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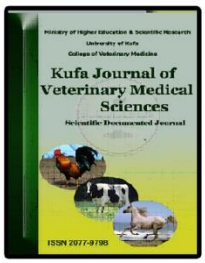

Analgesic and Anti- Inflammatory Effects of Hydro Alcoholic Extract of *(Syzygium aromaticum)* in Albino Mice

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Abstract

The study was carried out to evaluate the analgesic and anti-inflammatory properties of an hydro alcoholic *syzygium aromaticum* extract (SAE) .The analgesic activity was measured by using the hot plate test with Twenty four (24) mice divided randomly into four groups , T1, T2 received 100 , 200 mg /kg.bw of (SAE) while T3 dosed 50 mg /kg.bw of diclofanic acid and T4 used as control negative and dosed distilled water (D.W.) . The anti-inflammatory activity was measured by using formalin test with Twenty four (24) mice divided randomly into four groups , T1and T2 dosed with 100 and 200 mg /kg.bw respectively of (SAE) while T3 group dosed 0.3 mg/kg.bw of meloxicam and T4 dosed with (D.W.) , (all groups dosed orally before half hour of test) and then calculate the time of analgesia and anti-inflammatory effects . The result showed there is significant increase($p<0.05$) in time of analgesia and significant decreases ($p<0.05$) in number of licking in animal exposed to (SAE) comparing with that of control , also there is significant increase($p<0.05$) in time of analgesia and decrease in licking in animals received diclofanic acid and meloxicam respectively comparing with those received (SAE) and D.W the increase was dose dependent manner. In conclusion the hydro-alcoholic extract of clove E.C showed analgesic and anti-inflammatory effect .

Keywords : clove , hot plate test , formalin test .

التأثيرات المسكنة والمضادة للالتهاب للمستخلص المائي الكحولي لنبات القرنفل في الفئران البيضاء

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الخلاصة :
اجريت هذه الدراسة للتحقق من التأثير المسكن والتأثير المضاد للالتهابات للمستخلص المائي الكحولي لنبات القرنفل .تم قياس التاثير المسكن باستخدام اربعة و عشرون (24) من الفئران باستخدام اختبار اللوح الساخن مقسمة عشوائيا الى اربع مجموعات، جرعت كل من مجموعة 100) ,T1,T2 ، 200ملغم/كغم (من وزن الجسم على التوالي من المستخلص المائي الكحولي للقرنفل .في حين تم تجريع المجموعة الثالثة بجرعة 50) ملغم/كغم (من وزن الجسم من علاج حامض الديكلوفانيك ، واستخدمت المجموعة الرابعة كمجموعة سيطرة وجرعت بالماء المقطر . تم قياس التاثير المضاد للالتهاب باستخدام اربعة و عشرون (24) من الفئران باستخدام اختبار الفورمالين مقسمة عشوائيا الى اربع مجموعات، جرعت كل من مجموعة T1,T2 100) ,، 200ملغم/كغم (من وزن الجسم على التوالي من المستخلص المائي الكحولي للقرنفل .في حين تم تجريع المجموعة الثالثة بجرعة 0.3) ملغم/كغم (من وزن الجسم من علاج الميلوكسيكام ، واستخدمت المجموعة الرابعة كمجموعة سيطرة وجرعت بالماء المقطر) . جرعت المجاميع فمويا قبل نصف ساعة من بدأ الاختبار (ومن ثم تم حساب وقت تسكين الألم . اظهرت النتائج وجود زيادة معنويه ($p<0.05$) في وقت تسكين الالم وكذلك نقص معنوي ($p<0.05$) في عدد اللعقات المسجلة للحيوانات التي جرعت مستخلص القرنفل مقارنتا مع تللك التي جرعت ماء مقطر وكذلك وجود زيادة معنويه ($p<0.05$)في وقت تسكين الالم ونقص معنوي في عدد اللعقات المسجلة في الحيوانات المجرعه حامض الدكلوفانيك وعقار الميلوكسيكام مقارنتا مع تلك التي جرعت بمستخلص القرنفل وكذلك مجاميع السيطرة . نستنتج من الدراسة الحالية ان للمستخلص المائي الكحولي لنبات القرنفل تاثير مسكن ومضاد جيد للالتهاب .
الكلمات المفتاحية: القرنفل , اختبار الصفيح الساخن , اختبار الفورمالين.

