Quercetin action on pain modulation

Ação da quercetina sobre a modulação da dor

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ABSTRACT
Background: Quercetin is a flavonoid widely found in plant kingdom and target of studies in pharmacological area due to its potent antinociceptive effect compared to analgesics used in conventional therapies. The aim was to evaluate its antinociceptive activity and antinociception mechanism. Methods: For this, 40 Norvegicus Wistar rats were used, divided into 4 groups: Q50 (treated with quercetin 50 mg/Kg), Q100 (treated with quercetin 100 mg/Kg), Q500 (treated with quercetin 500 mg/Kg) and Positive control (PC) without quercetin treatment), who were submitted through the pain induction methods by tail immersion and formalin in the first step to assess antinociceptive action and in the second step, tail immersion method receiving antagonists from opioid, cholinergic and nitric oxide - L-arginine to evaluate the action mechanism. Results: Quercetin antinociceptive activity was verified at the dose of 50 mg/kg and 100 mg/kg in tail immersion test after formalin injection, with better performance at the dose of 50 mg/kg. There were no statistically significant results in paw opening and capsaicin tests. Quercetin demonstrated a possible influence on opioid and cholinergic pathway, which was not observed on the nitric acid - L-arginine pathway in view of parameters tested. Conclusion: Quercetin performed the best antinociceptive activity at a dose 50 mg/kg and there was a possible influence on opioid and cholinergic pathways.

Keywords: quercetin, antinociception, flavonoid, pain.

RESUMO
Antecedentes: A quercetina é um flavonóide amplamente encontrado no reino vegetal e alvo de estudos na área farmacológica devido a seu potente efeito antinociceptivo em comparação com os analgésicos utilizados em terapias convencionais. O objetivo era avaliar sua atividade antinociceptiva e seu mecanismo antinociceptivo. Métodos: Para isto, foram utilizados 40 ratos Norvegicus Wistar, divididos em 4 grupos: Q50 (tratados com quercetina 50 mg/Kg), Q100 (tratados com quercetina 100 mg/Kg), Q500 (tratados com quercetina 500 mg/Kg) e Controle Positivo (PC) sem tratamento com quercetina), que foram submetidos através dos métodos de indução de dor por imersão caudal e formalina na primeira etapa para avaliar a ação antinociceptiva e na segunda etapa, o método de imersão caudal recebendo antagonistas de ópioide, colinérgico e óxido nítrico - L-arginina para avaliar o mecanismo de ação. Resultados: A atividade antinociceptiva da quercetina foi verificada na dose de 50 mg/kg e 100 mg/kg no teste de imersão da cauda após a injeção de formalina, com melhor desempenho na dose de 50 mg/kg. Não houve resultados estatisticamente significativos nos testes de abertura da pata e capsaicina. A quercetina demonstrou uma possível influência na via opióide e colinérgica, que não foi observada na via ácido nítrico - L-arginina, tendo em vista os parâmetros testados. Conclusão: A quercetina realizou a melhor atividade antinociceptiva na dose de 50 mg/kg e houve uma possível influência nas vias opióides e colinérgicas.

Palavras-chave: quercetina, antinocicepção, flavonóide, dor.

1 INTRODUCTION

Quercetin is a flavonoid found in red onion (*Allium ceppa L.*) that has antioxidant, anti-inflammatory and anticancer functions. It is also possible to find such a compound in foods such as capers, yellow peppers, lemon, peeled apples, red grapes, broccoli, canned beers, among others foods, presenting a variable amount in each food[1].

Flavonoids are secondary and non-essential metabolites, widely distributed throughout the plant kingdom, which have fundamental functions in plant physiology, such as: protection against damage caused by ultraviolet radiation due to their spectroscopic properties in the ultraviolet region, visual signaling of flowers and fruits for pollination by insects[2], they can also act as catalysts in the light phase of photosynthesis and as regulators of iron presence involved in phosphorylation[3].

As the quercetin is a flavonoid and, also widely found in the nature, it is the target of studies in pharmacological area due to its potent antinociceptive effect compared to analgesics used in conventional therapies, where it is thought about the elucidation of its pain neuromodulation mechanism[4].

Pain perception is caused by a harmful stimulus, injury or even by diseases[5]. Pain is defined by the International Association for the Study of Pain (IASP) as an unpleasant multidimensional phenomenon, involving not only a sensory component, but also an emotional component, and which is associated with a concrete or potential tissue injury, or is described according to this injury[6].

