

# Anti-Inflammatory and Analgesic Activities of Aqueous Extract Date Palm (*Phoenix Dactylifera L*) Fruit in Rats

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**Abstract: Objective:** The purpose of this study is to evaluate the anti-inflammatory and analgesic activities of aqueous extract of date palm fruit in rats.

**Design and Setting:** The study was an experimental research carried out at the medical laboratory of University Sultan Zainal Abidin, Kuala Terengganu, Malaysia.

**Methods:** Four groups of rats were conveniently assigned with six rats per group based on law of diminishing return. The groups received different interventions of 500mg of aqueous extract of date palm, 300mg of aqueous extract of date palm, Paracetamol and normal saline. Hot tail flick test was used to evaluate the analgesic activity of the rats, while 2.5% croton-oil was used to assess the anti-inflammatory activity in each group.

**Results:** Statistical significant median difference of the analgesic and anti-inflammatory effect of date fruit aqueous extract (DFAE) was revealed at ( $p=0.001$ ,  $\chi^2=17.84$ ) and ( $p=0.002$ ,  $\chi^2=14.80$ ) respectively. In addition, the significant difference of the analgesic effect of DFAE lies between normal saline and 500mg DFAE ( $p=0.001$ ,  $\chi^2=15.250$ ). Likewise the significant difference of the anti-inflammatory effect of DFAE lies between group that received paracetamol and 300mg DFAE ( $p=0.027$ ,  $\chi^2=0.027$ ), paracetamol and normal saline ( $p=0.001$ ,  $\chi^2=15.717$ ), and 500mg DFAE and normal saline ( $p=0.031$ ,  $\chi^2=11.467$ ).

**Keywords:** Date Palm fruit, inflammation and pain.

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## 1. INTRODUCTION

Pain is an unpleasant, emotional and sensory experience as a result of tissue damage or injury (Woolf, 2010). Pain is part of the inflammatory process (Mironidou-Tzouveleki, 2010). Inflammatory process occurs in order to fight, remove and repair damaged tissue after injury while pain develops in order to protect the organs from further damage (Warren, 2007). The other three cardinal signs of inflammation are hotness, redness and loss of function. (Libby, 2007)

Anti-inflammatory conventional drug treatments are limited in their effectiveness in managing the incidence and outcome of many inflammatory disorders nevertheless NSAIDS and narcotics are the two pharmacological drugs that are available in the management of pain and inflammation with a serious adverse effect as revealed from a research study (Punchard et al., 2004). They also present a significant number of side-effects in patients and recently, it has been shown that non-steroidal anti-inflammatory agents may even attenuate the healing process (Mansouri et al., 2014).

Natural remedies have been a thing of interest to researchers attempting to find lasting and convincing remedy to diseases and disorders over the years. Date palm is an ancient plant used in folk medicine for the treatment of various diseases and disorders (El Hadrami & Al-Khayri, 2012). It is consumed as principal foods and ingredients that form the basis of the diet of the people of the middle east and the world at large (Al-Juraisy et al., 2010; Friedman et al., 2010). It has been reported to have high nutritional value and health benefits (Al-Farsi et al., 2007; Sadiq et al., 2013) because it is packed with essential nutrients, vitamins and minerals that are required for development, growth and overall well being (Ali et al., 2014; Al-orf et al., 2012). Date palm fruit is a commonly available fruit which is cheap and affordable; being a natural remedy comes with little or no side effect (Rahmani et al., 2014)

Literature has been reported that date palm possesses neuro-protective (Pujari et al., 2011), cerebro-protective (Kalantaripour et al., 2012), hepato-protective (Ragab et al., 2013) properties, and so much more. This research is primarily based in finding a remedy with the least or no adverse side effect in the management of pain and inflammation. However in understanding the anti-inflammatory and analgesic activity of date palm fruit is yet to be known and understood.

## 2. MATERIALS AND METHODS

### Confirmation of date fruit and preparation of aqueous extract:

The date palm was purchased from Hillwah Trading SDN BRD, Kuala Terengganu, Malaysia which has a storage system which can maintain the quality and hygiene of the fruit. The date was analyzed and confirmed by HERBARIUM UNISZA with herbarium number 0028.

The pit was separated from the date palm fruit flesh and washed; it was then put into an oven at 50<sup>0</sup>c and allowed to dry. The dried date palm fruit was then blended in a blender. About 300g of the blended date palm fruit was mixed with 1500ml of normal saline in a conical flask which was then placed in a water bath at 100<sup>0</sup>c for 30 minutes. It was then removed and then passed through a sieve and then through a filter paper. It was then put in a rotary evaporator at 40<sup>0</sup>c. The crude extract was then placed in an oven at 50<sup>0</sup>c and allowed to dry up. 300mg/kg and 500mg/kg will be prepared with normal saline in the ratio 1:2 (v/w).