Introduction:

For centuries till now and tomorrow the used of herbal plants in our life increasing specially in medicine and drug industries . The clove(*Syzygium aromaticum*)(SAE) is a tree with approximately 20 meters in height which is found in many countries around the world (1) , (2). The clove tree consist of leaves and buds which used commercially as food additives and in perfumery product (3). Clinically it have therapeutic effect against alimentary disturbance (4). Another studies conducted that the clove have been effective against microbial infections (5),(6). Furthermore it is a approved that the clove have a good cytotoxic and even anti-cancerogenic properties (7),(5). Another studies showed that plant give good antibacterial effect against bacteria cause oral infection which are accompanied with dental decay and periodontal diseases (8). The clove oil has been used skin disease and parasites (9) also has revealed anesthetic effects in fish (10). Many studies reported the analgesic effect in patients suffering from toothache and anal fissure (11). The analgesic , anesthetic and anti-inflammatory effect of eugenol(the main component of clove) have been certified by using experimental animals (3)(12)(13). In imitative medicine, the buds of (SAE) give pharmacological action against epilepsy (14).Thus the present work is carried out to look for the analgesic and anti-inflammatory effects of (SAE) in mice. In addition to the role of opioid system in analgesic effects of (SAE) was examined using diclofanic acid and for for nociception effect used meloxicam as comparative drugs.

MaterialsαMethods:

A-Preparation of clove hydro alcoholic extract: (SAE) powder was done according to (15) by using 1000 ml flask in which 50 grams of buds powder in the flask , after that up to1000 ml of 30% ethanol was added, by magnetic stirrer mixed and extracted at 40°C for 72 hours, and filtered with gauze to get rid the residue then extra filtrated by whatman paper and millipore paper (0.5mm) . Finally incubated at 40◦C. and the final extract was frozen at –20°C until use.

B- Animals: eighty four 48 male albino mice (24 for hot plate test and 24 for formalin test) , aged 6-8 weeks with weight range (25-30g), supplied from the animal house of the College of veterinary medicine of Al-Qasim Green

University in accordance with international ethical standards of research for work with laboratory animals. They were housed and maintained in a conventional animal facility, with controlled conditions of temperature (20 ± 5ºC). Standard pellet and diet were produced *ad libitum*.

C-Hot plate test: This test evaluate the thermal pain reflexes due to foot pad contact of mice with a heated surface, hot plate test is the most sensitive to centrally acting analgesics (16) . Twenty four 24 mice were randomly divided into four test groups of six mice each as show in (table 1). The hot plate sets at 55 ± 0.2 °C. The method set up according to (17).

D- Formalin test : To assessment of nociception effect of (SAE) we were taken Twenty four albino mice were divided equally into four groups (6 mice for each group). Solutions E.C extract at doses of (100 and 200 mg/kg B.W.), meloxicam (0.3 mg/kg B.W) as comparative drug and 1ml/kg D.W-as control). The procedure was done according to (18). and adapted by (19). And we were recorded the time of starting licking the injected paw.

E- Statistical Analysis: Statistical analysis was applied by one ways ANOVA with (LSD) to compare groups means .Probability level $P<0.05$ was considered statistically significant by using statistical package for social sciences (SPSS),Version

Results and Discussion:

A-Hot Plate Test: The results of the hot plate test were summarized in table (1).revealed significant analgesic activity ($p<0.05$) in animals dosed with (SAE) compared with control group the increase was dose dependent manner. The reference drug diclofanic acid also significantly delayed the reaction time comparing with control mice. The diclofanic acid showed significant analgesic activity ($p<0.05$) in comparison with that produced by (SAE) and D.W group .

Table (1): Change in pain reaction time after different periods of SAE extract and Diclofanic acid in mice (Hot plate test).

groups Times	100mg/kg (SAE extract) T1 M±S.E	200mg/kg (SAE extract) T2 M±S.E	diclofanic acid (50mg/kg) T3 M±S.E	Control (D.W) T4 M±S.E
Zero time	**2.3 ± 0.2** **C a**	**2.5 ± 0.4** **C a**	**2.4 ± 0.2** **D a**	2.2 ± 0.1 D a
After (30minute)	**4.8 ± 0.2** **A c**	**5.9 ± 0.2** **A b**	**6.8 ±0.2** **A a**	2.8 ± 0.3 C d
After (60minute)	**4.7 ± 0.12** **A a**	**5.6 ± 0.18** **A a**	**5.9 ± 0.4** **B a**	3.4 ± 0.3 A b
After (90minute)	**3.6 ± 0.4** **B bc**	**4.6 ± 0.2** **B a**	**4.4 ± 0.1** **C a**	3.3 ± 0.2 B b

-Different capital letters denote significant differences ($p<0.05$) within groups.

