Research Article

Synergistic effect of *Lemon* and *Ginger* juice for analgesic activity on Swiss albino mice

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https://doi.org/10.31024/ajpp.2018.4.2.12

Received: 28 January 2018 Revised: 22 February 2018 Accepted: 1 March 2018

Abstract

Objective: The present study was carried out to investigate the synergestic effect of *lemon* and *ginger* juice for central and peripheral analgesic activity on experimental animals using Acetic acid induce writhing, Hot plate and Tail immersion methods. **Material and methods:** The central analgesic activity was measured by Hot plate and Tail immersion methods in Swiss albino mice and peripheral analgesic activity was measured by acetic acid induced writhing in Swiss albino mice. Aspirin was used as the standard drug in the dose of 100 mg/kg. The *lemon* juice and *ginger* juice was administered at 0.8 ml per animal. Phytochemical analysis showed the presence of flavonoids, alkaloids, glycosides, saponins, tannins, sterols, carbohydrates, and resins. **Result:** All the doses showed significant central and peripheral analgesic activity i.e ($p<0.05$), which is comparable to that of the control and standard drug aspirin. The test and standard drugs significantly ($p<0.05$) reduced the number of abdominal constriction and stretching of hind limb induce by the injection of acetic acid in a dose dependent manner. The Hot plate and Tail immersion test useful in the elucidating centrally mediated antinociceptive responses, which focused mainly on changes above the spinal cord level. All the test and standard drugs significantly ($p<0.05$) reduced the pain as compare to the control group. **Conclusion:** The results of pharmacological tests performed in the present studies suggest that the *lemon* and *ginger* juice in combination gives Synergestic effect for analgesic activity.

Keywords: *Lemon* juice, *Ginger* juice, Analgesic

Introduction

Pain is interpreted as a suffering that results from the perception of painful stimuli. It's a common symptom and it indicates that something is wrong in the body and may give a clue to the nature of disease. Pain is a specific sensation with its own peripheral and central mechanisms independent of other five senses. Pain is not a disease but the most common medical complaint. It is usually as an indication of ill health. Most of the diseases have a component of pain. Pain can be defined as the effect produced in consciousness by the arrival of nerve impulses generated by noxious stimuli in the brain. Drugs, which alter the pain sensitivity or remove pain, are called as painkiller or analgesics (Ahmed et al., 2000). The control of pain is one of the most important parameter to which drugs are put.

Pain is defined by the International Association for the study of pain as, "an unpleasant sensory and emotional experience associated with actual (or) potential tissue damage (or) described in terms of such damage" (Al-Amin et al., 2006). Pain is an emotional component, in the same person, it may varies from time to time, which varies from person to person. Unrelieved acute pain can cause chronic pain. Long standing pain can cause anatomical and even genetic changes in the nervous system (Baskar et al., 2012). Pain is one of the most common reasons for which an individual takes the advice of a physician.

Nonsteroidal anti-inflammatory drugs (NSAIDs) reduce pain and edema by suppressing the formation of prostaglandins, by inhibiting the activity of the enzyme Cyclooxygenase (COX-1 and COX-2). Prostaglandins are important mediators of several components of GI mucosal defense, so suppression of synthesis of prostaglandins (PGs) by NSAIDs greatly reduces the resistance of the mucosa to

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injury as well as interfering with repair processes. The solution to this problem are selective COX-2 inhibitors which were responsible for suppressing the prostaglandin synthesis at sites of inflammation, and not in the GI tract.

Use of Aspirin is also increasing because of its utility in reducing the number of common disorders including stroke, myocardial infarction, Alzheimer's disease and cancer. However, use of aspirin is limited by their significant side effects upon the stomach and the kidney. Their side effects as well as their therapeutic actions are related to their ability to inhibit cyclooxygenase enzymes involved in the first step of the arachidonic acid cascade.

In addition, the damaging effect of some NSAID's upon the stomach and intestine is due to their acidic nature, as with indomethacin, ibuprofen, diclofenac, naproxene, aspirin.

The market for NSAIDs is expanding rapidly because of an aging population in developed countries are associated with the increase in the prevalence of diseases like arthritis (Cena et al., 2003).