### Drug dosage for required animal:

Doses of extract were selected and determined according to previous study of Elberry and colleagues' with some modification in which in their research they use date pollen suspension and extract which show a dependent dose (Elberry et al., 2011) while in this research date palm fruit aqueous extract was used to see if it will as well produce a dependent dose.

60ml orange flavored children paracetamol (Smithkline Beecham Philippines) purchased from Farmasi Baiduri Berhad SDN, Kuala Terengganu Malaysia was mixed with normal saline in fivefold. The syrup contains 50mg per ml of paracetamol. Therefore the diluted 300ml contain 3000mg of paracetamol. The dosage was calculated according to the weight of the rat, as the company dosage is 15mg/kg. Normal Saline (0.9% Sodium Chloride, Sigma-Aldrich, USA).

### Sample size and design:

This research is an experimental cross sectional research design. Four groups of rats were conveniently assigned with six rats per group based on law of diminishing return (Charan & Kantharia, 2013). This method is called the resource equation method. In this method a value E is measured which is the degree of freedom of analysis of variance (ANOVA).

$E = \text{Total number of animals} - \text{Total number of groups}$

Group A was given normal saline (0.9%, w/v), (Safi et al, 2011). This served as the negative control. Group B was given 300mg/kg rat of the aqueous extract of date palm fruit by oral gavage. Group C was given 500mg/kg rat of the aqueous extract of date palm fruit. Group D was given 15mg/kg of paracetamol by oral gavage. This served as the positive control. They were fed with the extract for three weeks.

### Selection of animals, caring and handling:

Sprague-Dawley, 60 days old rats, were housed six per cage in four cages and they were put under controlled condition of photoperiod controlled room (light: dark: 12h: 12h:), humidity (60+-5%), temperature (22+-2 degrees Celsius), with free access to food and fresh treated water which free from any harmful impurities. All experiments were conducted in

accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and were approved by the local ethical committee (University Sultan Zainal Abidin). Approval letter No.UniSZA.0/3/374-3 (01)

**Analgesic effect via hot tail flick test:**

The analgesic activity was determined in Sprague dawley rats according to the method of (Rizwan et al., 2003). All drugs were given orally for 21 days, on the last day the hot tail flick test was done whereby each rat was weighed with its tail marked about 2cm from the distal end. The rats were put in a restrainer with its tail outside the restrainer, water was heated at 50<sup>0</sup>C, and kept constant at that temperature, rat tail was inserted into the heated water, the time taken for the rat to flick its tail or its body sudden jerk due to pain was recorded as the first reading. Time gap for each test was 1min. reaction time was recorded based on the average of the readings. The maximum time was fixed to 15sec to avoid tissue damage. The percentage analgesia was calculated using the formula as below

$$\% \text{ analgesia} = \text{MPE} = \frac{(\text{TL} - \text{BL})}{(\text{ML} - \text{BL})} \times 100$$

Where MPE = Maximum possible effect

ML= Maximum latency or cut off time

TL = Test latency

BL = Basal latency or control latency (Swapnil and Smita, 2012).

**Anti-inflammatory effect via croton oil ear edema:**

Anti-inflammatory activity was determined according to Florentino and colleagues' (2013) .The rats were treated with paracetamol, aqueous extract of date palm fruit and vehicle, for three weeks. The inflammation was induced by the application of 2.5% (v/v) croton oil solution in acetone (2.5%) on the inner surface of the right ear; the same volume of acetone was applied to the left ear. After 6 hours the rats were sacrificed and segments of both ears were removed. The inflammation was measured by the difference between the weights of the segment.(Florentino et al., 2013). The percentage oedema inhibition was calculated using the formula below

$$I\% = [ 1 - \Delta Wt / \Delta Wc ] \times 100$$

Where I% = percentage edema inhibition

ΔWt = change in weight of the ear in the treated

ΔWc = change in weight in ear in the control (Boukhatem et al., 2014)

**Statistics and data analysis:**

The results were analyzed for statistical significance using Kruskal Wallis one way ANOVA; likewise pair wise comparison test was conducted to determine the significant between group comparisons. A p-value <0.05 was considered significant. Computer statistical package SPSS (Version 20) was used for analysis.

**3. RESULTS**

**Table 1: Comparison of medial ear weight between four groups and descriptive statistics of %I according to treatments**

Treatments	Dose	Median (IQR)	% I	$\chi^2$ (df)	p – value*
Normal saline	3ml	0.13(0.19)	--		
DFAE	300mg/kg	0.08(0.05)	38	17.84(3)	<0.001
DFAE	500mg/kg	0.05(0.02)	61.5		
Paracetamol	15mg/kg	0.015(0.05)	88.5		

DFAE –Date fruit aqueous extract, I – Edema inhibition \*Kruskal-Wallis one way Anova.