Pain involves emotions and other elements and it is an individual phenomenon, in which each person feels pain in their own way, there is no direct relationship between pain and its cause; the same injury can cause different pain in different individuals or in the same individual at different times; there is also pain without being able to find physical injury that gives rise to it[7].

When tissue is damaged, several inflammatory mediators are released, which directly activate nociceptors causing pain, or leading to sensitization of somatosensory nervous system. This process is characteristic of inflammatory pain, facilitating activation of the pain path until the healing process ends[8].

Medicinal plants use as an ally or substitute for conventional pharmacological therapies has become common over time. Medicines based on plants and derivatives of plant extracts are increasingly used due to the possibility of treating a wide variety of pathologies and disorders. However, the knowledge about its properties, effective doses and action mechanisms is still small, and this implies its therapeutic use [9,10].

In this context, the aim of this research was to evaluate through pharmacological tests the antinociceptive effect of quercetin, as well as the pain neuromodulation mechanisms.

2 METHODS

Samples

Wistar Norvegicus rats, 40 males, weighing between 180g and 200g, obtained from the State University of Maringá Central Bioterium, were acclimatized to 22 ± 2ºC, in a cycle of 12 hours clear/dark and treated with feed and water ad libitum. Animals were kept in the laboratory 1 hour before the experiments for acclimatization, and tests were conducted according to the Normative Resolution Animal Use Ethics Committee (CONCEA) nº 38, April 17, 2019.

Research steps

Animals were used for two moments of the study, to evaluate the antinociceptive effect and to analyze what is the possible mechanism of action involved, and there was a rest period of 10 days between one model and another.

Experimental groups

Animals were divided into 4 groups with the following subdivisions:

Positive control group (PC): Consisting of 10 animals in which nociception was induced by Formalin and Capsaicin test without receiving treatment.

Treat Group with 50 mg/kg of quercetin (Q50): Consisting of 10 animals in which nociception induction was performed by Formalin and Capsaicin test, treated with quercetin 50 mg/kg.

Treated Group with 100 mg/kg of quercetin (Q100): Consisting of 10 animals in which nociception was induced by Formalin and Capsaicin test, treated with quercetin 100 mg/kg.

Treat Group with 500 mg/kg of quercetin (Q500): Consisting of 10 animals in which nociception was induced by Formalin and Capsaicin test, treated with quercetin 500 mg/kg.

Treatment

Quercetin

The used dose for screening was 50 mg/kg, 100 mg/kg and 500 mg/kg and the administration route chosen for the study was oral.

Extracts Preparation

Preparation was carried out using quercetin powder diluted in water. For this, an average weight of 200 g per animal was considered, so for the Q50 group the dose of 50 mg/kg, 300 mg of quercetin were diluted in 15 ml of water. For the Q100 group, 100 mg/kg dose, 600 mg of the quercetin powder was diluted in 15 ml of water and for the Q500 group, 500 mg/kg dose, 3 g of the powder was diluted in 15 ml of water. Each animal received 0.5 mL of extract orally (administered by gavage).

Animal baseline

All animals underwent the tail immersion test 5 days before nociception induction procedures, thus constituting a baseline that demonstrated the natural pain threshold to these animals.

Tail immersion test

The test consisted of exposing the animal's tail in hot water with a temperature of approximately 51-55 °C, the tail removal time (indicative of pain) was recorded and the cut-off time to avoid injuries was about 180 seconds.

Pharmacological tests

Formalin-induced pain model

To confirm the possible quercetin antinociceptive effect, the procedure used was adapted to described by Hunskaar and Hole[11]. Animals received 50 µL of 2.5% formalin in sub plantar region of the right posterior paw. After formalin injection, animals passed the paw opening test and then the tail immersion test was performed. Animals received quercetin 30 minutes before the 2.5% formalin test.

Paw opening test

After formalin injection, footprints of the hind legs were obtained using a 5 megapixel digital camera, according to the method proposed by DeMedinaceli et al.[12], and modified by Lowdon et al.[13].

Animals were placed on a glass walkway 1 meter long by 10 cm wide and 70 cm high, where they walked to the stop point. The photos containing the footprints were filed in separate folders, stored and analyzed on computer, using the ImageJ program https://imagej.nih.gov/ij/index.html.