With the easy availability of the analgesic drugs there are many people suffering with symptoms of analgesic abuse. With the development of more and more synthetic drugs which have their unique adverse effects, it is necessary to give attention to find the possible remedies among indigenous herbal plants having the same effect. This has accelerated the global effort to harvest those medicinal plants that have substantial beneficial effects with least adverse effects (Debasis et al., 2011; Dubois et al., 2004).

Traditional medicine is widely used around the world and valued for a number of reasons. “Traditional medicines, of proven quality, safety, and efficacy, contribute to the goal of ensuring that all people have access to care.” For millions of people, herbal medicines, traditional treatments, and traditional practitioners are the main source of health care, and sometimes the only source of care (Ficker et al., 2003).

Lemon is very good dietary supplement. It is rich source of vitamins containing Vitamin C, Vitamin B (Thiamine, Riboflavin, Pyridoxine, Pantothenic acid and folat). It also contains Trace elements like Calcium, Magnesium, Zinc, Iron and Potassium. Its main chemical constituents include Flavanoids, Polysaccharides, sugars, volatile compounds, carotenoids and organic acids (Hardman et al., 1996).

Phytochemical composition of *Zingiber officinale* has been extensively studied in the past studies. *Zingiber officinale* is reported to possess essential oils, phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids, glycosides, saponins, steroids, terpenoids and tannin as the major phytochemical groups. These phytochemicals plays an important role in the medicinal property of this plant (Jagetia et al., 2004).

Ginger is extremely valuable in dyspepsia, flatulence, colic, spasms and other painful infection of stomach (Joseph et al., 2008). The pharmacological effects of *Zingiber officinale* have been reported to have hypolipidemic (Kaneria.,2007), anti-inflammatory (Nadkarni, 1927), antioxidant (Ojewole, 2006), hypoglycemic (Olaleye et al., 2000), analgesic (Penna et al., 2003) , anti-platelet (Penna et al., 2003), antiemetic (Rajagopal, 2006), antithrombotic (Shanmugasundaram and Venkataraman, 2005), anti-tumorigenic (Sharma, 1997), radio protective (Shukla and Singh, 2007), antimicrobial and antifungal (Thomson et al., 2002) actions. Considering the vast variety of actions of *ginger.*

Materials and methods

Drugs and chemicals

For the purpose of this work we selected Aspirin (100mg/kg), and Glacial acetic acid (0.7%).

Preparation of drugs and chemical solutions

In this study aspirin was taken as standard drug. Soluble form of aspirin was obtained from Nice, Cochin. It belongs to salicylates (nonsteroidal anti-inflammatory drugs). Glacial acetic acid is used to induce writhings in mice. 10 ml/Kg of 0.7% v/v acetic acid was used to induce writhings. 0.7% v/v acetic acid was prepared by adding 0.7 ml of acetic acid in 100 ml of distilled water. The solution was prepared freshly before each experiment.

Plant materials

Fresh lemons were purchased from local market in Nashik and were identified by Department of Pharmacognosy, at college of pharmacy for Women. The fresh lemons were squeezed by hand and then juice was filtered.

Ginger juice was prepared from fresh raw ginger rhizomes. It is washed, grated and pressed through clean muslin cloth.

Experimental animals

The animals were housed comfortably in a group of five in a single clean plastic cage with a metal frame lid on its top. They were housed under standard environmental conditions of temperature (24±1°C) and relative humidity of 30-70 %. A 12:12 h light dark cycle was followed. All animals had free access to water and standard pelletized laboratory animal diet ad libitum.

The experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethical Committee.

Experimental Protocol

The animals were divided into Five groups each consisting of five mice and received the following treatment.

Group I (Control): Animals received normal saline.

Group II (Aspirin): Animals received Aspirin (100 mg/kg)

Group III (*Lemon* juice): Animals received Lemon juice (0.4ml/ p.o.)

Group IV(*Ginger* juice): Animals received ginger juice (0.4ml/ p.o.)

Group V (*Lemon* juice + *Ginger* juice): Animals received Lemon juice + Ginger juice (0.8ml/ p.o.)

Acetic acid induced writhing

This test is used to identify the peripheral analgesic activity. Writhing is defined as a stretch, tension to one side, extension of hind legs, contraction of the abdomen so that the abdomen of mice touches the floor, turning of trunk (twist). Any writhing is considered as a positive response. Food was withdrawn 12 hours prior to drug administration till completion of experiment. The animals were weighed and numbered appropriately.

