



## Fatty acid Composition, Anti-inflammatory and Analgesic Activities of *Balanites aegyptiaca* Seeds in Rats

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### Abstract

The anti-inflammatory and analgesic effects of *B. aegyptiaca* seeds petroleum ether extract was investigated in Wistar albino rats. The anti-inflammatory effect was assessed on acute and chronic models by using carrageenan induced paw oedema and cotton pellet induced granuloma respectively. The analgesic activity of the petroleum ether extract of seeds was evaluated by acetic acid induced writhes. Oral administration of *B. aegyptiaca* seeds petroleum ether extract at dose rate of 2, 4 and 8ml/kg significantly ( $P < 0.05$ ) inhibited carrageenan induced paw oedema in rats. The inhibition was comparable to that observed by indomethacin used as a standard anti-inflammatory drug. In cotton pellet granuloma method, the formation of granuloma significantly ( $P < 0.05$ ) hindered by oral administration of the extract at dose rate of 4 and 8ml/kg compared to the control and standard drug diclofenac sodium. The petroleum ether extract also significantly ( $P < 0.05$ ) reduced the writhes produced by acetic acid compared to control and standard analgesic drug diclofenac sodium. Moreover, the acute toxicity study indicated that oral administration of seeds oil in rats did not have any side or toxic effects up to 20 ml/kg except of anorexia in rats received higher doses of the oil. Gas Chromatography analysis of seed oil revealed the presence of capric acid, palmitic acid and oleic acid. This study confirmed the traditional uses of *B. aegyptiaca* seeds as anti-inflammatory and as an analgesic agent which may be attributed to its fatty acids content.

**Keywords:** *Balanites aegyptiaca*, carrageenan, acetic acid, oedema.

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### Introduction

Medicinal plants are considered to be an important source of new chemical substances with potential therapeutic effects (Gupta *et al.*, 2006). The use of the medicinal herbs for

curing diseases has been documented in history of all civilizations. They contain active constituents that are used in the

treatment of many human diseases including inflammation (Das *et al.*, 2013).

Inflammatory diseases are currently treated with steroidal and non-steroidal anti-inflammatory drugs (NSAIDs). Despite their widespread use, NSAIDs are often associated with severe adverse effects; the most common being gastro-intestinal ulcers, bleeding and renal disorders (Nonato *et al.*, 2009 and Sivaraman *et al.*, 2010). Due of the deleterious side effects attributed to the prolonged use of NSAIDs and their ineffectiveness in some cases, the control of inflammation is still a major challenge (Nonato *et al.*, 2009).

*Balanites aegyptiaca* L. (Zygophyllaceae), is locally known *Hegleig* tree and its fruits called *lal'loub*. It is also known as 'Desert date' in English. *B. aegyptiaca* is a small to medium-sized semideciduous tree which attains a height of about 6 m. The tree is found in most and to sub humid tropical savannas of African, all over the Sahel and on many sites of the Sudan savanna, extending from the Atlantic coastline of Senegal to the red sea and Indian Ocean and the Arabian Peninsula (Maydell, 1986).

*Hegleig* is an extremely useful tree which has been utilized over thousands of years. All parts of the tree have medicinal uses including fruits, seeds, barks and roots (Elfeel, 2010; Maydell, 1986). The bark and roots are used as laxatives or tranquilizers (for colic). The bark is used against stomachaches, sterility, mental diseases, epilepsy, yellow fever, syphilis and as a vermifuge. Fruits and leaves, and especially the kernel-oil are applied for rheumatism and bark extracts for toothaches (Maydell, 1986). The oil exhibited anticancer activity, antimutagenic activity against *Fasciola gigantica* -induced mutagenicity, anthelmintic activity against hepatic worms (*Schistosoma mansoni* and *Fasciola gigantica*), antiviral activity against Herpes

simplex virus and antimicrobial activity against selected strains of Gram-positive bacteria, Gram-negative bacteria and *Candida* (Al Ashaal *et al.*, 2010). It is also reported that *B. aegyptiaca* have potent wound-healing, antioxidant (Annan and Dickson, 2008), hepatoprotective (Jaiprakash *et al.*, 2003), anti-inflammatory, analgesic (Gaur *et al.*, 2008) and antidiabetic activities (Mansour and Newairy, 2000).

