

## ANTI-NOCICEPTIVE EFFECT OF RHIZOME OF *ZINGIBER OFFICINALE*(GINGER),APPLE VINGER AND THEIR COMBINATION ON ANIMAL MODELS OF PAIN IN LABORATORY RATS.

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### ABSTRACT

This study was performed to investigate the anti-nociceptive effect of aqueous extract of *Zingiber officinale* rhizomes by three animal models of pain (hot plate test , tail flick and formalin test.

The results showed that the oral administration of aqueous extract of ginger caused (81%) increase in hot plate time,(100%)increase in tail flick time and (41%)decrease in number of licking and biting with respect to pre-administration number. While the rats that given apple vinegar only caused (79%)increase in hot plate time, (83%)increase in tail flick time and (47%)decrease in number of licking and biting with respect to pre-administration levels. When the ginger was mixed with vinegar, it showed only (85%)increase in hot plate test,(77%)increase in tail flick test and(63%)in formalin test.

### INTRODUCTION

The growth in patient use of complementary and alternative medicine has an impact on conventional medical practice (1).Clinical investigation of complementary and alternative medicine are made difficult by factors such as use of complex, individualized treatment and lack of standardization of herbal medicine(2).

Many types of practices have paid attention by World Health Organization, it issued a number of publications(3) and these deals with available experimental and clinical evidence for the effectiveness of several herbs in treatment of disease(4).Rhizome of *Zingiber officinale* used in treatment of toothache, rheumatic and muscular disorders, migraine headache, cold and flu, poor circulation in the hands and feet, headache .Comfrey cream when combined with *Zingiber officinale* rhizome for treating muscular disorders(5)

When 5-10% *Zingiber officinale* extract injected into the painful joint or reaction nodules in number of patients in China with rheumatic pain and chronic lower back pain. experienced full or partial relief of pain and a decrease in joint swelling(6)The ethanolic extract of the rhizome of *Zingiber officinale* reduced the carrageen-induced paw swelling and yeast induced fever in rats. This extract produced dose dependent inhibition of prostaglandin release using rat peritoneal leucocytes as a model (7).

Ginger administration was found to relieve pain and associated symptoms in patients suffering from rheumatic disorders (8),and also ginger used in treatment of migraine (9).Powdered ginger relieved pain to a varying degree in about three quarters of 38 patients with rheumatoid arthritis and osteoarthritis. Ginger extract was found in standardized, placebo-controlled clinical trial on 261 patients with knee osteoarthritis, to be moderately but significantly effective(10).

Ginger was found to be an effective antioxidant in a similar way to ascorbic acid (11).It had also antifungal activity(12)and as mixture of herbs,it stimulated upper gastro-intestinal tract(13).

### **Materials and Methods**

The rhizome of *Zingiber officinale* and apple vinegar were donated from Dr.Abdul-Basit Khalid Ahmed.Collage of Education. University of Basrah.

**Preparation of the aqueous extract:** the required quantity(4gm)of the powder of *Zingiber officinale* rhizome boiled in 200ml distilled water for five minutes, The clear supernatants were used(14).

**Animal Husbandry:-** laboratory rats were purchased in Education Collage /University of Basrah.

The technique used in breeding and maintaining rats was based on that described by (15).Rats were kept in opaque polypropylene cage with stainless steel lids(north kent plastic,U.K)and saw dust substrate was changed weekly.

The rats 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±3c) through the study period.Food and water were supplied *ad libitum*.

Foods were 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 and vitamins (1g/kg) of the mixture .These materials were mixed with water, suitable from were prepared(as pollute)and put in oven 40c to dry.(15).

### **Models of pain**

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(55°C) and the latency period for nociceptive responses which appear as licking ,flicking of the hind limb or jumping was measured in seconds.

Rats that showed nociceptive responses within 18 seconds were used in the experiment (16)and(17).

**Tail flick test:-**

Two centimeters of the end of rat tail was placed in a water bath at 50 °C(Scientific Technical Supplies, Frankfurt, Germany).Four groups,6 male rats each weighing (500gm).Group(1) received distilled water, group(2) received aqueous extract of ginger rhizome .group(3)received aqueous extract of *Zingiber officinale* rhizome.Group 4 received aqueous extract of *Zingiber officinale* mixed with vinegar. The nociceptive response appeared as flicking of the tail. The rats which showed a nociceptive response within 18 seconds were used(18).

