

## **Effect of the Aqueous Extract of Celery (*Apium graveolens*) Seeds, Apple Vinegar and Their Combination on Animal Models of Pain**

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### ***Abstract***

This study was conducted to characterize the analgesic effect of the aqueous extract of celery (*Apium graveolens*) seeds using three animal models of induced pain in mice: hot plate, tail flick and formalin tests. The analgesic effect of apple vinegar was also tested alone and in combination with the aqueous extract of celery.

The results showed that the oral administration of aqueous extract of celery caused (80.5%) increase in hot plate time, (102.9%) increase in tail flick time and (40.1%) decrease in number of lickings and bitings with respect to pre-administration number. Also the mice that given apple vinegar only caused (78.9%) increase in hot plate time, (82.2%) increase in tail flick time and (46.6%) decrease in number of lickings and bitings with respect to pre-administration levels. When celery extract was mixed with vinegar, it showed only (84.6%) increase in hot plate test, (76.9%) increase in tail flick test and (62.3%) in formalin test. It can be concluded that both the aqueous extract of celery and apple vinegar have analgesic effect in all the three pain models. Their combination did not enhance the effect of each other one hour after administration except a mild increase in formalin test.

**	/	*	**	/	*
(80.5%)			(%102.9)		
(40.1%)					
(82.2%)				(78.9%)	
( %4.6)				(46.6%)	
(62.3%)					(76.9%)

**Introduction**

The use of plants for healing purposes predates human history and forms the origin of much of the modern medicine. Interest in herbal medicine is increasing at the present time (Ernst, 2000). Herbal medicine is part of a wide variety of medical practices collectively called complementary or alternative medicine which are spreading worldwide (Anonymous, 1996). World Health Organization (WHO) has paid attention to these types of practices and issued a number of publications (Zhang, 1998). Among them is one that deals with the available experimental and

clinical evidence for the effectiveness of several herbs in treatment of disease (WHO monograph, 1999).

In folk medicine, celery (*Apium graveolens*) has a long history of medicinal and food use. It has been claimed to relieve indigestion, stimulate uterus and to act as anti-inflammatory (Bown, 1995). Celery is said to be useful in cases of hysteria, promoting restfulness and sleep (Grieve, 1984). An essential oil obtained from the plant is reported to have a calming effect on the central nervous system. Some of its constituents showed antispasmodic, sedative and anti-convulsant actions (Chevallier, 1996). It has been used in treating rheumatism and kidney complaints (Launert, 1980).

An experimental study on the aqueous extract of celery seeds showed prolongation in duration of pentobarbitone sleeping time in mice (Jawad *et al.*, 2000). Moreover, celery has an anti-nociceptive effect against both acetic acid induced writhing and hot plate induced thermal stimulation (Alkofahi, 1997).

Celery is one of ingested plants used in induce abortion in pregnant women (Ciganda and Labordes, 2003). A compound isolated from the seeds of celery (3-n-butylpht-halide) was shown to have cerebral–ischitic activity (Zhang, 2002). Consequently, the present study was intended to investigate the effect of celery seed aqueous extract on two more animal models of pain to characterize further, the analgesic effect of this plant. Apple vinegar is among agents traditionally mixed with medicinal plants or their extracts to dilute or potentiate their effects. Apple vinegar, itself, has been shown to have anti-inflammatory effect (Chauhan *et al.*, 1998). For these reasons; apple vinegar was also tested alone and in combination with celery seeds extract.

### ***Materials and Methods***

**Preparation of the Aqueous extracts:** the seeds of *Apium graveolens* were purchased from local markets and identified after culturing at the College of Science, University of Basrah. The seeds of the plant were powdered using electrical grinder. The required quantity (2 gm) of the powder of plant seed

were boiled in 100 ml distilled water for five minutes. The clear supernatants were used. (Ibrahim *et al.*, 1988)

**Preparation of apple vinegar (apple cider):** apples (*Pyrus malus*) were washed and left to dry. Then, apples were cut, without removing their cortex or seeds, to small pieces of similar size and put in a glass container. The large glass container was closed by a piece of cloth after adding tap water and left at room temperature for nine weeks. After these processes, the materials are converted to vinegar known by its odour and its flavor.

