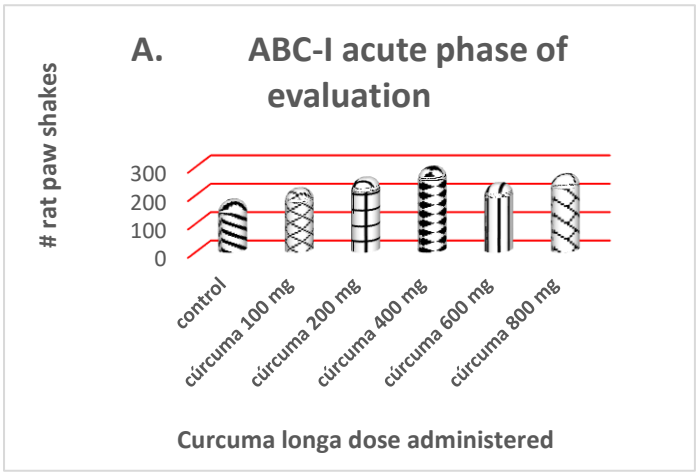
2. Results

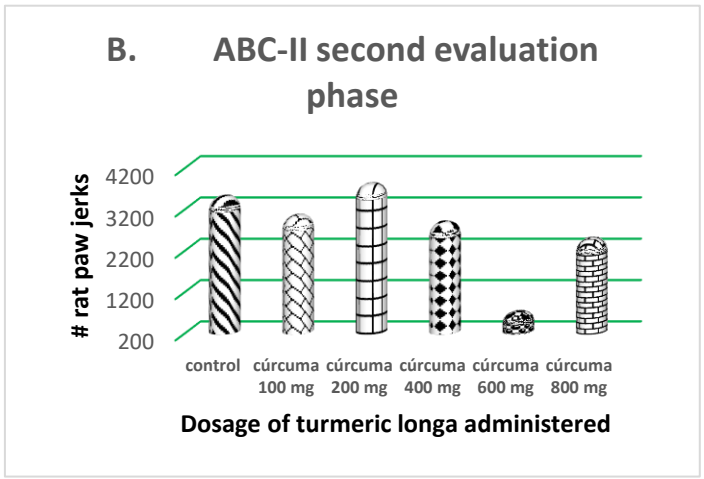
	ABC-I	E.E.	ABC-II	E.E.
control	**188.00**	**72.97**	**3484.50**	**1250.60**
100	**215.625**	**63.17**	**3025**	**956.84**
200	**254.375**	**77.23**	**3784.38**	**1267.99**
400	**292.50**	**113.91**	**2862.5**	**699.96**
600	**234.5**	**29.37**	**685**	**201.67**
800	**266.25**	**48.75**	**2467.5**	**272.5**

Table 3. Data obtained by applying the AUC formula of the evaluation time courses of each rat applying different doses of turmeric longa and subsequently 5% formalin. Data used to make comparison graphs of ABC A. and B.

The formula was applied to obtain the ACB (area under the curve) of the data obtained in the evaluations of each rat of each group and the data shown in table 3 were obtained. These data were used to make bar graphs to perform comparing the AUCs of each dose of turmeric used and selecting the dose for repeated administration

