The analgesic effect of Local Passiflora, Cinnamon and Chamomile plant ethanol extract in Swiss albino mice

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ABSTRACT
The study was performed to investigate the analgesic effect of the ethanol extracts of Passiflora, Cinnamon and chamomile in Swiss albino mice. Forty Swiss albino mice, and were divided into 5 groups (8 animals per each group). Analgesic activity of the plants was evaluated by using acetic acid induced writhing test, hot plate test and heat conduction test in albino mice. The study revealed that the dose (33 mg/kg) of Passiflora ethanolic extract produced (12 ±1.309) in heat conduction test (9.1250 ±1.246 sec.) in hot plate test and (7.875±1.246 stretch in 15 min.) in writhing test. The dose (8 g/kg) of cinnamon oil produced (10.875±1.246 sec.) in heat conduction test (10.25 ±1.035 sec) using hot plate test and (7±1.069 stretch in 15 min) in writhing test. The dose (13 g/kg) of chamomile oil produced (12±1.069 sec.) in heat conduction test (12.5±1.603) in hot plate test and (6.125±1.246 stretch in 15 min) in writhing test. All three plants showed analgesic effect. The strongest effect was shown by chamomile then cinnamon and last comes passiflora showing the weakest analgesic among them.

التأثير المسكن لنبات ورد الساعة البابونج والدارسين في الفئران

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الكلمات المفتاحية: التأثير المسكن، ورد الساعة، الدارسين، فص رذنك، فحص الصفيحة الساخنة، فحص التوصيل الحراري

الخلاصة
نفذت هذه التجربة لدراسة التأثير المسكن لكل من نبات ورد الساعة، الدارسين والبابونج في الفئران ولمقارنة التأثير المسكن لهذه النباتات مع بعضها. استخدم في هذه الدراسة 40 فارا وقسمت المجموعة الى □مس مجاميع صغيرة بواقع ثمان فئران في كل مجموعه. التأثيرالمسكن لهذه النباتات قيم □لال فحص الرذنك المحفز □لال حامض الأسيتيك، فحص الصفيحة الساخنة وفحص التوصيل الحراري في الفئران. كشفت الدراسة ان جرعة (33ملغ/كغم) من المستخلص الإيثانولي لورد الساعة احدثت تأثير (1.31± 12.0) ثانية في فحص التوصيل الحراري و (1.25±9.13) في فحص الصفيحة الساخنة و (1.25± 7.88) حكه في15 دقيقه في فحص الرذنك . اما جرعة (13غ/كغم) من زيت الدارسين احدثت (1.25±10.88) ثانيه في فحص التوصيل الحراري و (1.035±10.25) ثانية في فحص الصحن الساخن و (1.07±7) حكه في15دقيقه في فحص الرذنك . اما جرعة (8غ/كغم) من زيت البابونج احدثت (1.07±12.0) ثانيه في فحص التوصيل الحراري و (1.61±12.50) ثانية في فحص الصحن الساخن و (1.25± 6.13) حكة في15دقيقه في فحص الرذنك .في حين اظهر السيطرة السالبه (0.71 ± 3.75)ثانية في فحص التوصيل الحراري و6.13 ±1.13) في فحص الصفيحة الساخنة و(12.13±1.25) حكة في 15 دقيقة في فحص الرذنك. اظهرت النباتات المدروسة تأثير مسكن

للألم وكان التأثير الاقوى يعود لنبات البابونج يتبعه في ذلك الدارسين وكان في المرتبة الاخيرة نبات ورد الساعة الذي اظهر اقل فعالية في تسكين الالم من بين النباتات المدروسة.

INTRODUCTION

Athreatening condition in the body's tissues normally could cause pain that is an important sensation.[1] Like hunger or thirst, it is a motivational somatic state that drives appropriate behavioral responses, but chronic pathological pain can also completely dominate attention and consciousness and cause intolerable suffering.[2] Our knowledge of the representation of pain in the peripheral nerves, the spinal cord and the brain is increasing, and become aware that many neurochemicals involved and the critical interactions with other systems, such as the thermoregulatory, sympathetic and immune systems, that make pain an integrated physiological phenomenon.[3-5]

The clinical treatment of pain has improved with the advent of local and spinal analgesia, with the use of stimulation in the periphery, spinal cord and the brain, with the identification of new pharmacological tools such a serotonergic and adrenergic agonists, and combination of behavioral and organic therapies.[6,7] Our study was designed and carried out to verify the presence of analgesic activity in passiflora, Chamomile & Cinnamon, to prove their effectiveness as analgesic agents against pain in mice and to decide which one of the three plants most powerful analgesic effect.

