

STUDY THE ANALGESIC AND SEDATIVE EFFECT OF *Ocimum basilicum* ALCOHOLIC EXTRACT IN MALE RATS.

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ABSTRACT

This study was carried out on male rats to evaluate the analgesic and sedative effect of different doses of Alcoholic Extract of *Ocimum basilicum* leaves. The analgesic effect studied by using formalin test, 20 male rats divided to a four groups (5 each), first group exposed water orally only before injection of formalin, second group exposed orally with Diclofenac at a dose 0.71mg/kg B.W before injection of formalin, Third and fourth groups orally incubated with Alcoholic Extract of *Ocimum basilicum* leaves at a dose (50 and 100mg/kg BW) respectively. While the sedative effect studied by using pentobarbitone sleeping time test and open field test, in each test 15 male rats used and divided to a three groups one of them treated with distilled water and the other two groups treated with Alcoholic Extract of *Ocimum basilicum* leaves at a dose (50 and 100mg/kg BW) respectively. Results of formalin test showed a significant reduction in the mean value of nociceptive response in Diclofenac group specially at late phase while, groups treated with Alcoholic Extract of *Ocimum basilicum* leaves revealed a significant reduction in the nociceptive response value at the both phases (early and late phase) moreover the group treated with 100mg/kg showed the highest attenuation in the mean value of nociceptive response compared to 50mg/ kg treated group. Furthermore, the dose 100mg/kg produced the potent sedative effect in pentobarbitone sleeping time test and open field test. These results pointed to analgesic and sedative effect of *Ocimum basilicum* may duo to its consistence of the active analgesic and sedative compounds and its effect are in a dose dependent manner.

Key words: analgesic, sedative, *Ocimum basilicum*, alcoholic extract, male rats.

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INTRODUCTION

Traditional medicinal plants are a therapeutic resource used by the population of the African, Asian continent specifically for health care, which may also serve as starting materials for drugs (Iwn *et al.*,1999). A medicinal plant” is any plant which in one or more of its part contains substances that can be used for the therapeutic purposes or which are precursors for the synthesis of useful drugs (WHO ,2001).

Basil (*Ocimum basilicum*) of the family *Lamiaceae* The plant tastes somewhat like anise, with a strong, pungent, sweet smell. Typically called sweet basil or Holy basil. The plant grows in several regions around the world. The genus *Ocimum* is ranked high among some of the astonishing herbs for having enormous medicinal potentialities (Klimańkova *et al.*,2008). *Ocimum basilicum* has also been used in the treatment of a number of ailments like bronchitis, rheumatism and pyrexia (Keita *et al.*, 2000). The fixed oil of *O. basilicum* was found to possess significant anti-inflammatory (Chaurasia, and Vyas, 1977) along with anti-microbial (Rana *et al.*, 1997), analgesic and spasmolytic properties without any noticeable toxicity or hypoglycemic effects (Sethi, 1979) and is an effective anti-diarrheal, antioxidant, anti-depressant and anti-helmentic and enhances wound healing (Vohora and Rizwan, 1973). In traditional medicine, *Ocimum basilicum* has been used as an antioxidant, antiseptic, preservative, sedative, digestive regulator and diuretic. It also has been recommended for the treatment of headaches, coughs, infections of upper respiratory tract, kidney malfunction and to eliminate toxins (Evans *et al.*,2007). It is also known the leaves of basil are suitable for the treatment of cough and pain (Basilico and Basilico, 1999). The latter use was based on observational impression rather than on clinical or experimental studies. Therefore, the aim of the present study was to investigate the analgesic and the sedative effect of different doses the alcoholic extract of *Ocimum basilicum* in male rats. So the aim of this study is to evaluate the analgesic and sedative effect of alcoholic extract of *Ocimum basilicum* leaves and to investigate which dose of the extract is more potent.

MATERIAL AND METHODS

PLANT MATERIALS AND EXTRACT PREPARATION:

Fresh *Ocimum basilicum* leaves were purchased from a local market in al-kut city. Later these plant leaves were washed under tap water, and then dried in room temperature at shade. The dried leaves were grinding to a fine powder by an electrical grinder. The plant classification was done in the Ministry of Agriculture/ State Board for Seeds Testing and Certification S.B.S.T.C in Abu Graib /Baghdad. Organic solvent extraction of the *Ocimum basilicum* leaves was carried out by using ethanol (95% ethyl alcohol) which is considered as very effective in extracting the active ingredients of the plant according to method described by (Effraim *et al.*, 2000) this was done by using Soxhlet apparatus. 50 gm of plant leaves powder was put inside the thumble and 500 ml of 95% ethanol was put inside the flask. The extraction was carried out for 24 hours by heating temperature that kept the solvent at 50-60 C° until a clear and colorless solvent appeared in the extracting unit. After that, the extract was dried by using an electric oven at temperature 40-45 C° until dry extract was obtained. The dry extract was placed in an incubator under 38-40 C° for complete dryness of the sample .The final extract was kept frozen at –20 C° until use.