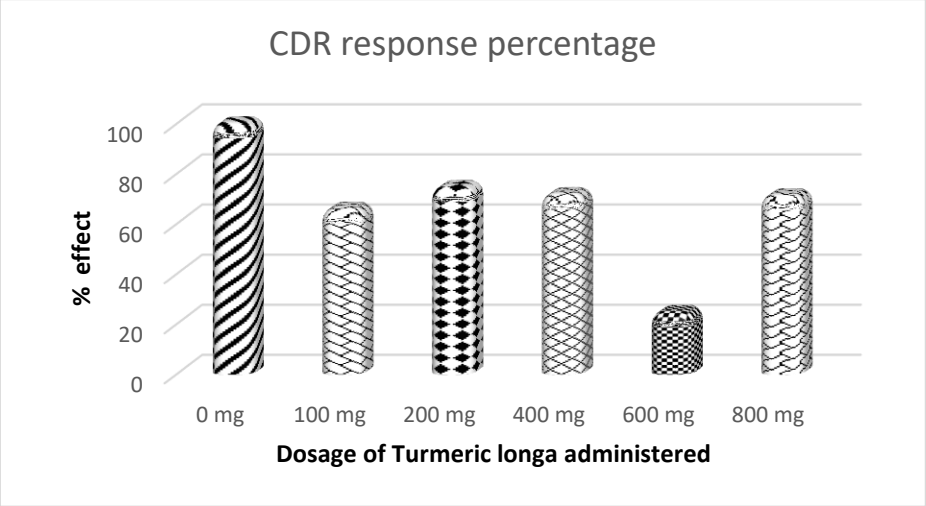
Next, the graphs of AUC I and II are shown applying formulas to obtain the data that allow us to select the dose to use of turmeric longa, graph A shows us the data obtained to perform the AUC of the first 5 min of Evaluation, In the 5% formalin model, from here with the data obtained we could say that the optimal dose to use would be 600 mg, since a decrease in AUC is observed compared to the other bars of each dose, although not against the control. In graph 2, the formula is applied to obtain the ABC from minute 10 to 60 of evaluation and it is where we can base ourselves for the selection of the dose to be used for the protocol of administration of turmeric repeatedly.





Graph 1. ABC – I and ABC – II data to perform the CDR, different concentrations of turmeric longa that help to select the dose to be administered in repeated administration protocols, graph A, corresponds to the first 5 min of evaluation and Graph B, from minute 10 to minute. 60 evaluation.

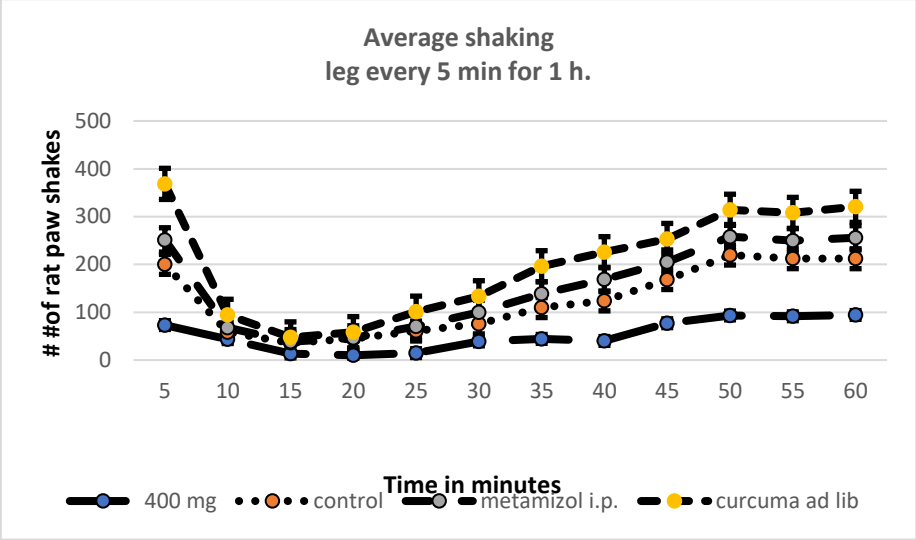
The data from the previous graphs (1 A. and B.) allowed us to select the 400 mg dose to administer turmeric for two weeks, because in graph B in the 400 mg bar (ABC-II 2862.5 ± SE 699.96), a decrease in AUC (area under the curve) is observed compared to the AUC bar of the control group. These data show us the analgesic effect; in the 600 administration bar (ABC-II 685 ± E.E. 201.67) the effect is more marked, but the E.E. is proportionally greater than in the 400 mg group and in the 800 mg bar, (AUC-II 2467.5 ± SE 272.5) if a decrease in AUC is observed, the error is lower, with a higher mortality of the rats in the group of rats those given the highest dose of 800 mg.