After 12 hrs fasting 25 healthy Swiss albino mice of either sex weighing 25-30 g were randomly divided into five groups of five animals each. Group I received 0.5 ml of normal saline (NS) (control group), Group II received 100 mg/kg of aspirin (standard group). Group III received lemon juice, Group IV received ginger juice, Group V received lemon and ginger juice in combination. All the drugs were given orally. After 1 hr all the animals received 10 ml/kg of 0.7% v/v acetic acid injection intraperitoneally. The writhing episodes were recorded for 30 minutes; stretching movements consisting of arching of the back, elongation of body and extension of hind limbs were counted (Umamageswari and Yasmeen, **2015).**

Percentage of inhibition was evaluated using following formula:

$$\text{Inhibition (\%)} = \frac{C - T}{C} \times 100$$

T = Treatment group

C = Control group

The results of Acetic acid induced writhing method in mice was tabulated in Table-1.

Hot plate method in rats

The paws of mice and rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The hot plate, which is commercially available, consists of a electrically heated surface. The temperature is controlled for 55° to 56 °C. This can be a copper plate or a heated glass surface. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded by a stop-watch. Food was withdrawn 12 hours prior to drug administration till completion of experiment. The animals were weighed and numbered appropriately. After 12 hrs fasting 25 healthy Swiss albino mice of either sex weighing 25-30 g were randomly divided into five groups of five animals each (Wolfe et al., 1999). Group I received 0.5 ml of normal saline (NS) (control group), Group II received 100 mg/kg of aspirin (standard group). Group III received *lemon* juice, Group IV received *ginger* juice, Group V received *lemon* and *ginger* juice in combination. All the drugs were given orally.

After 60 minutes, the animals are placed on the hot plate and the observations were recorded at the time interval of 15, 30, 60, 90 and 120 minutes. The results of Hot plate method in rats was tabulated in Table-2.

Tail immersion test in mice

The procedure is based on the observation that morphine-like drugs are selectively capable of prolonging the reaction time of the typical tail-withdrawal reflex in rats induced by immersing the end of the tail in warm water of 55 °C. The lower 5 cm portion of the tail is marked. This part of the tail is immersed in to the water bath of exactly 55 °C. Within a few seconds the rat reacts by withdrawing the tail. The reaction time is recorded in 0.5 s units by a stopwatch. After each determination the tail is carefully dried. The reaction time is determined before and periodically after oral administration of the test and standard substance. The cut off time is 15sec.

Food was withdrawn 12 hours prior to drug administration till completion of experiment. The animals were weighed and numbered appropriately (World Health Organnization, 2013).

After 12 hrs fasting 25 healthy Swiss albino mice of either sex weighing 25-30 g were randomly divided into five groups of five animals each.

Group I received 0.5 ml of normal saline (NS) (control group), Group II received 100 mg/kg of aspirin (standard group). Group III received *lemon* juice, Group IV received *ginger* juice, Group V received *lemon* and *ginger* juice in combination. The test and standard drugs were given orally. After 60 minutes, the observations were recorded at the time interval of 15, 30, 60, 90 and 120 minutes. The results of tail immersion test in mice was tabulated in table-3.

Statistical analysis

All the results are expressed as mean ± standard error of mean (SEM). The data were analyzed for statistical significance by one way analysis of variance (ANOVA) followed by Dunnett's test .Values of $p < 0.05$ were considered statistically significant.

Results

Analgesic activity by Acetic acid induced writhing test

The results presented in Table 1 shows that the Lemon and Ginger juice in combination exhibited significant (p<0.05) analgesic activity (46.07% inhibition respectively) compared to the control and also to that of aspirin, 100 mg/kg (48.12 %). Significant protection against writhing was observed in animals treated with aspirin, Lemon and Ginger juice in combination; where number of writhes after treatment were 30.4, 49.2, 37.4 and 31.6, respectively compared to 58.6 in the control group.