MATERIALS AND METHODS

Experimental animals

Forty Swiss albino mice of either sex were used in the study, they were aged differently, their weight ranging from 25 –30 grams. The animal house in the College of Pharmacy was used to keep all animals, and allowed free access to standard food and water. The experimental protocol was approved by the Ethical committee in the College of Pharmacy – University of Kerbala.

Plants

Ethanolic extract of Passiflora in a dose of 33 mg/kg, Oil of Cinnamon in a dose of 13 g/kg, Oil of Chamomile in a dose of 8 g/kg

Chemicals & Drugs

Acetyl salicylic acid (aspirin) powder (500 mg), (Novartis company), in a dose of (50 mg /kg), it was given (Intraperitoneal), one hour prior to the experiment.

Animals Grouping

Animals were divided into 5 groups (8 animals per each): 1. Group 1 (-ve control group), they were given normal saline. 2. Group 2 (+ve control group) (acetyl salicylic acid) , they were treated with acetyl salicylic acid in a dose of 50 mg / kg and was given (i.p) one hour prior to the experiment. 3. Group 3 (Passiflora group), they were treated with Passiflora in a dose of (1 drop in 1 ml dw) and was given (orally) one hour prior to the experiment. 4. Group 4 (Cinnamon group), they were treated with Cinnamon in a dose of (0.3 ml) and was given (orally) one hour prior to the experiment. 5. Group 5 (Chamomile group), they were treated

with Chamomile in a dose of (0.3 ml) and was given (orally) one hour prior to the experiment.

Extraction

Chamomile and cinnamon

Dried powder of the plant material (coarse powder) was placed into a distilling flask and added few pieces of porous earthenware. 200ml D.W was added to the flask and shake well. Another 200ml of water was added by rinsing the neck of flask. The distilling flask was connected with the still head of the apparatus. By the means until over flows, the condenser of the apparatus was connected with the cooling water (from the tap). The distilling flask was heated until the boiling starts. At the beginning of distillation, the time was recorded, and continued the distillation for one hourSwitch off heating. The graduated receiver was allowed to cool. [16]

Passiflora

The air dried,powdered plant with material was extracted petroleum ether and Ethanol using soxhlet apparatus. Each time before extracting with the next solvent,the material was dried in hot air oven below 40 c.Finally the material was macerated using hot water with occasional starring for 24 hr. and the water extracted filtered. [17]

Hot plate method

The animals were weighed and appropriately marked. After 15 minutes of drug administration the animals were individually placed on a hot plate and the basal reaction time was taken by observing hind paw licking or jump response (whichever appears first) was taken as end point. The reaction time in second was recorded at the interval of 15, 30, 45, and 60 minutes of drug administration with a cut off period of 15 sec. [22]

Acetic acid-induced writhing response method

The writhes were induced by intraperitoneal injection of 1.0% acetic acid (0.1 ml/10 g body weight). The tested plants will be administered orally to groups of mice 60 min before chemical stimulus. [23]

Heat conduction method

One hour after treatment with tested agents, the tip of tail was dipped up to 5 cm into hot water maintained at 58°C. The response time was noted as the sudden withdrawal of the tail from the hot water. Cut off time of 10 seconds was maintained to avoid damage to the tail for all groups. The time required for flicking of the tail, was recorded to assess response to noxious stimulus. [24]

Statistical Analysis

Results were expressed as Mean ± SD. statistical significance was calculated using one way analysis of variance (ANOVA) by SPSS software version 12.0 ($p>0.05$) was considered significance.

1. RESULTS

Heat conduction test

Table (3-1-2), time until pain response appeared ± standard error.

