Evaluation of Analgesic Activities of *Phoenix dactylifera* L. Leaflets Extracts in Mice

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**Abstract:** The objectives of the present study were to evaluate peripheral and central analgesic activities of *Phoenix dactylifera* L. (Date palm) leaflet extracts using, chemical and thermal methods induced pain in animals. The fresh and dried leaflets of El-Ammi variety were extracted in aqueous and methanol. The results of analgesic tests revealed that both extracts PLAE and PLME, of PD at doses of 25, 100 and 200 mg/kg ip in mice (n=6) for each dose significantly (P<0.0001) and dose dependently decrease in the number of writhes induced by acetic acid 1%. The maximum (100%) percent writhing inhibition was achieved at the dose 200 mg/kg, while the percentage writhing inhibition of the standard drug diclofenac sodium was 78.76%. Likewise, the results of hot plate test revealed that both extracts PLAE and PLME of PD increased in pain reaction time (% elongation). The highest increase occur with 200 mg/kg, i.p. at 180 minutes 160.47 % and 168.06 % respectively, while it was 49.69 % with the standard drug tramadol 40 mg/kg, ip at 30 minutes of treatment. Regarding the result of Maximum Possible Effect (% analgesia) it was 27.69% for PLAE and 34.86% for PLME at the dose of 200mg/kg in 180 minutes and 8.01% for tramadol in 30 minutes of treatment. The peripheral and central analgesic activities of both extracts were superior to diclofenac sodium and tramadol. Seeing that the mechanism of acetic acid, hot plate in induction of writhes and thermal pain and alleviation of the pain by the standard drugs, we believe that the peripheral and central analgesic activities of both extracts may be attributed to suppression of COX and LOX enzymes in the peripheral tissues. As well as, these extracts may abolish the release of endogenous inflammatory mediators, such as histamine, substance P, serotonin and bradykinin. Whilst, the potent central analgesic activity of leaflets extracts might be linked to opioid receptors stimulation alongside serotonin and noradrenaline reuptake inhibition in the brain. In conclusion, PD (date palm) leaflets extracts possess strong peripheral and central analgesic activities which might be related to the presence and synergistic action of various biogenic compounds. Further investigation is required.

**Key words:** Phoenix Dactylifera · Peripheral and Central Analgesic · Diclofenac · Tramadol

**INTRODUCTION**

Pain as defined by the International Association for Study of Pain (IASP) is an unpleasant sensory and emotional experience associated with actual or potential tissue damage and described in terms of such damage [1, 2]. The agents that selectively attenuate pain via acting in the CNS or peripheral pain mechanism without significantly changing consciousness are known analgesics [3] they involve non-steroidal anti-inflammatory drugs (NSAIDs) and opioid agents. The use of analgesic agent primarily targets the sensory mechanisms of pain. Disadvantages of using pain medications are the side effects which limited their uses [4]. Despite importance of opioid analgesics in severe pain, their uses in large doses may cause stupor, drowsiness, respiratory depression and on long term use they induce tolerance and dependence as well as withdrawal syndrome [5]. Different animal models employed for screening of analgesic agents [3, 6]. The models include: thermal (Hot-plat, tail-flick using radiant heat /immersion of the tail in hot or cold water); chemical (Acetic acid-induced writhing test and Formalin test); electrical (Stimulation of tooth pulp and grid-shock test) and mechanical model (Tail or paw pressure tests).
Medicinal plants used for the alleviation of pain in traditional medicine have been shown to possess promising analgesic activities in animal models and can be invaluable sources of new analgesic compounds. *Phoenix dactylifera* Linn (Date palm) belonging to family Arecaceae or palm family is a member of the monocotyledon plant [7]. The genus phoenix includes 17 species, all Phoenix species are dioecious. *Phoenix dactylifera* (PD) is a species of flowering plant and native to North Africa and Middle East of Asia [8]. Accumulating evidence has demonstrated that PD parts as fruit, leaves, pits, spathe possess antioxidant activity in rats [9] anti-inflammatory effect in mice [10]. Also, PD extracts have anti-hyperlipidemic [11] and anti-diarrheal activities in rats [12] and antimicrobial activity [13, 14] while, Phoenix paludosa leaves have analgesic, anxiolytic and antipsychotic activities [15, 16].