**Formalin Test:-**Briefly, each rat was placed in a transparent plastic cage and left for 5 minutes before formalin injection to allow habituation to the new environment 30 microliters of 2% formalin was injected s/c to the plantar region of hind paw of the rats the number of licking and/or biting of injected paw was recorded (19);(20).

All tests were performed before one hour after administration of the aqueous extract of ginger , vinegar or their combination. Each animal received a final volume a final 3ml orally. This consisted of 1.5ml distilled water or vinegar.

### Statistical analysis

The results were analyzed by one-way ANOVA and independent T-test by using spss (special program for statistical system) version 9.0. All data are expressed as mean and SD, the least significant difference (LSD) test was used to determine the differences between groups in ANOVA test (21).

## RESULTS

### 1-Hotplate test :-

Oral administration of aqueous extract of ginger resulted (81%) increase in hot plate time compared to pre-administration measurement ( $p < 0.01$ ). Similarly, apple vinegar resulted in (79%) increase in hot plate latency ( $p < 0.01$ ).

When ginger extract was mixed with vinegar, it caused (85%) increment in hot plate time (Table 1).

### 2- Tail flick test :-

Oral administration of aqueous extract of *Zingiber officinale* rhizome resulted (100%) increase in tail flick time compared to pre-administration measurement ( $p < 0.01$ ). When apple vinegar alone was used, it gave a statistically significant increase in tail flick time by (83%) with respect to pre-administration level. When ginger extract was mixed with vinegar, it increased tail flick time by (77%) (table 2).

### 3-Formalin test:-

Oral administration of aqueous extract of ginger rhizome resulted in (41%) decrease in the number of lickings and biting compared to control ( $P < 0.05$ ).

Apple vinegar alone resulted in (47%) decrease. When ginger extract was mixed with vinegar, it decreased the number of licking and biting by (63%) (Table 3).

## DISCUSSION

Pain is the most common medical complaint among civilized population (22). A search for effective, safe and cheap agent from natural sources, for example herbs of worthwhile. In addition, traditional herbal remedies are usually not used alone but usually mixed with other agents such as vinegar. This gives the basis for standing vinegar in our investigation.

In models utilizing thermally induced pain (hot plate and tail flick test) the aqueous extract of *Zingiber officinal* rhizome resulted 81% and 100% increase in hot plate and tail flick times respectively. This is agreed with that reported by (23) who found *Zingibar officinale* to have anti nocieptive effect, acetic acid-induced formalin test and hot plate test. Apple vinegar gave anti nociceptive effect comparable to that aqueous extract of ginger rhizome in three pain models used in this study.

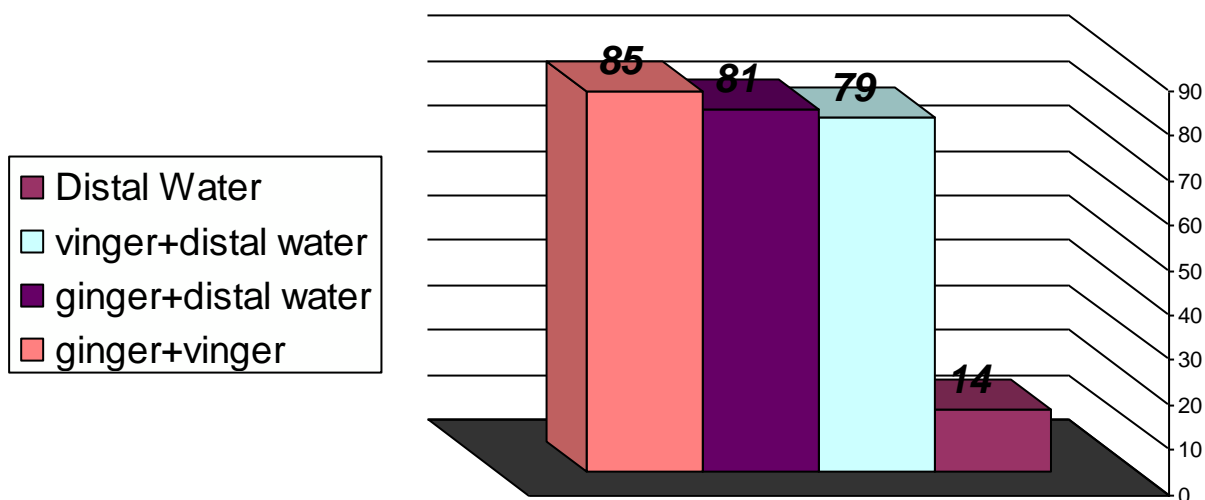
It increase the hot plate and tail flick latencies by 79% and 83% respectively. It also reduced the number of licking and biting in formalin by 47% in comparison to a 41% reduction by *Zingiber officinale* extract.