Table 1. Analgesic activity by Acetic acid Induced writhing in mice

Group	Dose	No. of Writhes in 30 min. (Mean ± SEM)	Inhibition (%)
Group I (Water)	-	58.6 ± 0.4	-
Group II (Aspirin)	100mg/kg	30.4 ± 0.24	48.12
Group III *(Lemon)*	0.4 ml/p.o	49.2 ± 0.58	16.04
Group IV *(Ginger)*	0.4 ml/p.o	37.4 ± 0.4	36.18
Group V *(Lemon + Ginger)*	0.8 ml/p.o	31.6 ± 0.4	46.07

N = 5, All values are expressed as mean ± SEM. Data were analyzed by using One way ANOVA followed by Dunnett's test. P< 0.05 against control group.

Eddy's hot plate method

As shown in Table 2, the *Lemon* and *Ginger* juice in combination produced significant analgesic activity. In this model, the combination prolonged significantly the reaction time of animal with relatively extended duration of stimulation. At the Combination dose the animals could withstand on the hot plate for 6.84, 7.08 and 7.45 seconds at 30, 90 and 120 min reaction time which was the highest and comparable with that of the reference drug Aspirin 100 mg/kg.

Table 2. Analgesic activity by Hot Plate method in mice

Groups	Dose	Time (min)				
		15	30	60	90	120
Group I (Water)	-	2.98±0.04	4.08±0.08	3.08±0.02	3.45±0.05	3.98±0.06
Group II (Aspirin)	100mg/kg	3.14±0.05	4.88±0.06	4.45±0.06	4.56±0.06	4.78±0.08
Group III *(Lemon)*	0.4 ml/p.o	3.18±0.08	5.12±0.05	5.67±0.06	5.78±0.07	5.67±0.06
Group IV *(Ginger)*	0.4 ml/p.o	3.32±0.05	6.04±0.08	6.54±0.08	6.79±0.08	6.18±0.04
Group V *(Lemon + Ginger)*	0.4 ml/p.o	3.42±0.04	6.84±0.06	7.04±0.09	7.08±0.06	7.45±0.05

N = 5, All values are expressed as mean ± SEM. Data were analyzed by using One way ANOVA followed by Dunnett's test. P< 0.05 against control group

Tail Immersion Test in Mice

In Tail immersion method all the test and standard drugs significantly (p<0.05) reduce the pain as compare to the control group. (Table-3) By applying Dunnett's test, it was shown that there is significant (p<0.05) effect of Group IV & Group V as compare to the standard at 90 and 120 minutes.

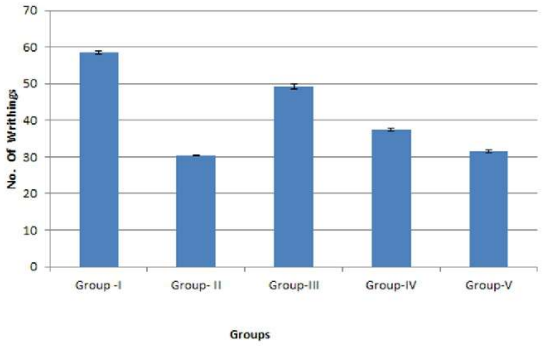

Figure 1. Analgesic activity by Acetic acid Induced writhing in mice. All values are expressed as mean ± SEM. Data were analyzed by using One way ANOVA followed by Dunnett's test (N = 5). P< 0.05; All groups were compared with control group.

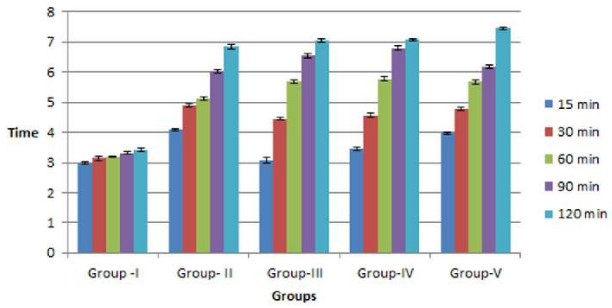

Figure 2. Analgesic activity by Hot Plate method in mice (N = 5). All values are expressed as mean ± SEM. Data were analyzed by using One way ANOVA followed by Dunnett's test. P< 0.05 All groups were compared with control group.