On the other hand researchers have been isolated huge numbers of active constituents from different parts of *B. aegyptiaca* as reviewed by Chothani and Vaghasiya, (2011). The most important constituent is found to be steroidal saponins (Elfeel, 2010). The kernels contained 45.0 to 46.1% oil and protein (32.4%), oil contains mainly palmitic, steric, oleic and linoleic acids which were the main fatty acids (Chothani and Vaghasiya, 2011). However, kernel contains a xylopyranosyl derivative of saponin present in mesocarp (Staerk *et al.*, 2007). Nine saponin have been also isolated from kernel cake of *B. aegyptiaca* (Chothani and Vaghasiya, 2011)

Scientific researches in the anti-inflammatory and analgesic activities of *B. aegyptiaca* especially on seeds oil extract have not yet been performed. The present study was designed to evaluate the anti-inflammatory and analgesic effects of *B. aegyptiaca* seeds petroleum ether extract using different models in rats and to determine the fatty acids composition of the seeds oil using Gas chromatography.

## Materials and Methods

### Plant materials:

Fruits of *B. eagyptiaca* were purchased from local market in Omdurman, Sudan. The fruits were then identified and authenticated by the botanists in Medicinal and Aromatic Plants Research Institute (MAPRI), National Centre for Research (NCR), Khartoum, Sudan.

### Preparation of the extract:

The fruits of *B. aegyptiaca* were opened and the kernels were separated and powdered using a blender. 200 gm of *B. aegyptiaca* seeds weighed and packed in soxhlet apparatus. The powdered seeds were extracted with petroleum ether (40 – 60) °C to extract oil. The solvent was then collected and evaporated under reduced pressure using rotary evaporator apparatus. The petroleum ether extract yielded pale yellow colour oil which was stored in dark bottles in room temperature till use (Harborne, 1984).

### Fatty acid composition of seeds oil of *B. aegyptiaca*:

Methyl ester of seeds oil of *B. aegyptiaca* was prepared according to the procedure of Christie, (1989); Christie, (1972) to determine fatty acids composition using Gas chromatography (GC- 2010, SHIMADZU-Japan).

### Experimental animals:

Wistar albino rats were obtained from Medicinal and Aromatic Plants Research Institute (MAPRI), National Centre for Research (NCR), and kept in plastic cages in the laboratory animal house in the College of Veterinary Medicine, Sudan University of Science and Technology. They were maintained under standard environmental conditions and provided with standard feed and water *ad libitum*. The animals were fasted overnight before the commencement of the experiment but were allowed with free access to water. All experiments were carried out using five animals in each group.

### Acute toxicity study:

According to Organization for Economic Co-operation and Development (OECD/OCDE) guidelines (OECD, 2001) the acute toxicity study was performed in Wistar albino rats of

either sex. Twenty rats (100 – 120 g) were divided randomly to 4 groups of 5 rats each. *B. aegyptiaca* seeds petroleum ether extract was administered orally to treated groups at a dose of 2, 4, 8, 20 ml/kg respectively and mortality was observed for 24 hours.

### Anti-inflammatory activity evaluation: Carrageenan induced paw oedema (for acute inflammation)

Anti-inflammatory activity was performed in albino rats of either sex (80 – 122 g) according to the method of Ramprasath *et al.*, (2004). Five groups of 5 rats per each were subjected to different treatments as follows:

Group 1: Control: Animals were kept untreated.

Group 2: Standard anti-inflammatory drug: Rats were administered orally with indomethacin at a dose of 10 mg/kg.

Group 3: Low dose: Rats were treated with petroleum ether extract of *B. aegyptiaca* seeds at a dose of 2 ml/kg orally.

Group 4: Medium dose: Animals were administered with petroleum ether extract of *B. aegyptiaca* seeds at a dose of 4 ml/kg orally.

Group 5: High dose: Rats were given petroleum ether extract of *B. aegyptiaca* seeds at a dose of 8 ml/kg orally.

Carrageenan was injected to all groups after one hour of the administration of the treatments into the sub planter tissue of the right hind paw at a dose of 0.1 ml (1% w/v in saline) to induce oedema. The paw volume was determined using a digital vernier calliper. The measurements were recorded at 0 h (before carrageenan injection) and 1, 2, 3 and 4 hours after carrageenan injection the % paw volume inhibition was measured using the following formula:

$$\% \text{ inhibition} = \frac{(P_{V_t} - P_{V_0})_{\text{control}} - (P_{V_t} - P_{V_0})_{\text{treated}}}{(P_{V_t} - P_{V_0})_{\text{control}}} \times 100$$

Where  $P_{V_0}$  = paw volume before administration of carrageenan and  $P_{V_t}$  = is the paw volume after administration of carrageenan.