ANIMALS:

Fifty mature male Albino Wister rats with average weight of 280-300g and age of 10-12 weeks were used in this study; all animals were obtain from the central animal house of collage of veterinary medicine university of Baghdad, animals in all stages of the experiments were housed in plastic cages in a conditioned room (22-25 °C) also had free access to standard pellet diet throughout the experimental period.

EXPERIMENTAL DESIGN:

a-STUDY THE ANALGESIC EFFECT OF *OCIMUM BASILICUM* ALCOHOLIC EXTRACT (FORMALIN TEST).

Formalin test was carried out in this experiment to investigate the analgesic effect of *Ocimum basilicum* alcoholic extract. Formalin test is important in studying the effect of analgesic after acute long-lasting pain (Dubuisson and Dennis, 1977; Francesca, 2004). 20 male rats divided to four

groups (each of 5 rats) first group (control group) exposed orally with distill water, second group exposed orally with Diclofenac 0.71mg/kg before injection of formalin, third group exposed Oral with *O.B.E* at a dose 50mg/kg B.W (Sirhan, 2011) before injection of formalin, fourth group exposed Oral with *O.B.E* at a dose 50mg/kg B.W before injection of formalin. 5% formaldehyde was used as described (Dubuisson and Dennis ,1977). Each rat received 10µl of formalin subcutaneously into the right hind paw by using insulin syringe with a 30-gauge needle. Immediately after formalin injection, animals were placed individually in open roof cage (50×50×30 cm) of flat floor to allow clear observation. Prior to this procedure, each rat was allowed for 5 minutes to adapt the testing box and left freely moving and exploring.

The formalin test which is sensitive for various classes of analgesic drugs has two distinct phases, reflecting different types of pain. The early phase (initial pain) that occurs about 5 minutes after formalin injection reflects a direct effect of formalin on nociceptors (neurogenic pain) whereas the late phase that occurs between 15 and 40 minutes following injection reflects tissue injury or inflammatory pain (Elisabetsky *et al.*,1995). An interphase period depression to pain response was noticed where a decrease in pain behavior occurred between (5) and (15) minutes after injection (auto analgesia). In the formalin test, several mediators such as histamine, kinin, serotonin and prostaglandins are released from damaged cells which take part in the inflammatory response and are able to stimulate nociceptors and induction of pain (Le Bars *et al.*,2001).

b- STUDY THE SEDATIVE EFFECT OF *OCIMUM BASILICUM* ALCOHOLIC EXTRACT.

i. PENTOBARBITAL SLEEPING TIME TEST:

This study was conducted according to that done by (Gilani and Janbaz , 1995) on Webster albino rats. 15 male rats were divided to three groups (5each), the rats of first group exposed orally with distill water and after 1 hour injected intra peritoneally with pentobarbitone 35mg/kg B.W. While, second group exposed orally with 50gm/kg B.W of *Ocimum basilicum* alcoholic extract and after 1 hour injected intra peritoneally with pentobarbitone 35mg/kg B.W. Third group, exposed orally with 100gm/kg B.W of *Ocimum basilicum* alcoholic extract and after 1 hour injected intra peritoneally with pentobarbitone 35mg/kg B.W. and the time of sleeping were recorded for each group.

ii. OPEN FIELD TEST:

The Open Field Test (OFT) is an experiment used to assay general locomotor activity levels and anxiety in rodents in scientific research (Hall and Ballachey, 1932) this test developed by Calvin S. Hall to test emotionality of rodents (Victor, 1969). The open field test (OFT) is a commonly used qualitative and quantitative measure of general locomotor activity and willingness to explore in rodents (Stanford, 2007). Changes in these measures are often used to assess the sedative or stimulant effects of pharmacological agents. This basic behavioral assessment is used in almost every study involving rodent behavior. 15 male rats used in this parameter divided to three groups (5each); first group exposed to 1 ml of distilled water only and considered as control group, Second group exposed 50mg/kg B.W of *Ocimum basilicum* alcoholic extract, third group exposed 100mg/kg B.W of *Ocimum basilicum* alcoholic extract. the measurement taken after 1 hour from exposure of extract for each rat individually in a wooden box which setup manually and it have surrounding walls to prevent escape, the floor of the wooden box marked in a grid for a 16 square, each square dimensions are (15x15cm). The observation recorded for five minutes as the following:

- a) Number of squares or lines cross that each animal moved over it.
- b) Number of jumping or trying to escape.