**Animal Husbandry:** Albino mice were purchased from Al-Kindi Vaccine Company/ Baghdad. The technique used in breeding and maintaining mice was based on that described by Yousif (1989). Mice were kept in opaque polypropylene cage measuring (30x12x11cm) with stainless steel lids (North Kent Plastic, U.K.) and saw dust substrate was changed weekly. The mice were housed in a separate room in light-controlled room (white fluorescent light in from 6.00-18.00 hr and darkness for the rest of the days and temperature (25±3 C°) throughout the study period. Food and water were supplied *at libitum*. Food was prepared in the laboratory by mixing crude protein (15%), ground Soya bean (6%), wheat flour (50%), wheat bran (25%), vegetable oil (2%), milk powder (2%) and minerals & vitamin (1 g/kg) of the mixture. These materials were mixed with water, suitable form were prepared (as pollute) and put in oven at 40C° to dry.

**Experimental design:** Three animal models of pain were used to test the analgesic effect: hot plate test, tail flick test and formalin test.

**Hot plate test:** Animals were placed on a metal plate (Lasso Company, India) maintained at (55C°) and the latency period for nociceptive responses which appear as licking, flicking of the hind limb or jumping was measured in seconds. Mice that showed nociceptive responses within 18 seconds were used in the experiment (Matsumoto *et al.*, 1996; Thoupradichote *et al.*, 1998).

**Tail flick test:** Two centimeters of the end of mouse tail was placed in a water bath at 50 C° (Scientific Technical Supplies, Frankfort, Germany). The nociceptive response appeared as flicking of the tail. Mice which

showed a nociceptive response within 18 seconds were used. (Matsumoto *et al.*, 1996).

**Formalin test:** Briefly, each mouse was placed in a transparent plastic cage and left for 5 minutes before formalin injection to allow habituation to the new environment. Ten micro liters of 2% formalin was injected s/c to the planter region of hind paw of the mice. The number of lickings and/or bitings of injected paw was recorded (Reanmongkol *et al.*, 1998; Takeshita and Yamaguchi, 1998).

Each experiment consisted of four groups, 6 male mice each weighing 20-25gm. Group (1) received distilled water, group (2) received aqueous extract of *A. graveolens* seed; group (3) received apple vinegar, group (4) received aqueous extract of *A. graveolens* mixed with vinegar. All tests were performed before one hour after administration of the aqueous extract of *A. graveolens*, vinegar or their combination. Each animal received a final volume of 0.2 ml orally. This consisted of 0.1ml test material mixed with either 0.1ml distilled water or vinegar.

**Statistical analysis:** Data were statistically evaluated using paired and unpaired Student t-tests by the aid of SPSS package. P values less than 0.05 were taken as statistically significant.

### **Results**

**1. Hot plate test:** Oral administration of aqueous extract of celery resulted in 80.5% increase in hot plate time compared to pre-administration measurements ( $p < 0.01$ ). Similarly, apple vinegar resulted in 78.9% increase in hot plate latency ( $P < 0.01$ ). When celery extract was mixed with vinegar, it caused 84.6% increment in hot plate time (Table 1).

**2. Tail flick test:** Oral administration of aqueous extract of *Apium graveolens* seeds resulted in (102.9%) increase in tail flick time compared to pre-administration measurements ( $P < 0.01$ ). When apple vinegar alone was used, it gave a statistically significant increase in tail flick time by 82.2%

with respect to pre-administration level. When celery extract was mixed with vinegar, it increased tail flick time by 76.9 % (Table 2).

**3. Formalin test:** Oral administration of aqueous extract of celery seeds resulted in 40.1% decrease in the number of lickings and bitings compared to control ( $p < 0.05$ ). Apple vinegar alone resulted in 46.6% decrease. When celery extract was mixed with vinegar, it decreased the number of lickings and bitings by 62.3%. (Table 3).

**Table (1) Effect of aqueous extract of celery (*Apium graveolens*) seeds, apple vinegar and their combination using hot plate test as a model (n=6).**

	<b>Control (0.2ml) Dist. water</b>	<b>(0.1ml) Aqueous extract of celery+ (0.1ml) Dist. water</b>	<b>(0.1ml) Apple vinegar+ (0.1ml) Dist. water</b>	<b>(0.1ml) Aqueous extract of celery with(0.1ml) vinegar</b>
<b>Before oral administration</b>	3 ± 0.6	3.6 ± 0.5	3.8 ± 0.6	3.9 ± 0.7
<b>One hour after administration</b>	3.4 ± 0.5	6.5 ±* 1.9	6.8 ±* 1.3	7.2 ±* 2.1
<b>% change with respect to pre- administration time</b>	13.3%↑	80.5%↑	78.9%↑	84.6%↑