Group	Dose	Mean ± SD
1.control	----------	**3.75**
2.aspirine	50.0 mg /kg	**13.88***
3.passiflora	33.0 mg/kg	**9.13***
4.cinnamon	13.0 g/kg	**10.88***
5.chamomile	8.0 g/kg	**12.00***

* P < 0.05 significantly different from placebo

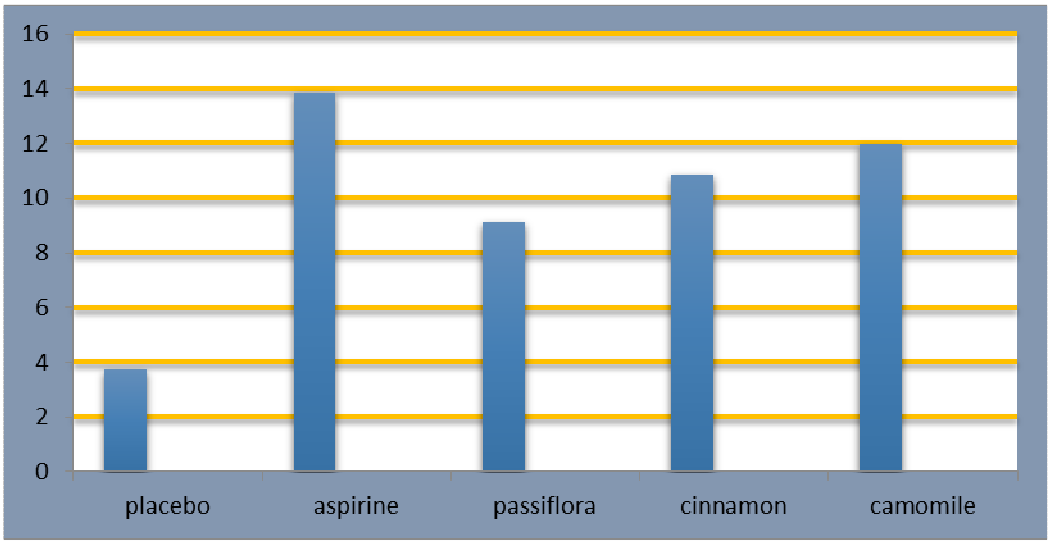

Figure (2-1-2), the difference in time of the response in different groups

3.2. Hot plate test

Table (3-2-3), time until pain response appeared ± standard error.

Group	Dose	Mean ± SD
1.placebo	--------	**6.125±1.125**
2.aspirine	50.0 mg /kg	**16.88 ±1.13***
3.passiflora	33.0 mg/kg	**12.0 ±1.125***
4.cinnamon	13.0 g/kg	**12.5 ±1.035***
5.chamomile	8.0 g/kg	**11.55 ±1.603***