Table 3. Analgesic activity by Tail Immersion test in mice

Groups	Dose	Time (min)				
		15	30	60	90	120
Group I (Water)		2.2±0.08	3.12±0.05	3.24±0.08	3.12±0.09	4.2±0.09
Group II (Aspirin)	100mg/kg	2.45±0.09	3.5±0.1	3.33±0.06	2.51±0.1	4.02±0.1
Group III *(Lemon)*	0.4 ml/p.o	2.78±0.09	4.01±0.09	3.89±0.05	3.26±0.15	4.1±0.08
Group IV *(Ginger)*	0.4 ml/p.o	2.9±0.06	4.45±0.08	4.23±0.08	4.22±0.09	5.01±0.07
Group V *(Lemon + Ginger)*	0.4 ml/p.o	3.09±0.02	5.12±0.06	4.54±0.09	4.23±0.08	5.48±0.06

N = 5, All values are expressed as mean ± SEM. Data were analyzed by using One way ANOVA followed by Dunnett's test. P< 0.05 against control group

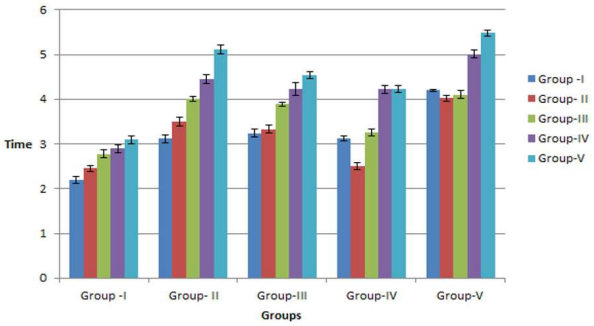

Figure 3. Analgesic activity by Tail Immersion test in mice (N = 5). All values are expressed as mean ± SEM. Data were analyzed by using One way ANOVA followed by Dunnett's test. P< 0.05; All groups were compared with control group.

Discussion

Three anti-nociceptive models; acetic acid-induced writhing reflex, tail immersion and hot plate models were used to evaluate the analgesic activity of combined juice of lemon and ginger. since tests of analgesic drugs commonly measure nociception and involves the reaction of animals to painful stimuli. The stimulus may be thermal (tail immersion or hot plate tests), chemical (acetic acid-induced writhing or formalin tests) or mechanical (tail or paw pressure tests). The hot-plate and tail-clip tests are useful in elucidating centrally mediated antinociceptive responses, which focuses mainly on changes above the spinal cord level.

In this study the synergestic analgesic effect was observed for this combined juice of *lemon* and *ginger,* which prolongs the hot plate latency and revealing the centrally acting nature of ombined juice. The tail immersion and hot plate models have been used to study centrally acting analgesics.

The significant increase in pain threshold produced by combined juice in these models suggests involvement of central pain pathways. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems. The analgesic effect produced by combined juice may be via central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leucotrienes, and other endogenous substances that are key players in inflammation and pain. Acetic acid-induced writhing reflex is a model of visceral pain which is highly useful for screening analgesic drugs. Acetic acid could induce writhing reflex in laboratory animals. Intraperitoneal injection of 0.7% glacial acetic acid produced abdominal writhing in this experiment. Acetic acid produces writhing reflex in animals by activating the chemo sensitive nociceptors. The acetic acid induced writhing involves the release of bradykinin and prostanoids at peripheral tissues, which acts on local peritoneal receptors. Also, it has been noted that the level of analgesia in acetic acid-induced models is indicated by the percent reduction in the number of abdominal constrictions.

Non steroidal anti-inflammatory drugs (NSAIDs) like aspirin will inhibit the enzyme cyclooxygenase at peripheral tissues and block the release of endogenous substances that exerts pain. The antagonism of prostaglandin receptors or suppression of the formation of the prostaglandins may be the possible reason for analgesic effects of combined juice against writhing.

Here in this research work we found that ginger juice is as effective as the conventional NSAIDs. The Ginger juice in combination with lemon juice is more effective for analgesic activity as compare to the Ginger juice only.

Conclusion

From the present study, we come to the conclusion that the combined juice of *lemon* and *ginger* posseses both peripheral and central analgesic activity in experimental animals.

Here we conclude that the combination product was more effective than the single drug, it may be due to different mechanism of actions of different drugs in combined products. But the chances of side effects of single drug is more as compare to the combination products. More study on combination drug therapy may overcome these problems.

Acknowledgement

The authors are thankful to the authorities of PRES's college of pharmacy, Chincholi, Nashik

For providing necessary facilities to carry out this research work.

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