Hence the present research was taken up to investigate peripheral and central analgesic effects of PD and to explain the possible mechanism of action in this aspect.

**MATERIALS AND METHODS**

**Collection of Plant Material:** The fresh leaflets of *Phoenix dactylifera* Linn, (El-Ammi variety) were collected from Misurata- Libya, washed thoroughly with running tap water, air dried under shade at room temperature for 40 days to avoid sun constituents degradation. The plant leaflets have been classified by the Department of Botany, Faculty of Science at the University of Misurata as (*Phoenix dactylifera*) leaflets.

**Extraction of Plant Materials:** Air dried leaflets of PD were cut in small pieces ground into a coarse powder in a suitable grinder and divided into four portions for preparation of the extracts as below. All the dried extracts were kept at a low temperature (4-8°C) in air tight container for further uses. The percentage yield was calculated using the formula:

\[
\% \text{ yield} = \left( \frac{\text{Weight of extracted material}}{\text{weight of original plant material used}} \right) \times 100
\]

**Preparation of Aqueous Extract (PLAE):** Two hundred fifty grams of the prepared powder was boiled for 30 minutes in a can using tap water (1:4 w/v). The extract was filtered and concentrated by evaporating the water using dehydrator machine at 70°C for 72 hours.

**Preparation of Methanolic Extract (PLME):** Two hundred fifty grams of the powder was extracted at room temperature, away of light for 48 hrs by macerated method using 700 ml of 99.8% methanol. The extract was concentrated by a rotary evaporator apparatus at temperature not exceeding 60°C. The extract was dried in oven drier at (45°C).

**Chemicals, Drugs and Equipments:** Chemicals and drugs included methanol (99.8%), acetic acid (Scharlan), diclofenac sodium and tramadol. All solutions were prepared immediately before use and the chemicals were of A.R. grade. Eddy's hot plat analgesiometer (Panlab Harvard apparatus), Dehydrator machine (Hummer, Germany), Soxhlet apparatus, analytical balance, oven, rotary evaporator, stainless-steel blender and mixer.

**Animals:** Adult healthy Swiss albino mice (n= 96, weighting 25±5 g) of either sex were used for fulfillment the aims of this study, (For evaluation of analgesic activities). All animals were housed in polypropylene cages having autoclaved wooden shaving beddings, in a room controlled conditions: temperature 24°C ± 2, relative humidity of 55 ± 5 and 12h light/ dark cycles. All animals were fed with laboratory chow and had free access to drinking water. All animals were obtained from the animal’ house of faculty of pharmacy Misurata University, Libya.

**Ethic Statements:** All treatments were in accordance with the animal care guidelines of the Institutional Animal Ethics Committee, Faculty of Pharmacy, Misurata University, Libya.

**Experimental Design**

**Evaluation of Analgesic Activity of PLAE and PLME**

**Peripheral Analgesic Activity (Acetic Acid):** The peripheral analgesic activity of extract was studied using acetic acid (1%) induced writhing model in mice [17, 18]. Forty eight animals were divided into eight groups (n=6 in each); control, standard and six test groups. The animals of three test groups received PLAE, at the doses of 25, 100 and 200 mg/kg, while the other three test groups received PLME, at the same doses. Standard group was administered standard drug (Diclofenac Na) at the dose of 4 mg/kg and the control group was treated with sterile distilled water (DW) at the dose of 1ml/100gm. The test samples, standard drug and DW were administered intraperitoneally 30 minutes prior intraperitoneal injection of 1.0 % acetic acid. After an interval of 15 min, the mice
were individually observed writhing (Constriction of abdomen, turning of trunk and extension of hind legs) for 30 min. The percentage protection against abdominal writhing was used to assess the degree of analgesia and was calculated according to the following formula [19, 20]:

\[
\% \text{ of writhing inhibition} = \frac{\text{mean number of writhes in control} - \text{mean number of writhes in test}}{\text{mean number of writhes in control}} \times 100
\]

\[
\% \text{ writhing} = \frac{\text{mean number of writhes in test}}{\text{mean number of writhes in control}} \times 100
\]