* P < 0.05 significantly different from placebo

3.3. Writhing test

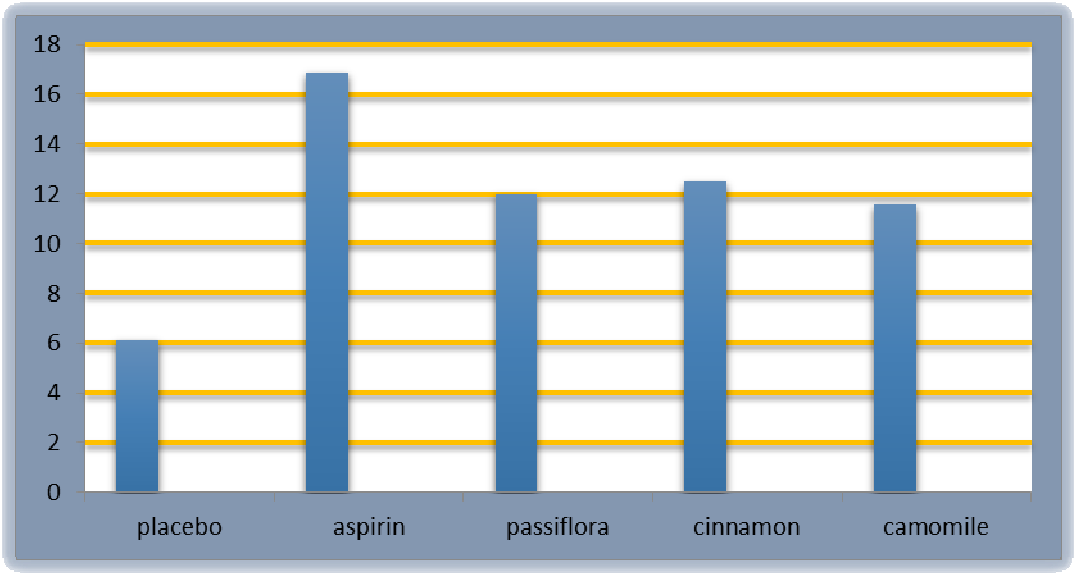

Figure (2.2.3), the difference in time of the response in different groups

Table (3.3.4), the difference in number of the abdominal streathes± standard error

Group	Dose	Mean ± SD
1.placebo	--------	**12.13 ±1.25**
2.aspirine	50 mg /kg	**5.0 ± 1.07***
3.passiflora	33 mg/kg	**7.88 ± 1.25***
4.cinnamon	13.0 g/kg	**7.0 ± 1.07***
5.chamomile	8.0 g/kg	**6.13 ± 1.25***

* P < 0.05 significantly different from placebo

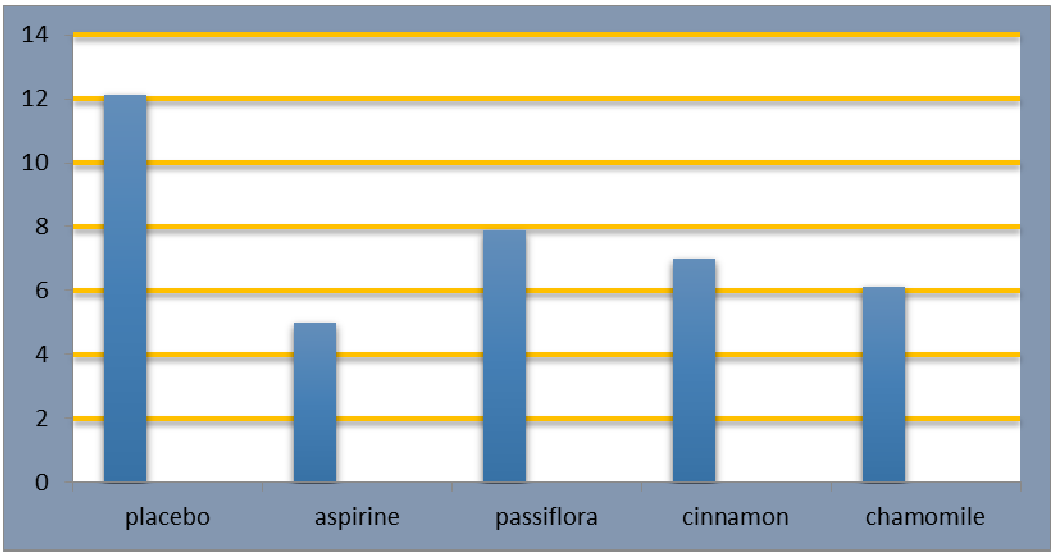

Figure (2.3.4), the difference in number of the abdominal stretches in different groups

4. DISSCUSION

Pain and inflammation are associated with pathology of various clinical conditions like arthritis, cancer, and vascular diseases.[8] In various traditional medicinal systems a number of natural products are used to relieve the symptoms of pain. The hot plate method has been found to be suitable for evaluation of centrally acting analgesics.[9&10] It is proposed that the

acetic acid acts indirectly by inducing the release of endogenous mediators which stimulate the nociceptive neurons sensitive to non-steroidal anti-inflammatory drugs (NSAIDs) and opioids.[7] Acetic acid-induced abdominal constrictions are useful experimental tools in the testing of new analgesic drugs because the abdominal injection of acetic acid in mice has been attributed to the release of arachidonic acid, which results the synthesis of prostaglandin via the cyclooxygenase enzyme.[6&7] The special nerve endings that sense pain is very sensitive to prostaglandin. When prostaglandin is released, the nerve endings respond to it through prostaglandin E2 receptor by picking up and transmitting the pain and injury messages to the brain and cause visceral writhing stimuli in mice.[5]