Pair wise comparison test with level of significant set at 0.05, shows that the significant difference lies between group that received paracetamol and 300mg DFAE ( $p=0.027, \chi^2=0.027$ ), paracetamol and normal saline ( $p=0.001, \chi^2=15.717$ ), and 500mg DFAE and normal saline ( $p=0.031, \chi^2=11.467$ ),

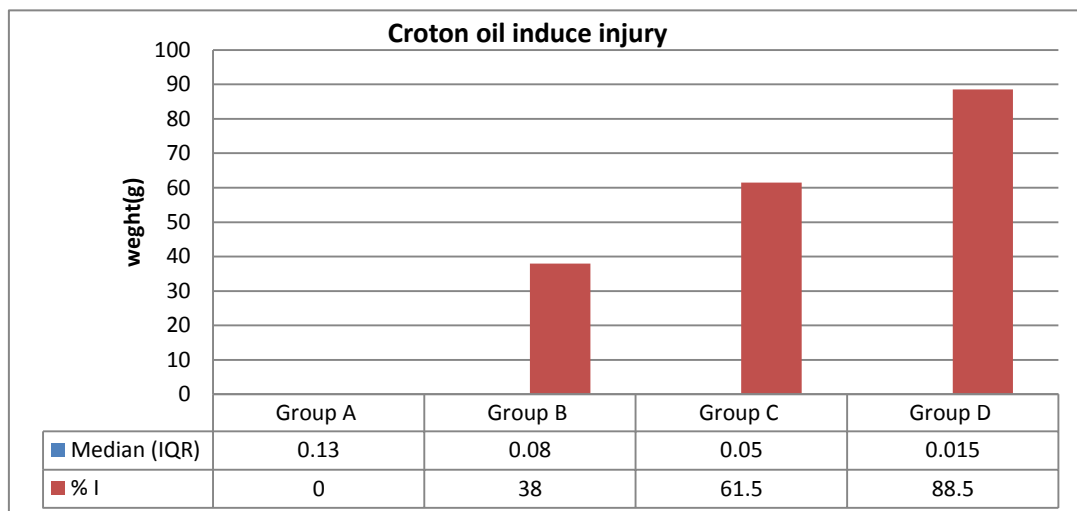


Fig 1: Determination of %I using croton oil induce edema

Table 2: Comparison of tail flick time between four groups and descriptive statistics of %analgesia according to treatments

Treatments	Dose	Median (IQR)	% A	$\chi^2$ (df)	p – value*
Normal saline	3ml	2.0(0.25)	--		
DFAE	300mg/kg	3.5(1.75)	11.54	14.80(3)	<0.002
DFAE	500mg/kg	6.5(2.75)	34.62		
Paracetamol	15mg/kg	4.0(2.00)	16.67		

DFAE –Date fruit aqueous extract, A - analgesia, \*Kruskal-Wallis one way anova test.

Pair wise comparison test , with level of significant set at 0.05, shows that the significant difference lies between normal saline and 500mg DFAE ( $p=0.001, \chi^2 = -15.250$ )

