

Analgesic activity of *Trigonella foenium graceum* (TFG) and *Emblica officinalis* (EO) extracts in white mice in comparison with diclofenac (Voltaren®)

Auroba M. S. Ibrahim Nibras N. A. Al-Abassi
Amer H. Chyad

Physiology & Pharmacology Dep. College of Veterinary Medicine, Baghdad
University, Baghdad, Iraq

Abstract:

Present study aimed to explore the ability of *Trigonella foenium graceum* (TFG) and *Emblica officinalis* (EO) extracts inducing the analgesic effect in comparison with diclofenac acid, Analgesic activity was assessed thermally as well as chemically induced pain by using hot plate test and formalin test. For hot plate experiment twenty albino mice were used of average weight 20g, randomly divided into four groups (5 each) and given TFG and EO extracts and diclofenac acid and mixture of TFG and EO orally at doses of 50mg/kg B.W, 250mg/kg B.W, 2mg/kg B.W and mixture of half doses of TFG and EO (25mg/kg B.W and 125mg/kg B.W respectively. TFG and EO extracts apart caused a significant increase in the reactive time to the thermal stimulus. For formalin experiment twenty five mice were used of average weight 20gm, randomly divided into five groups (5 each) and given TFG and EO extracts apart and mixture of half doses of both orally at same doses mentioned in hot plate experiment before the injection of 10 microliter (μ L) of 2% of formalin have showed a significant antinociceptive effect at the phases (early and late) and this may be attributed to their inhibitory effect on the nociceptive system and inflammatory mediators.

الفعالية التسكينية لخلاصتي نبتة الحلبة وفاكهة الاملا مقارنة بعقار الدايكولوفيناك (الفولتارين)

نبراس نائب العباسي

عروبة محمد سعيد

عامر حكيم جيايد

فرع الفلسفة والأدوية، كلية الطب البيطري، جامعة بغداد، بغداد، العراق

الخلاصة:

الدراسة الحالية استهدفت معرفة قابلية كلا من خلاصتي الحلبة والاملا في احداث الفعل التسكيني بالمقارنة مع الحمض الدايكولوفيناك (الفولتارين). تم استخدام 20 فارة بمعدل وزن 20 غرام في تجربة الصفيحة الساخنة ، قسمت عشوائيا الى اربع مجاميع بواقع 5 فئران لكل مجموعة جرعت فمويا بكلا الخلاصتين (الحلبة والاملا) وحمض الدايكولوفيناك وجرع 50ملغم لكل كغم ، 250ملغم لكل كغم و 2ملغم

لكل كغم على التوالي ومزيج من نصف الجرعة المذكورة لكلا الخلاصتين معا (الحلبة والاملا). اظهرت كل من خلاصتي الحلبة والاملا(عندما اعطيتا كلا على حدا) زيادة معنوية في زمن الاستجابة للألم وهذه الزيادة ممكن ان تعزى لدور كل من الخلاصتين بشييط البروستاغلاندينات. في اختبار الفورمالين تم استخدام 25 فارة ، قسمت عشوائيا الى 5 مجاميع وبواقع 5 فنران لكل مجموعة حيث تبين إن الإعطاء المتزامن لنصف الجرعة المذكورة اعلاه لكلا الخلاصتين واعطائهما كلا على حدا كان له الأثر الفعال على كلا الطورين (المبكر والمتأخر) والذي يمكن عزوه للتأثير المثبط لكلا الخلاصتين معا.

Introduction:

The exponentially increasing list of analgesic drugs and its side effects, primary of self – administration has led to a considerable ephasis on use of medical herbs like (1). *Trigonella foenium graceum* (TFG) from the family fabacea, commonly known as fenugreek. It has rich medicinal components in leaves were evaluated for potent antinociceptive, anti-inflammatory and antipyretic activities (2) . Alkaloid, glycosides, salicylates, coumarins, saponins, soluble fiber 30%, insoluble fiber 20% and phenols are the major components in the leaves (3) . *Emblica officinalis* (EO) of the family Euphorbiaceae, commonly known Amla. It has various medicinal application as analgesic , antipyretic , antitussive , antioxidant and cytoprotective and antimicrobial activities (4) . It primarily contains alkaloid , phenols , amino acid and carbohydrates . They are reports on

the antinociceptive activity of certain flavonoids which are polyphenols found frequently in fruits , vegetables and grain. Quercetin, a bioflavonoid is reported to attenuate hyperalgesia (5).