Therefore, it has been suggested that the inhibition of prostaglandin synthesis is remarkably an efficient antinociceptive mechanism in visceral pain.[8] In order evaluating the analgesic effect of the extract of the plants, several tests (acute and chronic) were employed. Additionally, it is necessary to apply tests with different parameters such as stimulus quality, intensity, and duration. By applying behavioral nociceptive test, it was possible to create a complete picture of the analgesic properties of a substance.[19&20]

This study which was performed to evaluate the analgesic activity of three plants passiflora, chamomile and cinnamon reveled that:

Passiflora

a. Heat conduction test:

Compared to the placebo the passiflora extract showed significantly higher inhibition of (9.13±1.25) with the placebo having (3.75 ± 0.71) but when comparing passiflora with the other two plant extracts and the control aspirin, the passiflora extract showed the least amount of inhibition of (9.11±1.25) compared to (13.88 ± 0.71) aspirin and (10.88 ±1.25) cinnamon and(12.0±1.07) chamomile which indicates that although it has central analgesic activity it is less compared to the other two plants extracts.

b. Hot plate test:

The results revealed that Compared to the placebo the passiflora extract showed significantly higher inhibition of (12.0 ± 1.31)while the placebo was(6.13 ±1.13) but compared with the other two plant extracts it had higher inhibition than cinnamon (10.25 ± 1.04)but less than chamomile (12.5 ± 1.61)and the aspirin (16.88 ± 1.13).

c. Writhing test:

The results revealed that as compared to the placebo the passiflora extract showed significantly higher inhibition of (7.88±1.25) while the placebo was(12.12±1.25) but on comparing passiflora with the other two plant extracts and the control aspirin, the passiflora extract showed the least amount of inhibition compared to (5.0±1.07)aspirin and(7.0±1.07)cinnamon and (6.13±1.25) chamomile.

Passiflora showed mild to moderate inhibitory effect in the mouse writhing assay (a useful test for evaluating mild analgesic, non-steroidal anti-inflammatory agents) suggesting that the extract analgesic activity may be peripherally mediated.[18]. From the results of the hot plate and heat conduction tests, the extract also had a mild to moderate effect in the various acute (phasic) pain models.[23] In the presence of heat and pressure centrally-acting analgesic drugs elevate the pain threshold of animals.

The effect of the extract on these pain models indicates that it may be centrally acting.[6] The results obtained suggest that the analgesic effect is mediated more via central than peripheral inhibitory mechanisms. The pharmacological activity of Passiflora is attributed primarily to the alkaloids and flavonoids.[18] The harmala alkaloids inhibit monoamine oxidase, which may account for part of their pharmacologic effect.

Chamomile

a. Heat conduction test:

Compared to the placebo the chamomile extract showed significantly higher inhibition of (12.0±1.07) with the placebo having (3.75±0.71) and when comparing chamomile with the other two plant extracts and the control aspirin the chamomile extract showed the highest amount of inhibition of compared to the other two plant extracts (10.88±1.25) cinnamon and (9.13±1.25) passiflora but less than the control aspirin (13.88 ± .071).

b. Hot plate test:

The results revealed that Compared to the placebo the chamomile extract showed significantly higher inhibition of(12.50 ± 1.60) while the placebo was(6.13±1.13) but compared with the other two plant extracts it had higher inhibition than cinnamon (10.25 ± 1.04) and passiflora (12.0 ± 1.31) but less than the aspirin (16.88 ± 1.13).

c. Writhing test:

The results revealed that Compared to the placebo the chamomile extract showed significantly higher inhibition of (6.13±1.25) while the placebo was (12.12±1.25) but when comparing chamomile with the other two plant extracts and the control aspirin the chamomile extract showed the higher amount of inhibition compared to cinnamon (7.0±1.07) and passiflora (7.88±1.25) but less than that of aspirin (5.0 ±1.07).