Graph 3. Percentage of response of the administered doses of the plant to select the dose of repeated administration of turmeric longa

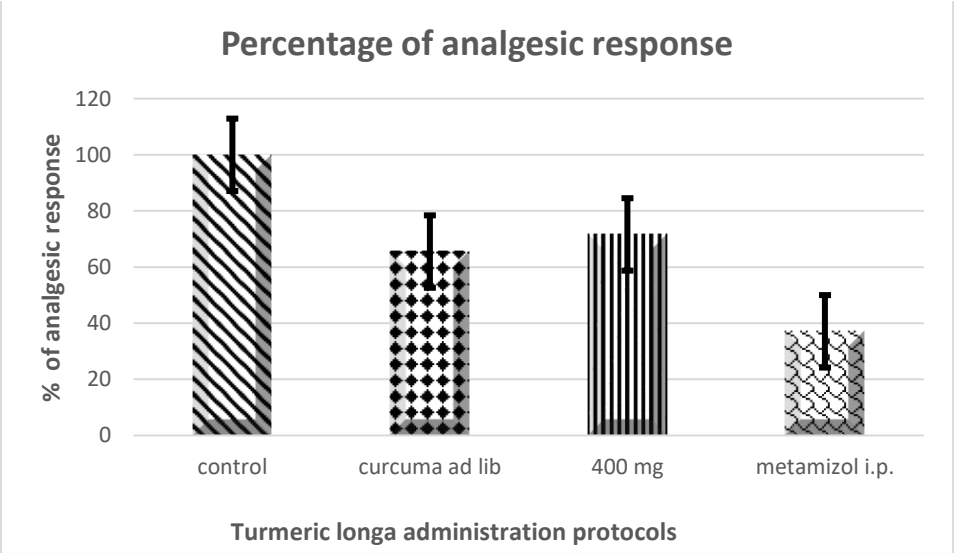
Graph 3. Here are shown the results of the evaluation of the experimental groups evaluating the analgesic effect, each group corresponds to groups made up of 6 ± 2 male Wistar rats. This graph shows the response percentage to the protocols where the 100% response is the group to which 9% saline solution was administered and then 5% formalin and we have 100% pain. The next dose of 100 mg of turmeric shows 65.83% pain, the 200 mg dose 74.45%, the 400 mg 71.63%, the 600 mg 24.61% and the 800 mg 71.61%

Once the dose was selected, the protocols established in table 2 were carried out and once the data was obtained, it proceeded to its analysis from where they were obtained to perform graphs 4 and 5, Evaluating the average of rat paw jerks for one hour, the control group with administration of 0.9% physiological solution, group to which turmeric 400 mg was administered orally for two weeks, group to administer turmeric ad libitum for two weeks and the group to which metamizole was administered 30 min Prior to the evaluation, each rat after following each protocol was evaluated with 5% formalin intraplantarly and the number of rat paw jerks was counted, obtaining the data to perform graphs 4 and 5 where the temporal courses are shown. of the evaluation of the protocols of repeated administration of turmeric buying them with metamizole and 0.9% physiological solution.



Graph 4. Time courses of the average rat paw jerks in the 5% formalin model applying the repeated administration protocols identifying the analgesic effect of turmeric longa. The group where 400 mg of turmeric longa was administered shows less jerking of the rat's paw, which corroborates the analgesic effect of the plant compared to the control group.

Graph 5 shows the percentage of the average rat paw twitching values, looking for the global analgesic effect, adding the rat paw twitching and making the average of these. When buying the control group with the turmeric group, a good analgesic effect is observed applying this protocol of administration of turmeric longa, when comparing the control group against the turmeric group at a dose of 400 mg, a good analgesic effect of the plant is also observed. , although not as effective as the administration of metamizole that we know is a widely known analgesic, but with several side effects that we would be avoiding using the plant.



Graph 5. Graph of the analgesic response percentage in the different administration protocols of turmeric longa and comparing them with a control group with administration of 0.9% physiological saline solution and with metamizole as a known analgesic.