RESULTS AND DISSCUSION

At the early phase of formalin test the oral exposure of O.B.E at a dose 100mg/kg B.W (T3 group) cause the higher significant attenuatetion to the nociceptive response value (33.82 ± 1.27) compared to C and T1group values (41.43 ± 1.12 and 38.20 ± 1.61). On the other hand, at the late phase of the formalin test, diclofenac group (T1) recorded the highest significant reduction in the nociceptive response value (20.63 ± 1.51) compared to the other groups. While (T3and T2 group) showed slight attenuatetion in nociceptive response values (37.12 ± 2.13 and 42.34 ± 2.24) respectively compared to T1 group value (20.63 ± 1.51).

Within group, result of the C group and T2 group showed that the late phase recorded a significant elevation in the nociceptive response when compared to the each group corresponding early phase. Inversely, T1 group showed a significant reduction in the nociceptive response value (20.63 ± 1.51)

at late phase when compared to early phase value (38.20 ± 1.61) of the same group. While, T3 group showed no significant difference between the nociceptive response value of early phase and late phase (33.82 ± 1.27 and 37.12 ± 2.13) respectively (table 1).

In addition to the sedative effect of *Ocimum basilicum* leaves extract (table 2) pointed out to an elevation in time of sleeping in both treated group. But the G3 group which treated with 100 mg /kg BW of *Ocimum basilicum* leaves extract recorded highest significant elevation in the mean value (81.40 ± 3.69) when compared to (G2 group) with mean value (57.60 ± 2.69) which treated with 50 mg /kg BW of *Ocimum basilicum* leaves extract and control group with mean value (48.40 ± 4.63) (table 2).

Table 1: Nociceptive response in male rats treated orally with the alcoholic extract of *Ocimum basilicum* leaves, Diclofenac. (Formalin test).

Groups \ Phases	Nociceptive response (Number of licking & flinching)	
	early phase (0-5)minutes	late phase (15-45)minutes
C (control group) Oral administration of distill water before injection of formalin	41.43 ± 1.12 Ab	56.58 ± 1.62 Aa
T1(treated group) Oral administration of Diclofenac at a dose 0.71mg/kg B.W before injection of formalin	38.20 ± 1.61 Ba	20.63 ± 1.51 Db
T2 (treated group) Oral administration of <i>O.B.E</i> at a dose 50mg/kg B.W before injection of formalin	36.23 ± 0.94 BCb	42.34 ± 2.24 Ba
T 3 (treated group) Oral administration of <i>O.B.E</i> at a dose 100mg/kg B.W before injection of formalin	33.82 ± 1.27 Ca	37.12 ± 2.13 Ca

- ❖ L.S.D. = 4.32, n =5.
- ❖ Figures represent mean \pm standard error.
- ❖ Different capital letters represent significant difference between groups vertically at $p < 0.05$.
- ❖ Different small letters represent significant difference within phase horizontally at $p < 0.05$.

Table 2: *Effect of alcoholic extract of Ocimum basilicum leaves (50, 100 mg/kg BW) on time of sleeping induced by pentobarbitone in male rats.*

Groups	Time of sleeping
G1 group Exposed orally with distill water and after 1 hour injected intra peritonealy with pentobarbitone 35mg/kg B.W.	48.40 \pm 4.63 b
G2 group exposed orally with 50gm/kg B.W of <i>O.B.E</i> and after 1 hour injected intra peritonealy with pentobarbitone 35mg/kg B.W.	57.60\pm 2.69 b
G3 group exposed orally with 100gm/kg B.W of <i>O.B.E</i> and after 1 hour injected intra peritonealy with pentobarbeton 35mg/kg B.W.	81.40\pm 3.69 a

- ❖ L.S.D. =17.8, n=5.
- ❖ Figures represent mean \pm standard error.
- ❖ Different letters represent significant difference between groups at $p<0.05$.

Furthermore, within open field test, treatment with 100gm/kg B.W of *O.B.E* (G3 group) recorded significant reduction in the mean values (5.80 \pm 0.58 and 1.40 \pm 0.24) of movement activity at the both behaviors measurement (no. of square which crossed and no. of escape jumping) when compared with G2 mean values (11.80 \pm 0.86 and 3.00 \pm 0.32) and G 1 mean value (17.40 \pm 1.03 and 7.20 \pm 0.58) respectively. While the G2 group which treated with 50gm/kg B.W of *O.B.E* recorded a slight significant reduction in the mean values of the both behaviors measurement (no. of square which crossed and no. of escape jumping) when compared with G 1 mean values (17.40 \pm 1.03 and 7.20 \pm 0.58) respectively.