### **Cotton pellet granuloma (for chronic inflammation)**

Twenty rats weighing between 80 – 166 g were allotted randomly into 4 groups of 5 rats each. The sterile cotton pellets (20 mg) were implanted under anaesthesia in shaved lumbar region of rats using small incision to induce chronic inflammation (Chouhan *et al.*, 2011).

Group 1 served as control, group 2 served as standard anti-inflammatory drug diclofenac sodium administered at a dose 10 mg/kg.

Group 3 and 4 served as test groups and received medium dose (4ml/kg) and high dose (8ml/kg) of the petroleum ether extract of *B. aegyptiaca* seeds respectively. All treatments were administered orally for 6 consecutive days from the day of cotton pellet implantation. On the 7<sup>th</sup> day, animals were killed by an over dose of anaesthesia. The cotton pellets were removed surgically, dried at 60 °C for 24h and weighed. The increase of pellets weight over 20 mg after dryness was taken as an index of granuloma formation.

### **Analgesic study**

#### **Acetic acid induced writhing test:**

Acetic acid induced writhing test was used to determine the peripheral analgesic effect of *B. aegyptiaca* seeds petroleum ether extract (Nwafor and Okwuasaba, 2003). Twenty five animals (90 – 156 g) were divided randomly into 5 groups of 5 rats per each.

Group1: Control: Animals received distilled water only (1 ml/100g).

Group 2: Standard analgesic drug: Rats were administered orally with diclofenac sodium at a dose of 10 mg/kg.

Group 3: Low dose: Rats were administered orally with petroleum ether extract of *B. aegyptiaca* seeds at a dose of 2 ml/kg.

Group 4: Medium dose: Animals were given petroleum ether extract of *B. aegyptiaca* seeds at a dose of 4 ml/kg orally.

Group 5: High dose: Rats were administered orally petroleum ether extract of *B. aegyptiaca* seeds at a dose of 8 ml/kg.

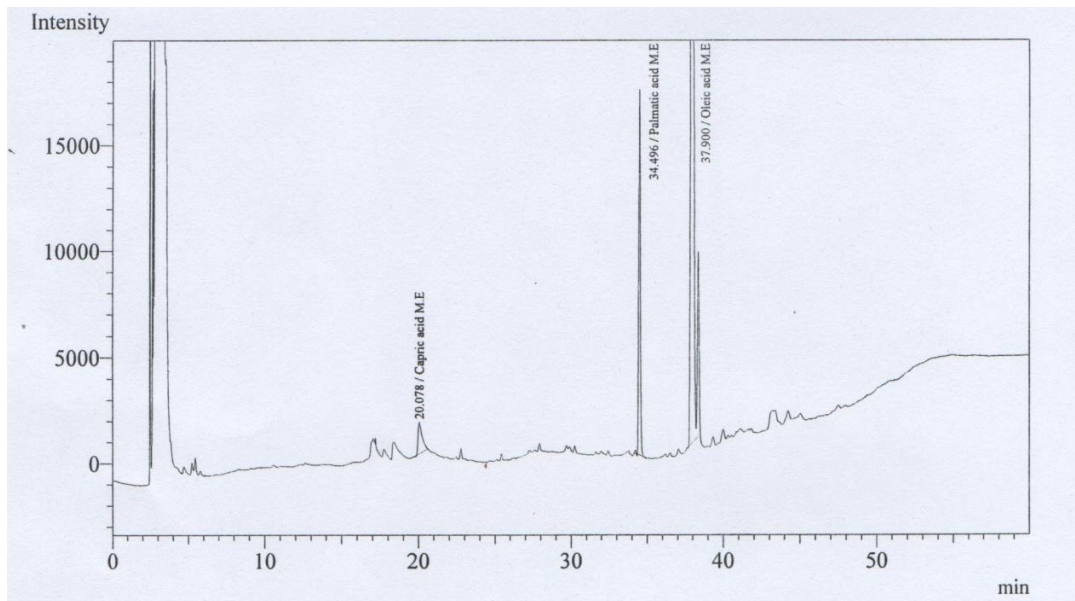
After one hour following the administration of the treatments, acetic acid (0.7%) at a dose of 0.1 ml/10g of body weight was injected intra-peritoneal to produce pain sensation. The number of writhing was calculated immediately after the injection of acetic acid for 20 min. The inhibition of writhing produced by the plant extract and standard drug was measured by comparing with the inhibition produced by the control group.