**Central Analgesic Activity (Hot-Plate):** The central analgesic activity of PD leaflets extract was assessed using Eddy's hot-plate analgesiometer [21]. Selected animals were divided into eight groups each comprising of six mice and subjected to different treatments. The first and second groups of animals were treated with sterile DW 1ml/100g and tramadol 40 mg/kg i.p., these agents used as control and reference standard groups respectively. Three groups of mice were treated with PLAE, of PD at a dose of 25, 100 and 200 mg/kg i.p., respectively. The other three test groups were treated with PLME of PD at a dose of 25, 100 and 200 mg/kg, i.p. respectively. The heated surface of a digital hot-plate analgesiometer was maintained at 55 ± 1°C. The mice were placed gently on the heated surface of the plate and the time required for paw licking or jumping (Pain reaction time PRT or pain latency period i.e. time between pain stimuli application and pain perception) was recorded with stop watch at 0, 30, 60, 90, 120 and 180 minutes. The reaction was taken as the interval from the instant the animal reached the hot plate surface until the moment animal licked its feet or jumped out. To minimize the damage to the animal paw, the cut-off time for latency of response was taken as 60 seconds. Evaluation: The mean reaction time for each treated group was determined and compared with that obtained for each group before treatment (Basal or time 0). Percentage increase (% elongation) in pain reaction time (PRT %), was calculated, using the formula [22] while, the Maximum Possible Effect (MPE) or percentage analgesia (% analgesia) was calculated using the formula as below [23].

\[
PRT \% = \frac{\text{PRT}_t - \text{PRT}_{t_0}}{\text{PRT}_{t_0}} \times 100
\]

where PRTt = pain reaction time at time t and PRT t = pain reaction time at time zero (0 h).

**Peripheral Analgesic Activity (Acetic Acid):** The effect of PLAE and PLME of PD, EL-Ammi cultivar on acetic acid-induced writhing in mice is presented in Table 1. The result showed that both extracts at the dose of (25, 100, 200 mg/kg) and the standard drug diclofenac sodium (4 mg/kg) significantly P<0.001 reduced abdominal number of writhes in mice when compared to the –Ve control group, reducing the mean number of writhing from 78.16 ± 4.85 in the –Ve control group to 0.00% at the dose of 200 mg/kg of both extract. The reduction was in a dose dependent manner. Also, the PLAE caused a dose dependent inhibition of writhing by 62.25, 98.72 and 100% respectively. In addition, the percentage writhing inhibition of PLME was dose dependent too, 87.84, 91.55 and 100% respectively. While the percentage of writhing inhibition for standard drug was found to be 78.76% at a dose of 4 mg/kg b.wt. The results were statistically significant as compared to DW and to diclofenac sodium at the level of P<0.0001.

**Central Analgesic Activity (Hot-Plate):** The results of central analgesic activity of both extracts PLAE and PLME on the hot plate method are presented in (Fig. 1 and 2). The hot plate test is useful in the elucidating centrally
Table 1: Analgesic effect of PLAE and PLME of PD on Acetic Acid-induced writhing in mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose mg/kg I.P.</th>
<th>Mean number of writhing (% writhing)</th>
<th>% writhing inhibition (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW+AA</td>
<td>1ml/100g</td>
<td>78.16±4.85 (100)</td>
<td>0.00</td>
</tr>
<tr>
<td>Diclo+AA</td>
<td>4</td>
<td>16.60±2.50 (21.23)</td>
<td>78.76 (P &lt; 0.0001)</td>
</tr>
<tr>
<td>PLAE+AA</td>
<td>25</td>
<td>29.50±4.26 (37.74)</td>
<td>62.25 (P &lt; 0.0001, 0.0036)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.0±0.51 (1.33)</td>
<td>98.72 (P &lt; 0.0001, 0.0001)</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.00±0 (0.00)</td>
<td>100 (P &lt; 0.0001, 0.0001)</td>
</tr>
<tr>
<td>PLME+AA</td>
<td>25</td>
<td>9.50±2.09 (12.15)</td>
<td>87.84 (P &lt; 0.0001, 0.0001)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>6.6±0.02 (8.44)</td>
<td>91.55 (P &lt; 0.0001, 0.0024)</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.00±0 (0.00)</td>
<td>100 (P &lt; 0.0001, 0.0001)</td>
</tr>
</tbody>
</table>