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 (24). It was found effective in patients with poly arthritis (25) and to have anti-inflammatory activity (24). We, in this 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.

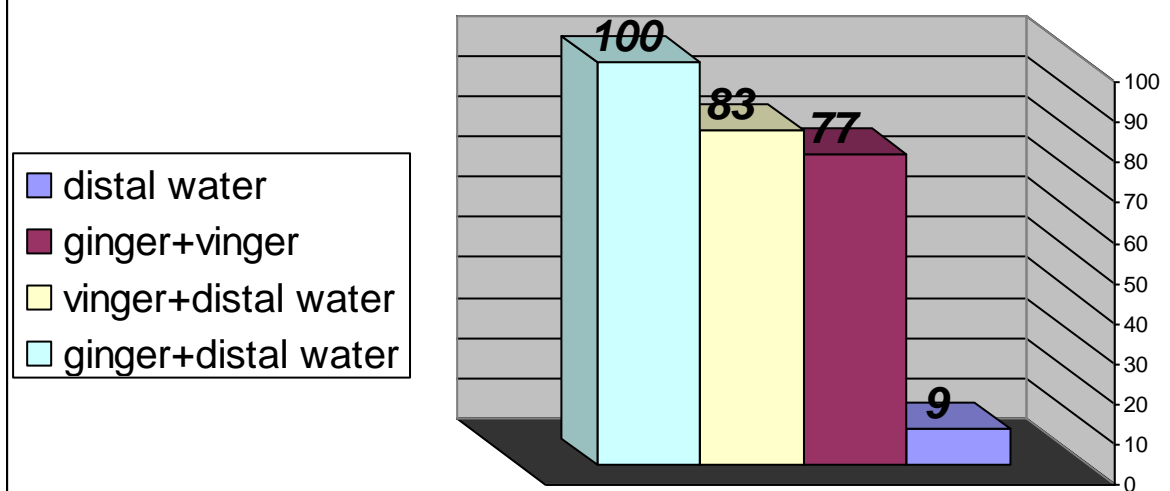
The aqueous extract of *Zingiber officinale* and apple vinegar together did not result in enhancement of the anti nociceptive effect to compared with those given alone in hot plate and tail flick tests. However , the combination resulted in a slight increase in the reduction of the number of licking and biting in formalin test from 41% and 47% to 63%. Among the constituents of *Zingiber officinale* is an aqueous extract which has calming effect on joint swelling ( 6 ) and relieve of pain, treated migraine headache ( 9). Treatment of knee osteoarthritis (10). Antioxidant,antifungal(11)and(12).