### **Statistical analysis:**

Data were expressed as the mean  $\pm$  SEM. Differences between experimental groups were compared by one way analysis of variance (ANOVA) followed by Duncan test. The results were considered statistically significant when  $P < 0.05$  (Gomez and Gomez, 1984).

### **Results**

Gas chromatography analysis of seed oil obtained from *B. aegyptiaca* petroleum ether extract revealed the presence oleic acid (22.61%), palmitic acid (4.90%) and capric acid (1.49%). The fatty acids composition of *B. aegyptiaca* seed oil is presented in Figure (1).



**Figure 1: Chromatogram-Gas Chromatography for *B. aegyptiaca* seeds oil**

In acute oral toxicity study, *B. aegyptiaca* seeds petroleum ether extract (2, 4, 8 and 20 ml/kg) was found to be safe. No mortality was observed in the animals received petroleum ether extract up to 20 ml/kg, except the observation of anorexia in rats received higher doses of the oil.

In this study, *B. aegyptiaca* seeds petroleum ether extract administered orally at 2, 4 and 8

ml/kg in rats significantly ( $P < 0.05$ ) reduced the paw oedema induced by carrageenan at 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> hours post carrageenan injection. The inhibition of rats treated with *B. aegyptiaca* seeds petroleum ether extract was comparable to indomethacin used as a standard anti-inflammatory drug (Table 1).

**Table 1: Effect of petroleum ether extract of *B. aegyptiaca* seeds on carrageenan induced rat paw oedema**

Groups	Dose	Increase in paw volume (mm)				Inhibition%			
		1h	2h	3h	4h	1h	2h	3h	4h
Control	-	1.98±0.07 <sup>a</sup>	2.02±0.04 <sup>a</sup>	1.96±0.06 <sup>a</sup>	1.73±0.03 <sup>a</sup>	-	-	-	-
Indomethacin	10mg/kg	1.11±0.07 <sup>b</sup>	0.89±0.11 <sup>b</sup>	0.70±0.09 <sup>b</sup>	0.51±0.05 <sup>b</sup>	43.5	55.9	63.5	70.4
Low dose	2ml/kg	0.97±0.06 <sup>b</sup>	0.98±0.02 <sup>b</sup>	0.91±0.08 <sup>b</sup>	0.71±0.06 <sup>b</sup>	51.0	51.2	53.4	59.0
Medium dose	4ml/kg	0.95±0.05 <sup>b</sup>	0.90±0.04 <sup>b</sup>	0.74±0.03 <sup>b</sup>	0.61±0.03 <sup>b</sup>	51.9	55.5	62.3	64.8
High dose	8ml/kg	0.98±0.02 <sup>b</sup>	0.88±0.02 <sup>b</sup>	0.72±0.05 <sup>b</sup>	0.52±0.01 <sup>b</sup>	50.1	56.5	62.8	69.8

Values are expressed as mean ± SEM, means within the same column with different superscripts are significantly different at  $P < 0.05$  (N=5).

The petroleum ether extract of *B. aegyptiaca* seeds was also examined for cotton pellet induced granuloma in rats (Table 2). Granuloma formation was inhibited significantly ( $P < 0.05$ ) in rats treated with standard drug, diclofenac sodium and petroleum ether extract of *B. aegyptiaca*

seeds for 6 consecutive days compared to the control group. The animals treated with petroleum ether extract of *B. aegyptiaca* seeds at a dose of 4 and 8 ml/kg showed 42.7% and 45.4% of inhibition respectively as compared to the control group, whereas



diclofenac sodium exhibited the highest inhibition rate (59.9%).

**Table 2: Effect of petroleum ether extract of *B. aegyptiaca* seeds on cotton pellet granuloma in rats**

Groups	Dose	Granuloma dry weight (mg)	Inhibition %
Control	-	38.8±2.3 <sup>a</sup>	-
Diclofenac sodium	10 mg/kg	15.4±0.9 <sup>c</sup>	59.9
Medium dose	4 ml/kg	22.0±2.1 <sup>b</sup>	42.7
High dose	8 ml/kg	20.9±0.9 <sup>b</sup>	45.4

Values are expressed as mean ± SEM, means within the same column with different superscripts are significantly different at  $P < 0.05$  (N=5).