DW= distilled water, AA= acetic acid, Diclo= diclofenac sodium, PLAE= palm leaf aqueous extract, PLME= palm leaf methanolic extract. Values are expressed as mean ± SEM (Standard Error Mean); n=6 mice.
d = Significant as compared to DW, s = Significant as compared to standard drug (diclofenac sodium)

mediate anti-nociceptive responses, which focuses mainly on changes above the spinal cord level. The results showed that the treatment of mice with tramadol 40 mg/kg, i.p. increased significantly the latency response in the hot plate test at 30, 60, 90, 120 and 180 minutes after treatment as compared to basal time of DW and basal time of tramadol. On the other hand by comparing the pain reaction time PRT (% elongation), basal time and post treatment, the PLAE and PLME at the doses of 25, 100 and 200 mg/kg, i.p. significantly and dose dependently increased the pain reaction time (PRT) in 30, 60, 90, 120 and 180 minutes after treatment as compared to basal time of PLAE, DW and tramadol. The Maximum Possible Effect (% analgesia) reveals that the PLME at the dose of 200 mg/kg 34.86% in 180 minutes had a better analgesic effect, while for PLAE at the same dose was 27.69% in the same time and tramadol 40mg/kg in 30 minutes after treatment it was 8.01% (Fig. 3).
DISCUSSION

Analgesic activity of the *P. dactylifera* leaflet extracts was evaluated by two widely used models namely acetic acid induced writhing and hot-plate method. In acetic acid-induced writhing test, PLAE and PLME of PD produced a significant ($p<0.0001$) inhibition of writhing response as compared to control group in a dose dependent manner and the maximum inhibition (100%) of writhing was found at 200mg/kg dose with both extracts, while the percentage writhing inhibition of the standard drug diclofenac sodium was (78.76 %). The current results are in consonance with Ashura *et al.* [15] and Jarayaman *et al.* [24] who reported that the ethanolic extract of leaves of *Phoenix paludosa* showed dose dependent decrease in the total number of writhing induced by acetic acid in mice. From our observation it can be suggested that the aqueous and methanolic extract of the PD leaflets, have potent analgesic activity. It has been postulated that acetic acid produce pain via stimulation of chemo sensitive nociceptors and acts indirectly by irritation of the visceral tissues, which lead to stimulating the release of endogenous mediators, such as serotonin, histamine, bradykinin, substance P and prostaglandin E2 (PGE2) and PGE2α in peritoneal fluids, as well as lipooxygenase products, which stimulate the nociceptive neurons sensitive to non-steroidal anti-inflammatory drugs (NSAIDs) [25-27]. Therefore, the results of the acetic acid induced writhing strongly suggests that the mechanism of PLAE and PLME may be linked partly to the inhibition of the enzymes cyclooxygenase (COX) and or lipooxygenase (LOX) in the peripheral tissues, thereby reducing these enzyme products as prostaglandins and leukotrienes. Furthermore, the constituents of these extracts may be act in synergistic manner and abolish the release of endogenous inflammatory mediators, such as histamine, substance P, serotonin and bradykinin.