Diclofenac acid (Voltaren) is one of non steroidal, anti-inflammatory drugs, the primary mechanism responsible for its anti-inflammatory, antipyretic, and analgesia action is inhibition of prostaglandin synthesis by inhibition of cycloxygenase (COX).

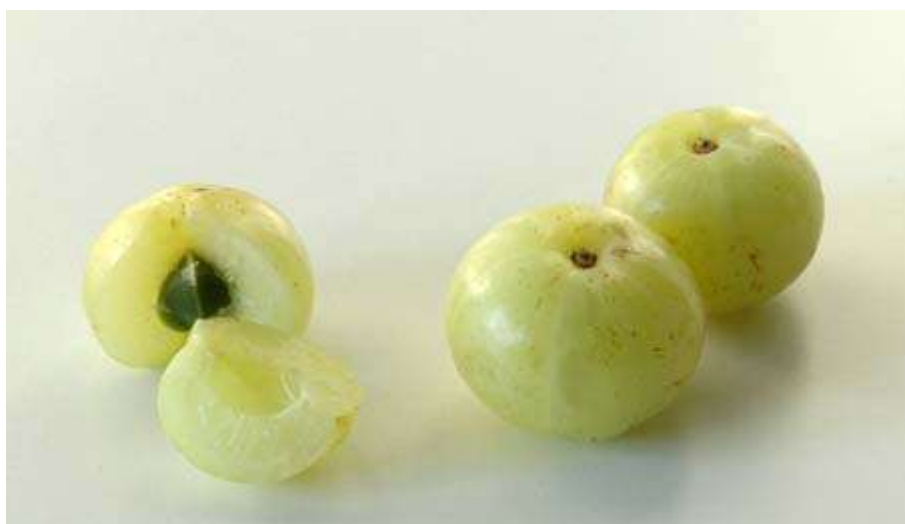
Materials and methods:

The green leaves of (TFG) and dried seeds of (EO) were purchased from local market and identified by national herbarium at Abu-Ghraib .The green leaves left to dry then ground into powder by electrical blinder. Both plants were extracted with hot 70% methanol.

Diclofenac acid (Voltaren) 25mg (S.D.I.-Iraq).



Pix (1) Ground leaves of *Trigonella foenum-graceum* (TFG).



Pix (2) Fruits of *Emblica officinalis* (EO).

Experimental animals:

Swiss albino mice (range of body weight =20-25g) were purchased from animal house of Sera and Vaccines Institute, acclimatized for seven days at standard conditions.

Experimental design:

Forty five Swiss albino mice were used to carry out analgesia test (hot plate test and formalin test).Twenty mice were used in hot plate test pretreatment values were taken then

mice were randomly divided into four groups (5 each) designated as ; A,B,C and D groups and treated orally with both plant extracts and drug as in the following:

Group A received aqueous solution of diclofenac sodium at dose of 2mg/ Kg B.W, left 30 min. before testing.

Group B received aqueous solution of (EO) methanol extract at dose 250 mg/ Kg B.W, left 45 min. before testing.

Group C received aqueous solution of (TFG) extract at dose 50mg/ Kg B.W, left 45 min. before testing.

Group D received half doses of aqueous solutions of (EO) 125mg/ Kg B.W. and (TFG) 25mg/ Kg B.W. extracts simultaneously that left one hour before testing.

The test was done by dropping the mice gently one by one on the hot plate whose temperature was maintained at $55 \pm 1^{\circ}\text{C}$. This temperature produces two behavioral components in mice that can be measured in terms of their reaction times, namely licking and flicking of the paw and jumping (6).

For formalin test, twenty five mice were used, divided randomly into five groups (5 each). At least 30 min. before performing formalin test, animals of all groups received oral treatment as in the following:

Group A: received distilled water and served as a control group.

Group B: received diclofenac sodium at dose of 2mg/ Kg B.W.

Group C: received (EO) extract at dose 250 mg/ Kg B.W.

Group D: received (TFG) extract at dose 50mg/ Kg B.W.

Group F: received half doses of both extracts, (EO) 125mg/ Kg B.W and (TFG) 25mg/ Kg B.W. extracts simultaneously.

Each mouse was placed in transport plastic cage and left 5 min. before formalin injection to allow adaptation of the new environment. Ten microliter of 2% formalin were injected intradermally into the

planter region of the right paw of all mice in each group. The injection of diluted formalin produced two pain-related behavioral components that can be measured in terms of nociceptive response namely flinching (is one pain related behaviors of formalin model characterized by spontaneous, rapid and brief shaking or lifting of the paw) and licking of the injected paw (7). The nociceptive response was measured immediately after formalin injection and continued for 60 min.