Data for each animal were identified individually, to allow follow-up over time. Measured parameters of the paws impressions of both animals, without pain induction by formalin and those who received formalin to induced pain were the length of the second finger to the fourth finger, and smaller openings were considered indicative of pain.

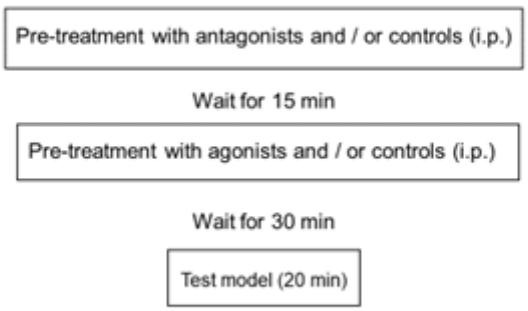
Capsaicin-induced pain model

Each animal received 20 µL of capsaicin solution (1.6 µg/paw), injected in tail region. After 30 minutes of treatment with the extracts, the test was performed by tail immersion method. Animals received quercetin 30 minutes before the capsaicin test.

Antinociceptive action mechanism analysis

Once the compound/dose that obtained the greatest effectiveness was chosen, possible action mechanism of antinociceptive property was studied. Neuromodulation pathways studied in the present study were opioid, cholinergic and nitric oxide (NO). Pharmacological model chosen to study the pathways and their possible influences on the quercetin antinociceptive effect was the model that have had the best performance between the doses tested. The adopted protocol was represented below, through the flowchart (figure 1).

Figure 1: Protocol flowchart used in study to determine the quercetin action mechanism.



A) Opioid system influence

Animals were pretreated with the μ-type opioid receptor antagonist, naloxone (1 mg/kg, i.p.), 15 minutes before quercetin administration. Positive control group received only naloxone.

After 30 minutes of animal's treatment with quercetin, the possible effect reversal was analyzed. Pain induction model used for this test was tail immersion.

B) Nitric oxide-L-arginine system influence

Animals were pretreated with L-arginine (NO precursor, 600 mg/kg, i.p.), 15 minutes before quercetin administration. Positive control group received only L-arginine.

After 30 minutes of animal's treatment with quercetin, the possible effect reversal was analyzed. Pain induction model used for this test was tail immersion.

C) Cholinergic system influence

Animals were pretreated with non-selective cholinergic antagonist atropine (1 mg/kg, i.p.), 15 minutes before quercetin administration. Positive control group received only atropine.

After 30 minutes of animal's treatment with quercetin, the possible effect reversal was analyzed. Pain induction model used for this test was tail immersion.

Euthanasia

Animals were anesthetized intra-abdominal with a solution in the proportion of 80 mg/kg of Ketamine Hydrochloride (Ketamine, 10 mL bottle) to 15 mg/kg of Xilasine Hydrochloride (Dopaser, 10 mL bottle) via intraperitoneal.

Animals were anesthetized with 80 mg/kg of Ketamine and 20 mg/kg of Xylazine, after checking the anesthetic status, they received 175 mg/kg of Pentobarbital (50 ml and 100 mg/ml per ampoule) intraperitoneally for lethal dose[14].

Statistical analysis

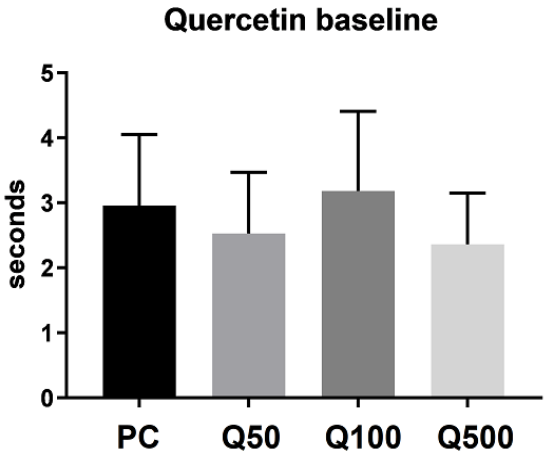
Results were presented as mean ± standard deviation for each experimental group of their respective level of the confidence limits to 95%. In cases of non-normal Gaussian distribution, analyzes were performed using Kruskal-Wallis test with Dunn's post-test.

3 RESULTS

Animal baseline

For the animals that belonging to quercetin group in baseline tests performed to detect the animals' natural pain threshold, as shown in figure 2, an average of 2.53± 0.94 seconds was observed at Q50 group. To Q100 group had an average of 3.18±1.22 seconds. In the Q500 group, a mean of 2.36±0.79 seconds was presented. For the PC group, the result was an average of 2.96 ± 1.09 seconds. In this test, results were not statistically significant.