The treatment of animals with *B. aegyptiaca* seeds petroleum ether extract produced a significant inhibition ( $P < 0.05$ ) in abdominal writhes induced by acetic acid especially at a dose of 4 (46.8 %) and 8 ml/kg (56.0%)

compared with the control group. Maximum writhing inhibition was 60.8% in diclofenac sodium group used as standard drug (Table 3).

**Table 3: Effect of petroleum ether extract of *B. aegyptiaca* seeds on acetic acid induced writhes in rats.**

Groups	Dose	Number of writhes	Inhibition rate %
Control	-	100.2±2.52 <sup>a</sup>	-
Diclofenac sodium	10 mg/kg	37.6±1.9 <sup>c</sup>	60.8
Low dose	2 ml/kg	94.2±2.42 <sup>a</sup>	6.0
Medium dose	4 ml/kg	53.2± 2.53 <sup>b</sup>	46.8
High dose	8 ml/kg	44.0±0.89 <sup>bc</sup>	56.0

Values are expressed as mean ± SEM, means within the same column with different superscripts are significantly different at  $P < 0.05$  (N=5).

### Discussion

Acute toxicity study showed that the petroleum ether extract of *B. aegyptiaca* seeds safe in rats when administered orally up to 20 ml/kg (OECD, 2001).

In the present study, the anti-inflammatory and analgesic activities of *B. aegyptiaca* seeds petroleum ether extract were evaluated using different models in rats. Carrageenan-induced inflammation is most commonly used as an experimental model for evaluating the anti-inflammatory potency of compounds or natural products (Parekar *et al.*, 2012). The results obtained in this study showed that injection of carrageenan into the rats' hind paw elicited a localized inflammatory response characterized by an increase of paw size (swelling) and pain as a result of increased vascular permeability, cell

infiltrations and inflammatory fluids. Control rats injected with carrageenan showed high paw oedema in the first hour and prolonged effect after 2 – 4 hours compared with standard anti-inflammatory drug, indometacin; this is in agreement with the finding of (Ravi *et al.*, 2009 and Parekar *et al.*, 2012). The probable mechanism of action of carrageenan-induced inflammation is bi-phasic; the first phase is attributed to the release of histamine, serotonin and kinins in the first hour, while the second phase is attributed to the release of prostaglandins and lysosome enzymes in 2 to 4 hours. The second phase is sensitive to most clinically effective anti-inflammatory drugs (Das *et al.*, 2013).

The petroleum ether extract of *B. aegyptiaca* seeds at a dose of 2, 4 and 8ml/kg remarkably

inhibited the first phase of inflammation as well as the second phase. The inhibition of oedema in rats treated *B. aegyptiaca* seeds petroleum ether extract was comparable to that observed by the standard anti-inflammatory drug, indomethacin. In agreement with our findings Gaur *et al.*, (2008) reported that the ethanol and petroleum ether extracts of aerial parts of *B. aegyptiaca* have significant anti-inflammatory activity on carrageenan-induced hind paw oedema in rats compared with the standard drug, indomethacin. Another study by Speroni *et al.*, (2005) showed similar finding in the investigation of the anti-inflammatory activity of two new saponins isolated from *B. aegyptiaca* in the carrageenin-induced oedema in the rats. The results indicated that the inhibitory effect of the extract on carrageenan induced paw oedema might be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandins synthesis.

In cotton pellet induced granuloma, oral administration of *B. aegyptiaca* seeds petroleum ether extract for 6 days at a dose of 4 and 8 ml/kg hindered significantly ( $P < 0.05$ ) the formation of granular tissue compared with the control. The inhibition of granuloma by *B. aegyptiaca* seeds petroleum ether extract at a dose of 4 and 8 ml/kg was considerably high but inferior to the inhibition observed by diclofenac sodium used as a standard anti-inflammatory drug. This reflects its efficacy in reducing an increase in the amount of fibroblasts and synthesis of collagen with mucopolysaccharide, which are natural proliferative events of granulation tissue formation. The potential mechanism involving anti-granulomatous effect of the extract could be due to the reduction of some or all key mediating cytokines leading to the inhibition of inflammatory cell recruitment, especially macrophage and mast cells, the

key initiators for granulation (Khumpook *et al.*, 2013).