-Different small letters denote significant differences($p< 0.05$) between group between periods.

L.S.D. =0.8

N= 6

The analgesic activity of (SAE) suggested the present of many component that acting on central nervous system or the peripheral nervous system or both in some cases. Therefore, it is always necessary to employ a method to distinguish between the two (20). Many previous Studies improved that pain generated by a thermal stimulus is mediated centrally (21) In this model, the effects of drugs on latency time of jumping responses or licking of the animal's front paws represents the analgesic effect on sensory receptor stimulation. By the other hand many studies have been founded deferent constituents in phytochemical analysis of clove buds. which include 15–20% essential oil eugenol , which is predominated by (70–85%), eugenyl acetate (15%) and β-caryophyllene (5–12%)(22),(23). Eugenol is a classic analgesic agent widely used by dentist due to its ability to relief tooth pain. The pain sensation is enhances by the acidic extracellular pH, and Ca2+ influx through activated voltage-or ligand-gated cation channels has been recognized to lower the intracellular pH in neurons(24). Eugenol action is inhibition of voltage-activated sodium and calcium channels (25),(26). In our study we thought that analgesic effect of (SAE)clove bud extract given may be due to the presence of high amount of eugenol. Therefore we are in agreement with Kamatou GP., et al 2012 (27) who recorded there is analgesic effect of clove buds when they studied the pharmacological, agricultural and other applications of eugenol from clove extract. Also we are in agreement with Monika Mittal., et al 2014 (28), who conducted the phytochemical and pharmacological action of clove.

B- Formalin Test: The results of formalin test listed in table (2) showed that there was significant reduction ($p<0.05$) in nociceptive response between different treated groups T1, T2, Meloxicam and control group manifested by reduction in the number of liking of injected limb, also between early and late phases for all treated groups.

Table (2) Analgesic responses of SAE extract on (No. of licking) in mice (formalin test)

groups / phases	T1 100 mg/kg (SAE extract) M±S.E	T2 200 mg/kg (SAE extract) M±S.E	T3 Meloxicam (0.3mg/kg) M±S.E	T4 D.W 1ml/kg M±S.E
acute phase (First 5 min.)	**27.6 ± 2.6** **A b**	**20 ± 0.77** **A c**	**19.5 ± 0.76** **A c**	35 ± 2.6 A a
Chronic phase (15-45min.)	**10.1 ± 0.73** **B ac**	**7 ± 0.73** **B bc**	**5.6 ± 1.1** **B b**	13.3 ± 0.9 B a

-Different capital letters denote significant differences ($p< 0.05$)within groups.

- Different small letters denote significant differences ($p< 0.05$) between groups.

L.S.D. = 4.4
N= 6 mouse.

The response to formalin presented by early and late phase, The early phase, beginning after five minutes of formaline injection and is likely due to direct chemical stimulation of nociceptors (acute pain). The following phase until 40 min, starts approximately 15 min after formalin injection and propose that peripheral inflammatory processes are implicated (29).Inflammation results in increased expression and enzyme activity of cyclooxygenases (COX) 1 and 2. cyclooxygenases 2 in turn produces inflammatory mediators such as prostaglandin.(30). These enzymes are involved in the inflammation and carcinogenesis processes, therefore it is recognized that potential COX-2 inhibitors can be considered anti-inflammatory or cancer chemo preventive agents (27).We thought the anti-inflammatory effect that produced from clove buds extract may be due to present of high amount of eugenol and acetyleugenol which have great effect on COX-2 .And that corresponding with Leem, H ,et al 2011(31) who conducted The clove buds oil exhibited strong inhibitory activity against COX-2 (58.15%) and 15-LOX (86.15%) enzymes at 10 μg/mL and 25 μg/mL, respectively. Other researchers Daniel., et al 2009(3), were studied The anti-inflammatory activity of eugenol by using carrageenan-induced paw edema tests in rats at 200 and 400 mg/kg of clove buds oil, and founded reduce in volume of pleural exudates without changing the total blood leukocyte count indicating the anti-inflammatory potential of eugenol.

In conclusion, the previous results of formalin test and hot plate test suggested extracts induced antinociceptive effect is probably due to an inhibitory effect of this extracts on central mechanism and peripheral mechanism**.**

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