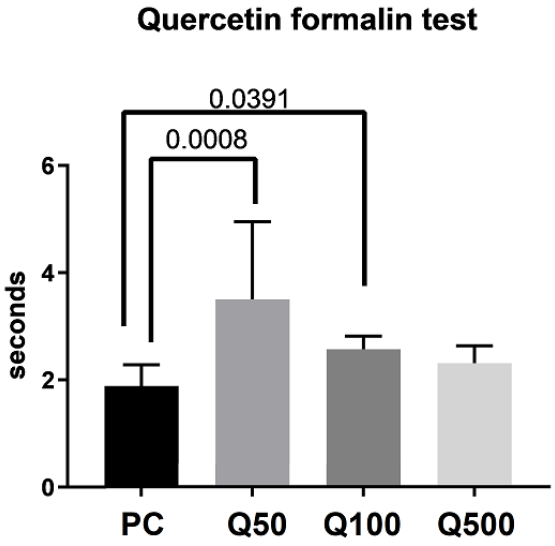
Figure 2: Natural pain threshold Representation of the PC groups (untreated), Q50 (quercetin treated with 50 mg/kg), Q100 (quercetin treated with 100 mg/kg) and Q500 (quercetin treated with 500 mg/kg) by the tail immersion method for the animals' baseline.



Tail immersion method test

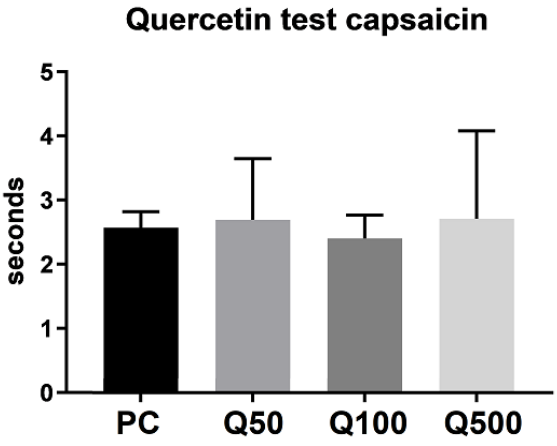
To the formalin test, as shown in figure 3, the Q50 group have presented an average of 3.50±1.45 seconds, compared to the PC group which presented an average of 1.89±0.39 seconds, showing statistical significance (p<0,001). Also, it was confirmed to antinociceptive activity of quercetin that have obtained the best performance between the tested doses. To the Q100 group, an average of 2.57±0.25 seconds was obtained, also obtaining statistical significance (p 0.039) against PC group. For the Q500 group, the mean was 2.31 ± 0.32 seconds, with no statistical significance.

Figure 3: Pain threshold representation of the PC groups (untreated), Q50 (quercetin treated with 50 mg/kg), Q100 (quercetin treated with 100 mg/kg) and Q500 (quercetin treated with 500 mg/kg) by tail immersion method after formalin injection.



For animal quercetin treated, to the capsaicin test, as shown in figure 4, the Q50 group was detected an average of 2.69±0.95 seconds. In the Q100 group, an average of 2.40 ± 0.36 seconds was obtained. For the Q500 group quercetin treated, an average of 2.71±1.37 seconds was found. The PC group had an average of 2.57±0.25 seconds. In this test, results were not statistically significant.

Figure 4: Pain threshold representation of the PC group (untreated), Q50 (quercetin treated with 50 mg/kg), Q100 (quercetin treated with 100 mg/kg) and Q500 (quercetin treated with 500 mg/kg), by the tail immersion method after capsaicin injection.