Table 3 : *Effect of alcoholic extract of Ocimum basilicum leaves (50, 100 mg/kg BW) on the movement activity in open field test.*

Behavioral measurements Groups	No. of square which crossed	No. of escape jumping
G1 group (Control group) Exposed orally with distill water before 1h from test	17.40±1.03 a	7.20±0.58 a
G2 group Exposed orally with 50gm/kg B.W of <i>O.B.E</i> before 1h from test 35mg/kg B.W	11.80±0.86 b	3.00±0.32 b
G3 group Exposed orally with 100gm/kg B.W of <i>O.B.E</i> before 1h from test 35mg/kg B.W	5.80±0.58 c	1.40±0.24 c

- ❖ L.S.D of (no. of square which crossed) bar = 3.84
- ❖ L.S.D of (no. of escape jumping) bar = 1.23
- ❖ n =5.
- ❖ Figures represent mean ± standard error.
- ❖ Different letters represent significant difference between groups at p<0.05.

The results of the present study demonstrate that alcoholic extract of *Ocimum basilicum*, have a significant effect on pain in the current antinociceptive model in rat (formalin test), these results also showed that the analgesic effect of alcoholic extract was in the range of 50 to 100mg/kg. But the dose of (100mg/kg) was the most effective and more potent and seems to qualified the effect of analgesic agent (Diclofenac). In formalin test, the centrally acting drugs such as narcotics inhibited both phases equally, while peripherally acting drugs only inhibited the second phase (Shibatta *et al.*, 1989). It is also well known that the formalin model may involve sensorial C-fibers (Heapy *et al.*, 1987) in early phase and a combined process generated by peripheral inflammatory tissue and functional changes in the dorsal horn in late phase (Dalal *et al.*, 1999).

In fact, the effect of the extract on both phases of formalin test showed that they contain active analgesic principles acting both centrally relates to antagonistic action of the nociceptors (neurogenic pain) and peripherally (inhibitory actions or released prostaglandins) (inflammatory pain). On the other hand, our result showed that diclofenac inhibit the nociceptive stimuli in both

phase in the formalin test especially in late phase due to its well known analgesic and anti-inflammatory effect (Rang *et al.*, 2003). While, the sedative effect of *Ocimum basilicum* alcoholic extract was confirmed by the significant increase in pentobarbitone sleeping time in a sleeping time test and suppression in the movement activity in open field test specially after pretreatment with the alcoholic extract at the dose 100gm/kg B.W.

There is always a question that which of the active components of whole extract of a medicinal plant is more important. There are many reports about the beneficial effects of *Ocimum basilicum* and their compounds. In *Ocimum basilicum* it has been reported that three main terpenes including linalool, 1,8-cineol, and eugenol comprise the most important components of the extract (Ismail, 2006). However, the centrally acting of *Ocimum basilicum* may contribute to the modulation of glutamergic and GABAergic transmission which is one mechanisms of action of monoterpenes (Szabadiss and Erdelyi, 2000).

The second major terpene is cineole present in the essential oil of *Ocimum basilicum*. It has been reported that 1,8-cineole exerts anticonvulsant activity, potentiates pentobarbitone sleeping time, and has an inhibitory effect on locomotor activity (Santos and Rao, 2000).

Also it's well documented that the most abundant constituent of the *Ocimum basilicum* alcoholic extract was linalool. Linalool may have analgesic effect through suppression of voltage-gated currents (Narusuye *et al.*, 2005). Additionally, it has been reported that linalool has a locomotor inhibitory action as well as hypnotic action (Robbers *et al.*, 1996).

Eugenol also reported is a one of the *Ocimum basilicum* essential oil (Aoshima and Hamamoto ,1999).Reported that eugenol action through potentiation of binding of GABA to its receptor and by increasing the affinity of these receptors to bind GABA. , it has been reported that eugenol has anesthetic, sedative, and muscle relaxant effects (Boissier *et al.*,1967).Thus, when GABA is a one of the inhibitory neurotransmitters (Goodman and Gilmans, 2001), the current study suggested that the activation of GABA neurotransmitter by eugenol may attributed to inhibition of the nociceptive response at the both phases in formalin test with a degree of sedation which clarified in pentobarbitone sleeping time test and open field test.