Amount of aqueous solution of treatment materials were individually adjusted according to body weight of animals and given orally via stomach tube.

Results:

In studying the analgesic effect, treatment of mice with TFG and EO extract caused significant increase at $P < 0.05$ in the pain reactive time to the thermal stimulus in comparison with the pretreatment values. While given EO extract caused an obvious significant increase in the pain reactive time to the thermal stimulus after treatment. The pain reactive time reached 20.40 ± 0.50 after being 9.80 ± 0.86 second see table (1). Simultaneous gavage of TFG and EO extracts at dose of 25mg/ Kg B.W and 125mg/ Kg B.W respectively has significantly prolonged the pain reactive time to the thermal stimulus while treatment with (TFG) extract also shown significant increase in the pain

reactive time but lesser than EO extract.

It has been difficult to separate the influence of anti-inflammatory activity from that of analgesia in standard animals tested by using hot plate test so the formalin was applied for this purpose. Intra dermal injected of diluted formalin solution into the right paw of hind foot of mice biphasic nociceptive response (licking and flinching of the injected paw). An early phase during first 5 min. Following injection of formalin and late phase starting (15) min. after injection and lasting for (45) min.

A significant difference at $P < 0.05$ has been found between the two

phases of formalin test (early and late) in group C when EO extract was given before injection of diluted formalin solution , the mean of numbers of licking and flinching reaction was 9.80 ± 0.37 and 14.80 ± 0.80 at early and late phase respectively.

While giving (TFG) extract group increase in the mean of the number of licking and flinching reactive time but lesser than EO extract .The administration of the mixture of EO and TFG extracts at doses of 125mg/ Kg B.W and 25mg/ Kg B.W respectively have shown significant nociceptive effect at both phases of formalin response more than when given alone table (2).

Table (1) The pain reactive time to the thermal stimuli (in seconds) in mice treated orally with the extract of **TFG**, **EO** a part or as a mixture of both to gather and (**votaren**) (hot plate test).

Groups of mice	Pre-treatment	Post-treatment	L.S.D
Treated orally with voltaren at a dose of 2mg per kg B.W.	b 8.00±0.44 B	a 16.20±1.24 B	3.04
Treated orally with EO extract at a dose of 250mg per kg B.W.	b 9.80±0.86 A	a 20.40±0.50 A	2.30
Treated orally with TFG extract at a dose of 50mg per kg B.W.	b 9.80±0.37 A	a 14.80±0.80 B	2.31
Treated orally with both extracts of EO at a dose of 125mg per kg B.W. and TFG at a dose of 25mg per kg B.W.	b 8.40±0.60 AB	a 16.60±0.74 B	2.21
L.S.D	1.79	2.59	

Figures represent mean \pm standard error

Number of group animals =5

Different Big letters at same groups mean significant difference at level 5% between groups.

Different small letters at same row mean significant difference at level 5% between groups.

Table (2) Nociceptive response in mice treated orally with the extracts of **TFG**, **EO** a part or both together as a mixture and (**voltaren**) before S/C injection of 10 micro liters of 2% formalin into the planter region of the right paw (formalin test).

Groups of mice	Nociceptive response (number of licking and flinching)		L.S.D
	Early phase (0-5) minute after injection	Late phase (15-45) minute after injection	
Treated orally as a control group with D.W before injection of formalin.	○○○○○ a 23.20 \pm 1.23 A	a 20.29 \pm 1.20 A	4.04
Treated orally with voltaren at a dose of 2mg per kg B.W before injection of formalin.	a 22.80 \pm 1.59 A	b 9.20 \pm 1.11 BC	3.48
Treated orally with EO extract at a dose of 250mg per kg B.W. before injection of formalin.	a 19.61 \pm 1.33 AB	b 14.80 \pm 2.03 B	5.73
Treated orally with TFG extract at a dose of 50mg per kg B.W before injection of formalin.	a 20.50 \pm 1.42 AB	b 15.38 \pm 1.16 B	4.25
Treated orally with both extracts of EO at a dose of 125mg per kg B.W and TFG at a dose of 25mg per kg B.W before injection of formalin.	a 17.20 \pm 1.01 B	b 11.40 \pm 1.72 B	5.25
L.S.D	3.95	4.40	

Figures represent mean \pm standard error.

Number of group animals=5

Different Big letters at same column mean significant difference at level 5% between groups.

Different small letters at same row mean significant difference at level 5% between groups.