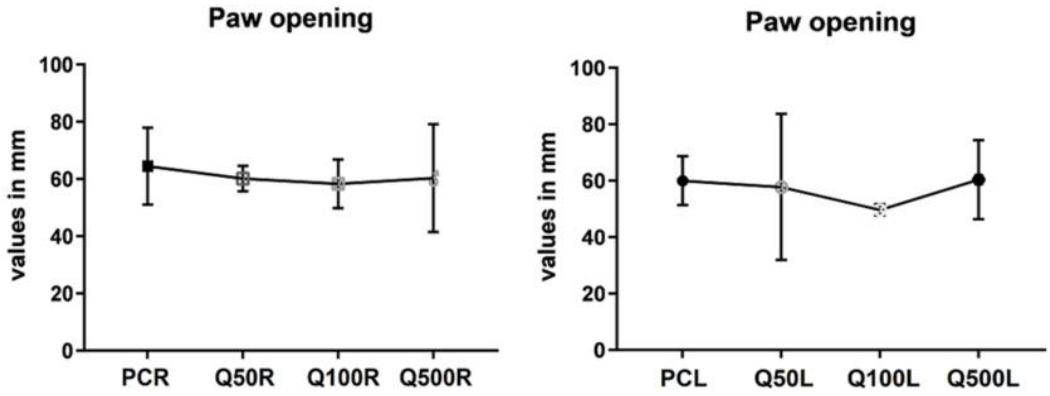


Paw opening test

For the animals the paw opening test, after applying of quercetin extract and performing the formalin test, as shown in figure 5, with respect to the rats right paw treated with quercetin, an average of 60.15± 4.42 mm was obtained for the Q50 group. In

the Q100 group, the average found was 58.29±8.50 mm of opening. For the Q500 group, the average was 60.32±18.84 mm. The animals in the control group, which were not treated, had an average of 64.48±13.44 mm of paw opening. However, the values found were not statistically significant.

Figure 5: Measure of the opening of the right (upper) and left (lower) hind paw after formalin injection in the right hind paw in groups Q50 (quercetin treated with 50 mg/kg), Q100 (quercetin treated with 100 mg/kg), Q500 (quercetin treated with 500 mg/kg) and CP (untreated).



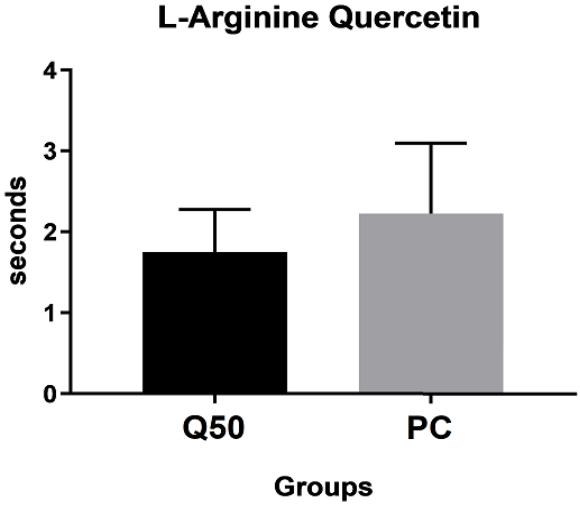
Regarding the animals left paw, in the Q50 group where quercetin was administered at a dose of 50 mg/kg, an average of 57.75±25.85 mm of opening in paw was observed. In the Q100 group treated at a dose of 100 mg/kg of the extract, the average was 49.63±1.95 mm. The value found for the 500 mg/kg dose to the Q500 group treated was an average of 60.38±13.99 mm of paw opening. For the control group, that is, who did not receive treatment, result obtained was an average of 59.99±8.63 mm. However, these paw opening values also did not demonstrate statistical relevance.

Antinociceptive action mechanism analysis

Possible influence on the nitric oxide-L-arginine system

In the study of the influence possibility the of system via L-arginine-nitric oxide, as shown in figure 6, the average for Q50 group, which obtained the best performance, was 1.75±0.52 seconds and the average to PC group was 1.23± 0.41 seconds, without statistically significant difference. As the values between the groups were close, it can be said that quercetin did not influence the nitric oxide production from L-arginine, indicating that it does not influence the path in question.

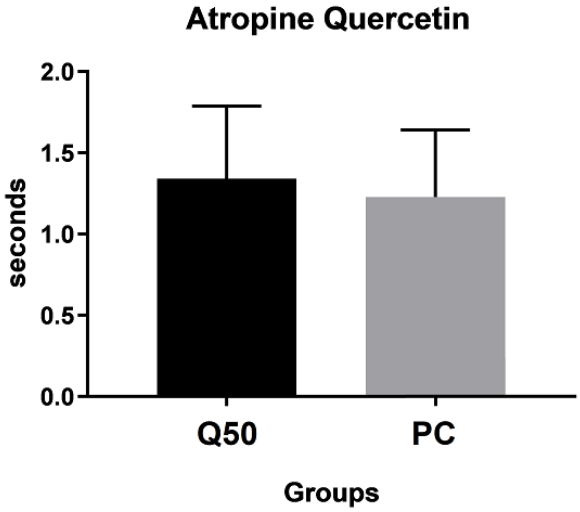
Figure 6: Pain threshold representation Q50 groups (pretreated with quercetin and then treated with L-arginine) and PC (treated only with L-arginine) by the tail immersion method.