Hot plate is useful in the elucidating centrally mediated anti-nociceptive responses, which focuses mainly on changes above the spinal cord level [28]. In the hot plate model the paw of mice are very sensitive to temperature at 50-55 ±1°C [29]. In this model used, the data illustrated in (Fig. 2) showed that pretreatment of mice with PLAE and PLME of PD, El-Ammi cultivar resulted in significant and dose dependent increased in pain reaction time (PRT). The highest increase (%) elongation) occurs with 200 mg/kg of both extracts at 180 minutes (160.47 and 168.06%) respectively. The highest increase in reaction time with tramadol 40mg/kg was 49.69 % at 30 minutes. Increased in reaction time or latency period indicates the level of analgesia of drug or extract [30]. In the current study the maximum possible effect MPE (% analgesia) revealed that the PLME at the dose of 200 mg/kg 34.86% in 180 minutes had a better analgesic effect and PLAE at the same dose and time was 27.69%. While, the MPE of tramadol 40mg/kg was 8.01% in 30 minutes after treatment Figure 4. This is in line with the study done by Maryam *et al.* 2015 on anti-inflammatory and analgesic activities of aqueous extract date palm fruit in rats, which reveals that oral administration of 500 mg/kg of the extract is effective in the reduction of pain than 300 mg of the same extract and 300 mg paracetamol [31]. Vyawahare and his colleagues
2009 [32] reported that the methanolic extract of PD fruit at doses of 100 and 300 mg/kg produced significant increase in the reaction time in the hot plate at 60 and 120 min after administration to mice (P<0.01), which was most marked at a dose of 300 mg/kg, whereas PD extract 30 mg/kg showed a significant increase in reaction time at 60 min (P<0.05) but was insignificant at 120 min after the administration. Opioid drugs act via binding with four distinct receptors like mu (µ), delta (δ), kappa (κ) and orphan (ORL-1) in pre and post synaptic membrane as well as inhibiting neurotransmitter release [33, 34]. The µ-receptor stimulation is associated with analgesia and has been shown to be potent in regulating thermal pain [35]. Stimulation of µ-receptor is also associated with non-analgesic events such as respiratory depression (µ), physical dependence, euphoria, hypotension, miosis and constipation [36]. The δ-receptor is the strongest binding site of the endogenous enkephalins. Morphine and other commonly used opioid analgesics also bind to δ-receptor and act as an agonist much like do with µ-receptor. The kappa receptor induces analgesia without the dangerous and unwanted side effects that the mu and delta receptors are associated with. However there are not any selectively strong agonists to this receptor as of now. Tramadol is a centrally acting analgesic agent that is structurally related to morphine. It is mainly used for the alleviation of moderate to severe pain [37]. Tramadol consists of two enantiomers (+ and -); which contribute to analgesic effect. Both enantiomers have two distinct but complementary mechanisms of action; the (+) tramadol is a selective for µ-opioid receptor and inhibits serotonin reuptake whereas the (-) tramadol mainly inhibits noradrenaline reuptake [38, 39]. Tramadol has rarely been associated with respiratory or cardio-vascular depression in humans, even in large doses and this set it apart from other opioid receptor agonists. In addition, a minimal incidence of constipating effects and low likelihood for development of tolerance and dependence make this a valuable agent for clinical use [40, 41].

In the previous studies the phytochemical screening of P. dactylifera leaves showed the presence of many bioactive constituents like, alkaloids, flavonoids, phenols, saponin, terpenoids, carbohydrates and tannins [9, 42]. These compounds can exhibit extensive pharmacologic and other activities. Saponins, triterpenoids [42, 43] and tannins [44] showed the analgesic properties. Flavonoids and tannins inhibit number of enzymes such as cyclooxygenase, lipoxygenase, phosphodiesterase, xanthine oxidase and C-2-ATPases [45, 46]. Other researcher reported that, the anti-nociception effects were attributed to alkaloids [47]. The analgesic activity of PLAE and PLME of PD leaflets is superior to tramadol. Therefore, taking all these data together we believe that the probable mechanisms of potent central analgesic activity of leaflets extract is mediated by stimulation of certain opioid receptors and inhibition of neurotransmitters reuptake in the brain. These effects may be attributed to the presence of various bioactive constituents and their synergistic action.

CONCLUSIONS

On the basis of these findings, it might be inferred that aqueous and methanolic extracts of phoenix dactylifera leaflets have stronger peripheral and central analgesic activity than certain NSAIDs and opioid agents. These activities were related to the presence of flavonoids, alkaloids, tannins, steroids, terpenoids and other biogenic compounds. The potent activity might be due to synergistic action of the constituents.

Conflicts of Interest: The authors report no declaration of interest.

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