The extract showed Potent inhibitory effect in the mouse Wrething assay (a useful test for evaluating mild analgesic, non-steroidal anti-inflammatory agents). This suggests the peripherally mediated analgesic effect of the extract.[23] The extract also showed a significant analgesic effect in the various acute (phasic) pain models, mainly the hot plate and heat conduction tests.[26] The pain threshold of animals was elevated in the presence of heat and pressure centrally-acting analgesic drugs, indicating that it may be centrally acting.[11] Centrally-acting analgesic drugs such as narcotic analgesics, inhibit both phases of pain in this model, while drugs that act peripherally, such as aspirin or indomethacin, only inhibit the late phase.[11&12] From the results obtained, the effect is clearly mediated via peripheral and central inhibitory mechanisms.[9]

Flavonoids, alkaloids, and essential oil, which were extracted during the preparation of extract, could be account for the promotion of the anti-nociceptive effect of chamomile.[10] This is consistent with those reported for the effects of many flavonoids, alkaloids, and essential oils.[11] For instance, the benzodiazepine like activity of flavonoids apigenin and chrysin of chamomile is reported, and is thought to contribute to the sedative effect of chamomile.[12] Benzodiazepines are known to be an inhibitory agent for anxiety, pain, and potentiate GABA-induced chloride current.[20] The anti-nociceptive activity was also reported for luteolin 7-O-glucoside from Verbascum salviifolium and hesperidin from Aloysia triphylla.[24] In addition, the alkaloid laetispicine from Piper laetispicum has been found to

reduce the number of writhes in mice in a dose-dependent manner.[12] Furthermore, the analgesic activity of 1-nitro-2-phenylethane, the main component of Aniba canelilla essential oil, has been reported.[11&12] Based on these observations, it can be said that the anti-nociceptive effect of in the present study is partly related to these active ingredients.

Cinnamon :

a. Heat conduction test

Compared to the placebo the cinnamon extract showed significantly higher inhibition of (10.88±1.25) with the placebo having(3.75±0.71) and when comparing cinnamon with the other two plant extracts and the control aspirin the cinnamon extract showed less inhibition compared to the chamomile plant extracts(12±1.07) and the control (13.88±0.71)but aspirin higher than passiflora (9.13±1.25).

b. Hot plate test:

The results revealed that Compared to the placebo the cinnamon extract showed significantly higher inhibition of(10.25 ± 1.04) while the placebo was(6.13±1.13) but compared with the other two plant extracts it had the least inhibition than chamomile(12.5 ± 1.61) and passiflora (12.0 ± 1.31) and less than the aspirin (16.88 ± 1.13).

c. Writhing test:

The results revealed that Compared to the placebo the cinnamon extract showed significantly higher inhibition of (7.0 ±1.07) while the placebo was (12.12±1.25) but when comparing cinnamon with the other two plant extracts and the control aspirin, the extract showed higher inhibition compared to passiflora (7.88±1.25) but less inhibition than chamomile (6.13±1.25) and aspirin (5.0 ±1.07).

Cinnamon showed moderate inhibitory effect in the mouse writhing assay (a useful test for evaluating mild analgesic, non-steroidal anti-inflammatory agents) suggesting the peripherally mediated mechanism.[15] For the hot plate and heat conduction tests ,the extract also showed a mild to moderate effect.[13] In the presence of heat and pressure, the pain threshold of animals has been elevated by using the centrally acting analgesic drugs.[16] The effect of the extract on these pain models indicates that it may be centrally acting.[13&14] The results obtained mediated more via peripheral than central inhibitory mechanisms. Cinnamaldehyde is a specific TRPA1 (mammalian transient receptor potential (TRP) ion channel) activator. It has been shown to excite a subset of sensory neurons highly enriched in cold-sensitive neurons and elicit nociceptive behavior in mice.[14&15]

5. CONCLUSION

This study indicates that the analgesic properties of all three plant extract in Swiss albino mice which showed peripherally and centrally- mediated inhibitory mechanisms. The plant are placed in order of their analgesic activity in having the strongest analgesic activity has been shown by chamomile while cinnamon showed less activity and passiflora showed the least analgesic activity of the three plant extract. To sum up, this study provides a rational for the use of theses plant in pain and inflammatory disorders in folk medicine. Further studies are needed to assess the analgesic effect of the plants on humans.

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