Possible Cholinergic system influence

The test that aimed to verify the influence on the cholinergic system, specifically on muscarinic receptors, as shown in figure 7, the average for Q50 group was 1.34±0.45 seconds and for the PC group 1.23 ± 0.41 seconds. Considering that the value of both did not show a statistically significant difference, it is possible that quercetin also influences the system in question due to reversion the effect presented.

Figure 7: Pain threshold representation of the Q50 group (pretreated with quercetin and then treated with atropine) and PC (treated with atropine only) by the tail immersion method.

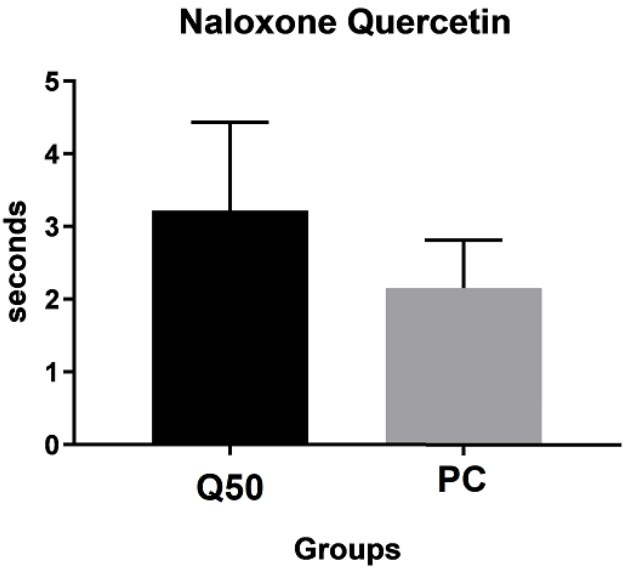


Possible opioid system influence

To verify the influence on opioid system, as shown in figure 8, the mean for Q50 group, pretreated with naloxone, was 3.22±1.21 seconds and to the PC group was

2.15±0,66 seconds, with no statistical significance, indicating a possible influence of quercetin on the opioid system.

Figure 8: Pain threshold representation of the Q50 group (pretreated with quercetin and then treated with naloxone) and PC (treated with naloxone only) = 2.15 ± 0.66 s by the method tail immersion.



4 DISCUSSION

Studies have reported that quercetin demonstrates antinociceptive activity in animals studied in some test systems[15].

Martínez, et al.[16], have worked on antinociceptive activity of the plant Tilia americana var. Mexican and quercetin (active compound alone) and carried out experiments using organic and aqueous extracts of the vegetable, as well as quercetin doses of 30-100 mg/kg i.p. To evaluate antinociception, the functional pain-induced impairment model in rats (PIFIR) was used, with the use of uric acid and the formalin-induced pain model.

Authors have found that both plant aqueous extract and quercetin produced significant dose-dependent antinociceptive activities for equally models of pain induction used, with quercetin being the dose of 100 mg/kg i.p. was the one that showed the most significant results.

In the investigate by Toker, et al.[17], in order to consider the antinociceptive and anti-inflammatory activity of main flavonoids compounds presented in Tilia argentea plant isolated: kaempferol and quercetin, authors orally administered isolated compounds at a dose of 50 mg/kg to animals, using the antinociception evaluation as contortion test induced by p-benzoquinone.

Both compounds have been shown to have potent antinociceptive and anti-inflammatory activity at the used dose. In this work, despite to use of different pain induction models and different tests, result have found to corroborate the findings of Toker, et al.[17], demonstrating that the quercetin orally administration at a dose of 50 mg/kg have presented antinociceptive effects.

Anjaneyulu and Chopra[15], aiming to explore quercetin antinociceptive effect in control animals and animals with streptozotocin-induced diabetes (STZ), administered in diabetic animals oral quercetin at doses of 50 and 100 mg/kg, and submitted all mice (control and diabetics) to thermal hyperalgesia test by tail immersion test (warm water). Authors observed that quercetin at a dose of 100 mg/kg produced a marked increase in latencies of tail movements in diabetic and non-diabetic mice, indicating the antinociceptive activity of this flavonoid.