In this study, the analgesic activity of *B. aegyptiaca* seeds petroleum ether extract was also investigated using acetic acid induced writhing test in rats. The extract exhibited significant inhibition of abdominal writhes ( $P < 0.05$ ) especially at the medium and high doses (4 & 8 ml/kg) compared to the control and standard analgesic drug, diclofenac sodium. The low dose (2ml/kg) was found to be ineffective in reducing abdominal writhes compared with the control. Acetic acid is well-known to induce indirect release of prostaglandin as well as lipooxygenase products into the peritoneum which stimulate the nociceptive neurons sensitive to the non steroidal anti-inflammatory drugs. For that reason, the results of this study suggest that the mechanism of this action might be partially due to inhibition of lipooxygenase and/or cyclooxygenase in the peripheral tissues, thereby reducing the production of prostaglandins and interfering with the mechanism of transduction in primary afferent nociceptors (Prabhu *et al.*, 2011).

In this study, Gas Chromatographic analysis of *B. aegyptiaca* seeds oil revealed the presence of oleic, palmitic and capric acids. Researchers reported that the oil of *B. aegyptiaca* seeds contains palmitic, stearic, oleic, and linoleic acids as the main fatty acids (Chothani and Vaghasiya, 2011), this is in agreement with our results in the presence of oleic and palmitic acid. The higher content of fatty acids could be responsible for the anti-inflammatory effect of *B. aegyptiaca* petroleum ether extract (Chouhan *et al.*, 2011).

Others have speculated that the anti-inflammatory of seeds extract could be strongly due to the presence of steroids (Elfeel, 2010) which are well known to have potent anti-inflammatory and analgesic activities (Mungole and Chaturvedi, 2011).

Meda *et al.*, (2010) reported that *B. aegyptiaca* has antioxidant properties, which may be responsible for its anti-inflammatory activity (Sokeng *et al.*, 2013).

In conclusion, *B. aegyptiaca* seeds petroleum ether extract possess potent anti-inflammatory and analgesic effects in different models used during this study. The inhibitory effect of this extract could be attributed to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandins synthesis. Moreover, the phytoconstituent, high fatty acids content and antioxidant property of the plant may have a valuable role in its anti-inflammatory and analgesic activities.

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## محتوى الاحماض الدهنية و الاثر المضاد للالتهاب و الفعالية كمسكن للالم لمستخلص بذور الهجليج في الفئران

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فى هذه الدراسة تم التقصي عن محتوى الاحماض الدهنية و الاثار المضادة للالتهاب والمسكنة للالم لمستخلص البتروليوم ايثر لبذور الهجليج فى الجرذان. الاثر المضاد للالتهاب تم تقويمه فى نموذج للالتهاب الحاد و نموذج للالتهاب المزمن باستخدام الكراجنان لاحداث الوذمة وكرات القطن لاحداث الورم الحبيبي على التوالي. الاثر المسكن للالم تم تقويمه بواسطة حمض الخليك لاحداث الانقباضات. الاعطاء الفموي لمستخلص البتروليوم ايثر لبذور الهجليج بجرعة 2، 4 و 8 مل/كجم ثبط معنوياً الوذمة المحدثة بالكراجنان فى الجرذان. وجد ان التنشيط مشابه لذلك الذي لوحظ بواسطة عقار الاندوميثازين المستخدم كعقار معيارى مضاد للالتهاب. فى طريقة الورم الحبيبي بكرات القطن، أعيق معنوياً تكوين الورم الحبيبي بالاعطاء الفموي للمستخلص بجرعة 4 و 8 مل/كجم مقارنة بالمجموعة الضابطة والعقار المعيارى ديكلوفيناك صوديوم. مستخلص البتروليوم ايثر ايضاً قلل معنوياً الانقباضات المحدثة بحمض الخليك مقارنة بالمجموعة الضابطة و العقار المعيارى المسكن للالم، ديكلوفيناك صوديوم. ايضاً الدراسة السمية الحادة اوضحت ان الاعطاء الفموي لزيت البذور فى الجرذان لايسبب اي اثار جانبية او سامة حتى 20مل/كجم باستثناء قلة الشهية فى الجرذان المعطاة الجرعات العالية من الزيت. تحليل الغاز اللوني لزيت بذور الهجليج اظهر وجود حامض الكابريك ، حامض البالمتيك و حامض الاوليك. هذه الدراسة أكدت صحة الاستخدام التقليدي لبذور الهجليج كمضاد للالتهاب ومسكن للالم والذي يمكن ان يعزى لمحتواه من الاحماض الدهنية.