Discussion:

The hot plate test is commonly used to assess analgesic effect or other centrally acting drugs (8). Treatment of mice with TFG and EO extracts apart showed a significant increase in the reaction time to the thermal stimulus in comparison with pretreatment values. Given EO group has shown an obvious significant increase in the pain reactive time. This increase may be due to activity of some of its chemical constituents in particular Quercetin, a bioflavonoid reported to attenuate thermal and chemical hyperalgesia [7], also flavonoids, rutin which belongs to quercetin group has an inhibitory effect on 5-lipoxygenase pathway (the main pathway for production of chemical mediators important in pain and inflammatory processes) (9). TFG treated group also shown a significant increase in the pain reactive time. This increase may be due to activity of its chemical constituents including: phenols, glycosides and alkaloids (2). These compounds have been reported to have a role in relieving pain through elevation of pain threshold or diminishing inflammatory symptoms by inhibiting some chemical mediators such as: prostaglandin.

Other mechanisms can be suggested either by depressing ability of nociceptors to bind the chemical mediators or by decreasing conducting rate of nerve impulses along nerve fibers responsible for propagation and transport of impulses.

The early phase of formalin test seemed to be caused predominantly by C-fiber activation due to the peripheral stimulus, while the late phase of appeared to be dependent on the combination of an inflammatory reaction in the peripheral tissue and functional changes in the dorsal horn of the spinal cord. these functional changes seemed to be initiated by the C-fiber barrage during the early phase (10). The TFG and EO extracts revealed antinociceptive effect during the early stage and late phase of formalin test while both the extracts showed more significant nociceptive effect when given together, as do centrally acting antinociceptive drugs (11) we can not exclude the possibility that the extracts induced antinociception in the late phase is partly mediated by peripheral mechanism like diclofenac acid which is used in this experiment and showed attenuation of the late phase

nociceptive responses in the formalin test and which came in agreement with conclusion of (12) that the non-steroidal anti-inflammatory drugs like (indometacin and diclofenac acid) and steroidal dexamethasone and hydrocortisone inhibited only the late phase. The TFG and EO extracts significantly inhibited the behavioral changes caused by acute nociceptive stimuli (hot plate and formalin test), these suggest that TFG and EO extracts induced antinociceptive is due to an inhibitory effect of this extracts on central mechanism and peripheral mechanism.

References:

1. Biwal S., Das. MC and Nayak P. (2003). Antinociceptive activity of seeds of *Trigonella foenum graceum* in rats. Indian J. Physio-pharmacol;47(4):479-480.
2. Javan M., Ahmadiani A., Semnani-ans E. and Kamalinejad M. (1997). J.Ethanopharmacol Pp. 275-283.
3. Ahmadiani A., Javan M., Semnani-ans E. and Kamalinejad M. (2001). J.Ethanopharmacol Pp. 275-283.
4. Khan K.H. (2009). Roles of *Emblica officinalis* in Medicine- A Review. Botany Research International 2(4):218-228.
5. Kumar N.P and Annamalai A.R. (2009). Antinociceptive activity of *Emblica officinalis* in high fat diet-fed/low dose streptozotocin induced diabetic neuropathy in rats. Indian Journal of Experimental Biology Pp.737-742.
6. Eddy N.B. and Leimbach D. (1953). J.pharmacol. Exp. Ther. Cited by Bars, D. L., Gozariu, M. and Samuel, W. (2001). Animal models of Nociceptive. Pharmacological Reviews. 53 (4) 597-652.
7. Dubuisson, D. and Dennis, S. (1977). The formalin test: A quantitative study of analgesic effects of morphine, meperidine and brain stem stimulation in rats and cats. Pain. 4 (2): 161-174.
8. Beirith, A.; Santos, A.R.S.; Rodriguez, A. (1998) Eur. J. Pharmacol. 345,233.
9. Ficarra, R.; Ficarra, P.; Tommasini, S.; Calabro, M.L. and Ragusa, S. (1995). Leaf extracts of some cordial species: analgesic and antiinflammatory activities as well as their chromatographic analysis. Farmaco. 256-50: 245 Italy.
10. Tjolsen, A.; Berge, O.F.; Hunskaar, S.; Rosland, J.H. and Hole, K. (1992). The formaline test: an evaluation of the method. Pain, 51:5-17.
11. Shihata, I.M. (1951). A pharmacological study of *Anagallis arvensis*, M.Sc. Thesis Faculty of Vet. Medicine, Cairo University.
12. Hunskaar, S. and Hole, K. (1987). The formaline test in mice: Dissociation between inflammatory and non-inflammatory pain. Pain. 30 (1):103-114.