Like the authors mentioned above, in the present study, the route of administration, doses and test used coincide. However, the results obtained in the present study showed that the dose of 50 mg/kg was one that produced significant antinociceptive activity, differently from that found by Anjaneyulu and Chopra [15].

In studies on antinociceptive properties of quercetin, Willain Filho, et al.[18], found that this compound at a dose of 60 mg/kg i.p. have had antinociceptive activity in acetic acid-induced pain test, and it also inhibited nociception induced by formalin, glutamate and capsaicin (10-60 mg/kg i.p).

Result found by Willain Filho and collaborators[18], corroborates the quercetin effect in inhibiting nociception in formalin-induced pain model found at the present study. As for the capsaicin pain model, results found were not statistically relevant. A possibility to the absence of results for this model may be the differences in methodology used, while the authors above performed capsaicin application on animal's paw, in this work application was performed on tail, in addition the quercetin administration was performed orally and not intraperitoneally.

The paw opening test performed immediately after the formalin injection into the animal's paw, presents results linked to formalin nociception model. Sousa, et al.[19], and Silva, et al.[20], report that formalin test is widely used to evaluate the analgesic drugs action, being considered a reliable model of tonic pain to the inflammatory type.

In addition, they clarify that the nociceptive response to formalin occurs in a biphasic manner: there is a short initial period, called phase I lasting 5 to 10 minutes (neurogenic or acute pain) attributed to direct nociceptors activation. Phase II, in other

hand, consists of a period between 20 to 40 minutes to sustained activity associated with release of local endogenous mediators, which generate local inflammatory response, responsible for sensitizing primary afferents and medullary neurons and subsequent nociceptors activation, resulting from a inhibition of nociceptive transmission through supraspinal and spinal circuits.

Thus, the absence of results with statistical significance in this work about the paw opening test, could be attributed to a quercetin antinociceptive action in phase II to the formalin test, a phase which was not observed to verify the opening values of paws.

Regarding the action mechanisms involved regarding antinociception, a possible influence on nitric oxide system - L-arginine, which, according reported by Riedel and Neeck[21], is involved in nociception processes, was analyzed. However, there are controversies regarding the nitric oxide (NO) role in painful processes, some studies report that NO influences the nociception development, and may have a nociceptive or antinociceptive effect, depending on its concentration, action location and sensitivity model to pain[22].

In this study, due to the fact that the groups threshold (figure 6) was lower when compared to the same groups when they passed the baseline test (figure 2), the possibility of NO production from L-arginine, consequently causing a painful condition and as the group results Q50 and PC in figure 5 were similar, it is believed that quercetin does not act in this way.

It was verified also the possible influence on cholinergic system, specifically in muscarinic receptors, due to studies showing high density of this receptor type in dorsal spine surface areas, having an antinociceptive effect triggered by direct agonists responsible for cholinesterase inhibition[23].

Starting from the context in which animal has mechanism where acetylcholine performs analgesic activity[24], when there is a harmful stimulus, neurotransmitter can be prevented from occupying its receptors if there is the atropine presence, a muscarinic antagonist, resulting in blocking antinociceptive action[25].

At the present study, comparing pain detection threshold for the groups according figure 6, with the groups threshold in figure 2, it can be noted that groups that were treated with atropine had lower threshold and when comparing to Q50 and CP groups from figure 7, it is observed that values are close and with no statistically significant difference, indicating that atropine may have blocked the quercetin action and, therefore, there is the possibility of flavonoids acting through this route.

Finally, a possible influence on opioid system was evaluated. It is known that endogenous opioids can modulate pain under stress conditions, known as endorphins, are divided into three classes: encephalins that interact with δ receptors, dynorphins with κ and β-endorphins interacting with μ receptors[26]. μ receptors have direct relationship with nociception functions, with morphine as the first identified agonist and naloxone as the antagonist[27].

5 CONCLUSION

Assuming that naloxone can block antinociceptive response generated by an endogenous opioid resulting from harmful stimulus, as seen in figure 8, and that the group treated with quercetin showed a similar result, it can be said that naloxone reversed the effect antinociceptive caused by quercetin, therefore, there is the possibility that quercetin acts via opioid route.

The results of this research suggest that quercetin has antinociceptive activity at a dose of 50 mg/kg and from pharmacological tests it was observed that it can exert this effect through the opioid and cholinergic pathway.

Conflicts of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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