Flavonoids were also documented (Sirhan, 2011), as a main compound in *Ocimum basilicum* alcoholic extract. The flavonoids are known for their antinociceptive and /or anti-inflammatory activity (Meyre-Silva *et al.*, 1999). Flavonoids which belongs to Qurectine group has an inhibitory effect on 5-

LOX (LIPOOXYGENASE) pathway (the main pathway for production of chemical mediators important in pain and inflammatory process) (Ficarra *et al.*, 1995). In our study the extract has inhibitory effects on peripheral antinociceptive (late phase) this effect may be due to components such as flavonoids in *Ocimum basilicum* extract.

Another compound in *Ocimum basilicum* extract are Tannins and Caffeic acid (Sirhan, 2011) , Tannins and Caffeic acid were reported had anti-inflammatory effect by inhibiting of prostaglandins release (Neradil *et al.*, 2003; Seeram *et al.*, 2005), so depending on the above fact these compounds may attributed the peripheral antinociceptive effect in the current study.

Sweet Basil contain large amounts of (E)-beta-caryophyllene, BCP is the only product identified in nature that activates CB2 selectively; it interacts with one of two cannabinoid receptors (CB2), blocking chemical signals that lead to inflammation, without triggering cannabis's mood-altering effects (Rsc.org, 2012).

On conclusion, the analgesic and sedative activities of *Ocimum basilicum* alcoholic extract could be contribute to terpenes, flavonoids, Tannins, Caffeic acid and (E)-beta-caryophyllene as the major constituents.

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دراسة التأثير المسكن والمهدئ للمستخلص الكحولي لنبات الريحان في ذكور الجرذان .

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المستخلص

أنجزت هذه الدراسة على ذكور الجرذان لتقييم التأثير المسكن والمهدئ لجرع مختلفة من المستخلص الكحولي لأوراق نبات الريحان. تمت دراسة التأثير المسكن باستخدام اختبار الفورمالين ، استخدم ٢٠ جرذاً ذكراً قسمت إلى أربع مجموعات (٥ جرذ لكل مجموعة) ، جرعة المجموعة الأولى عن طريق الفم الماء فقط قبل الحقن بالفورمالين ، أما المجموعة الثانية فقد جرعة عن طريق الفم بالدايكلوفيناك بجرعة 0.71 ملغم/كغم من وزن الجسم قبل الحقن بالفورمالين ، وجرعة المجموعة الثالثة والرابعة عن طريق الفم بالمستخلص الكحولي لأوراق الريحان بجرعة (٥٠ و ١٠٠ ملغم/كغم من وزن الجسم) على التوالي. بينما التأثير المسكن فقد تمت دراسته باستخدام اختبار وقت التنويم للبتوباربيتون واختبار الميدان المفتوح، في كل اختبار استخدم ١٥ جرذ قسمت إلى ٣ مجاميع احدهما تعرضت بالماء المقطر فقط بينما المجموعتين الأخرى تعرضت بالمستخلص الكحولي لأوراق الريحان وجرعة (٥٠ و ١٠٠ ملغم/كغم من وزن الجسم) على التوالي.

أظهرت نتائج اختبار الفورمالين وجود انخفاض معنوي في قيمة معدل الاستجابة للألم لمجموعة الدايكلوفيناك خاصة خلال الطور المتأخر من الاختبار بينما المجاميع التي عولجت بالمستخلص الكحولي لأوراق الريحان فقد أظهرت انخفاضاً معنوياً في قيم الاستجابة للألم خلال كلا الطورين من الاختبار (الطور المبكر والمتأخر) فضلاً عن ذلك فإن المجموعة التي عولجت بـ ١٠٠ ملغم/كغم من المستخلص أظهرت أدنى انخفاض بقيمة معدل الاستجابة للألم مقارنة بالمجموعة المعالجة بـ ٥٠ ملغم/كغم.

وفضلاً عن ذلك فإن المجموعة المعالجة بـ ١٠٠ ملغم/كغم أدت إلى أعلى تأثير مهدئ في اختبار وقت التنويم للبتوباربيتون واختبار الميدان المفتوح. أشارت هذه النتائج إلى أن التأثير المسكن والمهدئ للمستخلص الكحولي لأوراق الريحان يعود إلى احتوائه على مركبات فعالة ذات تأثير مسكن ومهدئ وهذا التأثير معتمد على الجرعة.

الكلمات المفتاحية: تأثير مسكن ، تأثير مهدئ ، مستخلص كحولي ، نبات الريحان ، ذكور الجرذان.