Data are expressed as mean ±SD of hot plate time (in seconds), Significant difference with respect to pre-administration time: \* $P < 0.01$  (Student -t-test)

**Table (2) Effect of aqueous extract of celery (*Apium graveolens*), vinegar and their combination on using tail flick test (n=6).**

	<b>Control (0.2ml) Dist. water</b>	<b>(0.1ml) Aqueous extract of celery+ (0.1ml) Dist. water</b>	<b>(0.1ml) Apple vinegar+ (0.1ml) Dist. water</b>	<b>(0.1ml) Aqueous extract of celery with(0.1ml) vinegar</b>
<b>Before oral administration</b>	3.7 ± 0.4	3.5 ± 0.7	4.5 ± 0.6	3.9 ± 0.8
<b>One hour after administration</b>	4 ± 0.5	7.1 ±* 0.93	8.2 ±* 0.99	6.9 ±* 2.1
<b>%change with respect to pre- administration time</b>	8.1%↑	102.9%↑	82.2%↑	76.9%↑

Data are expressed as mean ±SD of tail flick time (in seconds) Significant difference with respect to per-administration time:\* P<0.01 (Student –t-test).

**Table (3): Effect of aqueous extract of celery (*Apium graveolens*), apple vinegar and their combination on formalin test (n=6).**

	<b>Control (0.2ml) Dist. water</b>	<b>(0.1ml) Aqueous extract of celery+ (0.1ml) Dist. water</b>	<b>(0.1ml) Apple vinegar+ (0.1ml) Dist. water</b>	<b>(0.1ml) Aqueous extract of celery with (0.1ml) vinegar</b>
<b>Number of lickings and bitings</b>	15.9 ± 1.2	9.4 ±* 1.1	8.5 ±* 0.6	6 ±* 0.9
<b>%change with respect pre-administration number</b>		40.1%↓	46.6%↓	62.3%↓

Data are expressed as mean ±SD of number of lickings and bitings of the injected paw.

Significant difference with respect to per-administration time:\* P<0.01 (Student –t-test).

### Discussion

Pain affects hundred of millions of peoples throughout the world (Ashburn and Stats, 1999). It is one of the most common reasons why patients seek medical care (Arnold and Jeffery 2002). A search for effective, safe and cheap agent from natural sources, for examples herbs, is of worthwhile. In addition, traditional herbal remedies are usually not used alone but usually mixed with other agents such as vinegar or honey. This gives the basis for studying vinegar in our investigation. In models utilizing thermally induced pain (hot plate and tail flick tests), the aqueous extract of *Apium graveoulens* resulted in 80.5 and 102.7% increase in hot plate and tail flick times respectively. This is agreed with that reported by Alkofhi (1997) who found *A. graveoulens* to have anti-nociceptive effect in both acetic acid-induced writhing test and hot plate test.

Apple vinegar gave analgesic effect comparable to that of *A. graveoulens* aqueous extract in three pain models used in this study. It increased the hot plate and tail flick latencies by 78.9% and 82.7% respectively. It also reduced the number of lickings and bitings in formalin test by 46.6%, in comparison to a 40.1% reduction by *A. graveoulens* extract.

Vinegar, made by fermenting the juice of sweat fruits or grains such as apple, grape, dates or barely, is not only a diluted acetic acid but contains more than thirty important nutrients, minerals, vitamins, essential amino acids and several enzymes, and large amount of pectin (Chauhan *et al.*, 1998). It was found effective in patients with polyarthritis (Camara and Danao, 1999) and to have anti-inflammatory activity (Chauhan *et al.*, 1998). We, in the present study, report it to have a significant analgesic effect against the three types of pain stimuli; two forms of thermal stimulation and one by local chemical irritation through the use of formalin.

Unfortunately, using the aqueous extract of *A. graveoulens* and apple vinegar together did not result in enhancement of the analgesic effect of each given alone in hot plate and tail flick tests. However, the combination resulted in a slight increase in the reduction of the number of lickings and biting in formalin test from 40.1% and 46.6% to 62.3%.



Among the constituents of *A. graveoulens* (celery) is an essential oil which has a calming effect on the central nervous system. Others have antispasmodic and antimicrobial activities; it can also be treating rheumatism and induce abortion in women (Launert, 1980; Chevallien, 1996; Krishno and Banerjec, 1999; Cigarda and Lavorda, 2003). Therefore, future investigations should aim to identify the constituents that are responsible for this analgesic effect in celery as well as apple vinegar.

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