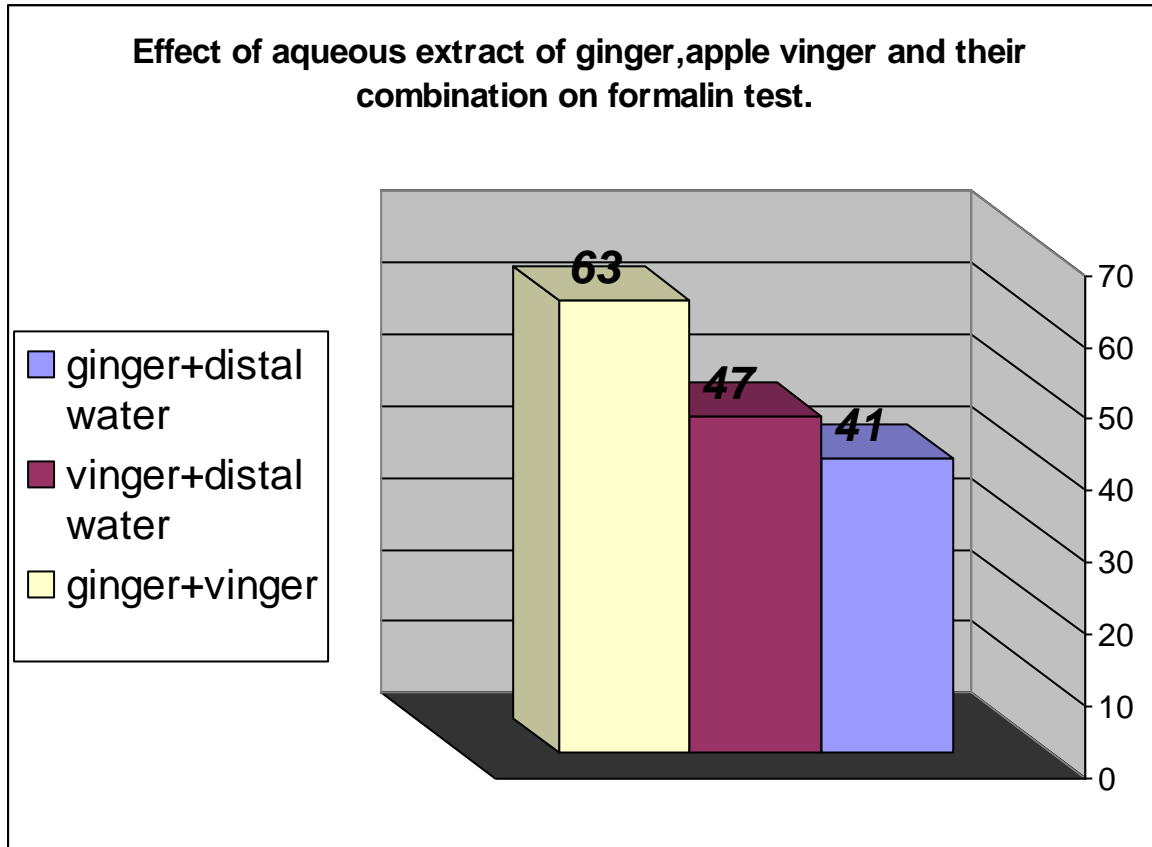
Therefore , future investigation should aim to identify the constituents that are responsible for this analgesic effect in ginger as well as apple vinegar .

**Effect of aqueous extract of *Zingiber officinale* rhizome ,apple vinger and their combination using hot plate test as a model.**



**Effect of aqueous extract of ginger (*Zingiber officinale*) vinger and their combination using tail flick test.**





**التأثير المضاد للآلم لريزومات الزنجبيل وخل التفاح ومزجهما معا على اختبارات الألم في الجرذان المختبرية.**

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### الخلاصة

أجريت محاولة دراسة التأثير المضاد للألم لريزومات الزنجبيل وخل التفاح ومزجهما معا. هذه المواد أعطيت عن طريق الفم إلى الجرذان وفحصت باستخدام النموذج الحيواني للألم (فحص الصفحة الحارة) و (فحص رفرقة الذنب) و (فحص الفورمالين). النتائج بينت ان اعطاء المستخلص المائي لريزومات الزنجبيل بسبب 79% زيادة في وقت الصفحة الحارة و 83% في وقت رفرقة الذنب و 47% يقل عدد اللطع والعص مقارنة فيما قبل الاعطاء في اختبار الفورمالين. لذلك نستنتج ان المستخلص المائي للزنجبيل وخل التفاح يمتلكان تأثيرا مضادا للألم في اختبارات الألم. والمزج بينهما لايزيد على تأثيرهما بمفردهما، ماعدا تأثير طفيف في اختبار الفورمالين.

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