It is observed the control group to which only 0.9% saline solution is administered and we obtained an average of rat paw jerks in 1 h after the administration of 5% formalin, the value corresponds to 73.82 with an S.E. of 11.33, the next bar corresponds to the administration protocol of turmeric ad libitum where the value is 47.63 ± 7.67, the next bar corresponds to the administration of turmeric at a dose of 400 mg for two weeks and the average of rat paw twitching was observed after the administration of 5% formalin intraplantar and the value is 52.88 ± 8.47 and finally the metamizole administration bar prior to intraplantar 5% formalin administration where the value is 27.39 ± 4.66. making a comparison of the values, the best analgesic effect is of metamizole, after the turmeric ad libitum protocol, later it is that of turmeric with the dose of 400 mg, all compared with the control group. of turmeric compared to the control group both in turmeric ad libitum and in the dose of 400 mg orally of turmeric, obviously less than metamizole that we know is a very good analgesic. In graph 4 the effect of turmeric 400 mg is seen, more effective, and in graph 5 it is less with metamizole as a positive control of the analgesic effect.

3. Discussion:

Montes and *et. al.*, [9]. They review several articles where it is mentioned how the curcumin extract of the curcuma longa plant helps in the control of dental pain, it has been effective against periodontitis, stomatitis and pediatric mucositis. Which supports the results found in our study. Various authors Fadus *et. al.* [10]., Mesa *et. al.* [11]., Akram M. *et. al.* [12].García Araiza *et. al.* (2017) [13]. confer anti-inflammatory properties that has to do with the inhibition of the synthesis of prostaglandins that, as we know, in one of the mechanisms that modulate pain and inflammation, thus supporting the results obtained in our study, it would be necessary to identify the effects collaterals of the plant that according to Fadus *et. al.*[10]. refer to gastrointestinal damage, Inhibition of sperm motility in vitro, inhibition of Heptacidin synthesis, iron chelation, increase in liver enzymes, suppression of platelet aggregation, contact dermatitis and urticaria

The treatment time of turmeric longa seda for two weeks according to the standardized protocols in previous experiments and metamizole only in one dose because metamizole is already known, it is a drug whose analgesic effect is well characterized and proven and is being administered in a single dose as a positive control before the analgesic effect and the difference or similarity of the analgesic effect compared to curcuma longa is seen.

4. Materials and Methods

The experiments were carried out in male Wistar rats (250 g of average weight) provided by the Bioterio of the Health Sciences Area of the Autonomous University of Zacatecas. The animals were kept in a special room at room temperature and alternating light-dark cycles of twelve hours (the light was turned on at 7:00 h). All animals had access to water and food ad libitum. The behavioral experiments were carried out between 8:00 a.m. and 3:00 p.m. under controlled temperature conditions (22 ± 2 ° C). At the end of the evaluation the animals were sacrificed by cervical dislocation or in a CO2 chamber. The experiments were carried out in accordance with the Ethical Guidelines and Standards for the Investigation of Experimental Pain in Animals [14]., and established in the Official Mexican Standard for the Use and Care of Laboratory Animals [15].

Drugs and reagents

The drugs used in this project were: metamizole sodium (Laboratorios Aventis Pharma, Mexico), formaldehyde (Sigma Chemical Co., USA), The drugs were dissolved in sterile isotonic saline solution (Laboratorios Pisa, Mexico), turmeric longa extract powder to dilute in 0.9% saline solution.

Formalin Model

The Formalin model is an inflammatory pain model. The rat was placed in a transparent acrylic (Plexiglas) observation chamber 20 cm in diameter and 30 cm in height to allow it to adapt to the new environment. After 30 minutes, the rat was removed for subcutaneous injection of 50 µL of 5% formaldehyde in the dorsal region of the right hind paw, using a 1 mL syringe with a 30G gauge needle. Subsequently, the rat was placed back inside the observation chamber. Two 30X30 cm mirrors were placed at the rear of the cylinder, forming an angle of 90º between them to facilitate the observation of the injected leg. Immediately after the injection of formaldehyde, the rat exhibited nociceptive behavior manifested as paw twitching. The number of shakes was recorded for 5 minute periods for one hour. Formalin induces a biphasic response, an acute Phase I (0-10 min) or neurogenic followed by a short period of quiescence (10-15 min), which is followed by a prolonged tonic inflammatory response (15-60 min) or Phase II. Nociception was evaluated as the

number of rat paw twitches [16]. The i.p. of metamizole was made 30 min before the injection of formalin.