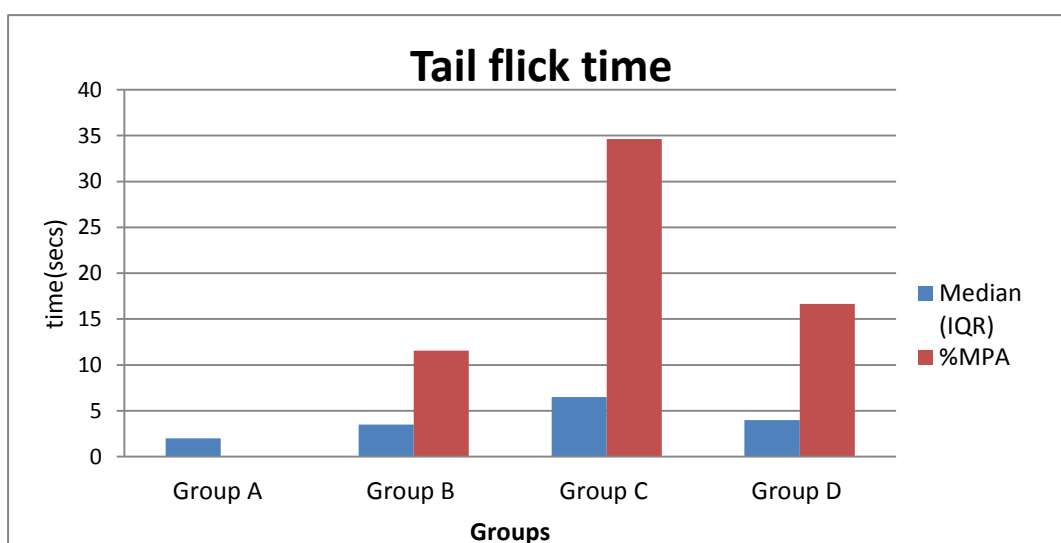


Fig 2: Determination of %analgesia using tail flick test

#### 4. DISCUSSION

Pre treatment with 500mg of aqueous extract of date palm fruit resulted in dose dependent reaction in croton oil evoked ear edema and differed significantly ( $P < 0.001$ ) among different groups of rats (table 1 and fig 1). The result showed that significant difference lies between group of paracetamol and 300mg ( $p = 0.027$ ), group of paracetamol and normal saline ( $p = 0.001$ ) and group of 500mg and normal saline ( $p = 0.031$ ). The descriptive statistics show that paracetamol tend to reduce the inflammation by 43.9% higher than 500mg, 132.9% higher than 300mg while 500mg tend to reduce the inflammation by 61.8% higher than 300mg however 500mg produce a dependent dose. There is every possibility with increase dose of the extract % inhibition can be more than that of paracetamol.

This is in line with the study done by (Mohamed & Al-Okbi, 2004) on the anti-inflammatory effect of 500mg methanolic and water of edible date palm fruit and 500mg of methanolic extract of date palm seed on adjuvant arthritis, which reveals that oral administration of both extract reduce the paw inflammation as well as the swelling throughout the experiment. Likewise it is in line with the study by Elberry and colleagues as they evaluate the anti-inflammatory effect of date palm on ATP which shows that date palm possesses anti-inflammatory activity as it reduce the size of the anterior and posterior lobes of the prostate as well as decreasing the proinflammatory cytokines in an atypical prostatic hyperplasia (ATP) induce rat (Elberry et al., 2011). These finding also support the finding of Mukherjee *et al.*, (2014) as they reported that date palm possesses not only anti-inflammatory effect but also anti-oxidant effect. Research have shown that this effect could be due to the present of antioxidant in date palm (Mukherjee et al., 2014).

Pretreatment with 500mg of date palm fruit aqueous extract resulted in dose dependent reaction in hot tail flick test and differed significantly ( $P < 0.002$ ) among different groups of rats (table 2 and fig 2). The result shows that the significant difference lies between normal saline and 500mg DFAE ( $p = 0.001$ ). Likewise, the descriptive statistics shows that the %analgesia of 500mg is 200.2% higher than 300mg, 108.4% higher than paracetamol, while paracetamol was 44.2% higher than 300mg.

This findings support the study of Alkuran and colleagues as they evaluated the effect of consuming date palm fruit on pregnant women which showed that women who consume date palm fruit four weeks prior to delivery tend to have ease pain during labor as it reduced the pain of labor (Al-kuran et al, 2011). This is also in line with a study done by shabani and colleagues in which they investigated the effect of date extract consumption on thermal hyperalgesia of neuropathic pain in diabetic rats which showed that date extract can not only decrease the thermal hyperalgesia but also can prevent pain resulting from diabetic neuropathy (Shabani et al., 2013).

#### 5. CONCLUSION

The outcome of the study revealed that 500mg of DFAE is effective in the reduction of pain than 300mg and paracetamol while paracetamol tend to inhibit edema more as compared to 500mg and 300mg but the percentage edema inhibition of paracetamol is comparable to that of 500mg of the extract. The effectiveness of the extract could be due to the presence of micronutrients in the date palm fruit especially vitamin C and E as compared to paracetamol, as well as due to the presence of antioxidants present in the fruit as compared to paracetamol. It can be concluded that feeding or oral administration of aqueous extract date palm fruit in high dose proves to be more effective in the reduction of pain and inflammation of an acute injury in rats. Being the available analgesic and anti-inflammatory drugs are associated with serious adverse effects; this research can bring another option to develop safer free from adverse effect and more effective drug in the management of pain and inflammation. By determining the histological severity of the injury can improve and provide deeper understanding of pathophysiology of pains as well as shade more light on the safety and efficacy of the drug candidate and hence develop better pain management modalities and approaches in the future.

#### ACKNOWLEDGEMENT

I wish to thank the Kano State Government of Nigeria and my husband Auwal Muhammad Abdulkareem for the courage and support in the smooth conduct of this study. Likewise, to my humble supervisor Professor Nordin bin Simbak for all the corrections given during the course of the study.

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