For the **CDR** (dose response curve), groups of male Wistar 6 ± 2 rats were made to which the protocols were applied as follows:

# rats	Average weight Rat	Turmeric longa dosage	Dose to be administered daily for two weeks calculated with respect to weight	Mixed in 0.9% physiological solution
6±2	250 g	0 mg/kg	0	1 mL
6±2	250 g	100 mg/kg	25 mg	1 mL
6±2	250 g	200 mg/kg	50 mg	1 mL
6±2	250 g	400 mg/kg	100 mg	1 mL
6±2	250 g	600 mg/kg	150 mg	1 mL
6±2	250 g	800 mg/kg	250 mg	1 mL

Table 1. Table of experimental groups to perform the dose response curve, to evaluate the analgesic effect, and select the dose to be used as a protocol.

The rest of the experimental groups were as follows: First group, turmeric longa 400 mg was administered orally for two weeks and subsequent evaluation with 5% formalin. The second group of turmeric ad libitum (mix 5 gr in 500 mL of distilled water for free use as water for use), for two weeks and subsequent evaluation with 5% formalin. a group administered metamizole 30 min. prior to the evaluation with 5% formalin, a control group to which only 0.9% physiological solution was administered for two weeks and subsequent evaluation with 5% formalin

Group of male Wistar rats 250 g	Group 1	Group 2	Group 3	Group 4
Wistar male rat Groups of 6 ± 2	Turmeric longa 400 mg / kg per day for 2 weeks	Turmeric longa ad libitum for 2 weeks	Metamizole 500 mg i.p	Control with 0.9% physiological solution (control without analgesic)
	Evaluation of each rat with the intraplantar administration of formalin at 5% obtaining rat paw twitch values.	Evaluation of each rat with the intraplantar administration of formalin at 5% obtaining rat paw twitch values.	Control with analgesic Evaluation of each rat with the intraplantar administration of formalin at 5% obtaining rat paw twitch values.	Evaluation of each rat with the intraplantar administration of formalin at 5% obtaining rat paw twitch values.

Table 2. Table experimental groups

5. Conclusions

Based on the method carried out, the optimal dose of administration of turmeric longa of 400 mg / kg per day was found, once the experiments and the analysis for the selection of the dose had been carried out, that daily dose was administered for two weeks obtaining a good analgesic of turmeric longa in the ad libitum administration protocol and in the daily dose of 400 mg per day for two weeks compared with the control group, so that supports the possible use of panta to moderately alleviate some pain without the need for use of already known analgesic drugs and thus avoid polypharmacy and some side effects of drugs that belong to the group of analgesics.

This section is not mandatory but can be added to the manuscript if the discussion is unusually long or complex.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, X.X. and Y.Y.; methodology, X.X.; software, X.X.; validation, X.X., Y.Y. and Z.Z.; formal analysis, X.X.; investigation, X.X.; resources, X.X.; data curation, X.X.; writing—original draft preparation, X.X.; writing—review and editing, X.X.; visualization, X.X.; supervision, X.X.; project administration, X.X.; funding acquisition, Y.Y. All authors have read and agreed to the published version of the manuscript." Please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and Mexican Official Norm NOM-062-ZOO-1999.

approved by the Institutional Review Board (or Ethics Committee) of NAME OF INSTITUTE (protocol code XXX and date of approval)." OR "Ethical review and approval were waived for this study, due to REASON (please provide a detailed justification)." OR "Not applicable." for studies not involving humans or animals. You might also choose to exclude this statement if the study did not involve humans or animals.

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Conflicts of Interest: the authors declare don't exist conflicts of interest or state "The authors declare no conflict of interest." Authors must identify and declare any personal circumstances or interest that may be perceived as inappropriately influencing the representation or interpretation of reported research results. The authors declare that under no circumstances or personal interest is there an inappropriate influence on the representation or interpretation of the research results. For this Study there was no funding for any research project, only the salaries of teachers who dedicate part of their time to management and research. There was an intervention of the Autonomous University of Zacatecas in the design of the study; in the collection, analysis or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Sample Availability: All data is available through the authors for verification or analysis of who is interested and are available from the authors.

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