Iraqi Journal of Veterinary Medicine Vol. 34, No. 1, 2010

Study the antinociceptive effect of the Zingiber officinale ethanolic extract and Vitamin C in rats.

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Accepted 31/3/2010

Summary

The present study was designed to investigate the antinociceptive activity of seperate and combined treatments of ethanol extract of *Zingiber officinale* rhizomes and vitamin C on visceral nociception induced by intraperitoneal injection of 2% acetic acid which produced writhing in rats. The preliminary chemical tests were performed on the extract and revealed the presence of flavanoids, alkaloids and tannins. Visceral nociceptive responses including the latency time to beginning of the first writhe, number of writhes per hour and the inhibition percentage of writhing. Seperate i. p. injection of ethanol extract of *Zingiber officinale* at dose of 25 and 50 mg/kg BW caused significant ($P<0.01$) increase in latency time and significant ($P<0.01$) reduction in writhing number. Seperated i. p. injection of vitamin C at dose of 10 mg/kg BW caused significant ($P<0.05$) reduction in writhing number, whereas, i. p injection of vitamin C at dose of 15 mg/kg BW produced significant prolongation in latency time and reduction in writhing number ($P<0.01$). Combined treatment with ethanol extract of *Zingiber officinale* at dose of 25 mg/kg BW and vitamin C at dose of 10 mg/kg BW. i.p. produced significant suppression ($P<0.01$) in pain responses as compared with the effect of the same doses when used each seperately. It is concluded that both ethanol extract of *Zingiber officinale* rhizomes and vitamin C are able to suppress visceral pain, and vitamin C potentiates the antinociceptive effect of the ethanol extract of *Zingiber officinale* rhizomes.

دراسة التأثير المضاد للألم لمستخلص الزنجبيل الكحولي وفيتامين C في الجرذان

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الخلاصة

صُممت هذه الدراسة لتقصي الفعالية المضادة للالم الاحشائي، المحدث تجريبياً عند حقن حامض الخليك 2% داخل البريتون والذي ينتج عنه سلوك الالتواء من الألم ، لكل من المستخلص الايثانولي لرايزومات نبات الزنجبيل وفيتامين ج عند اعطائهما بشكل منفصل او سوياً في الجرذان. اجريت بعض الفحوصات الكيميائية الاولية على المستخلص الايثانولي وبينت وجود الفلافونيدات، القلويدات والعفصيات، تم متابعة مجموعة من الاستجابات الناتجة عن الألم الاحشائي والتي تضمنت الوقت اللازم لاظهار اول التواء ناتج من الألم، عدد مرات الالتواء الناتجة من الألم خلال ساعة والنسبة المئوية للتثبيط في عدد مرات الالتواء الناتجة عن الألم. ادى الاعطاء المنفصل للمستخلص الايثانولي لرايزومات نبات الزنجبيل عن طريق البريتون عند جرعتي 25 و 50 ملغم/ كغم من وزن الجسم الى زيادة معنوية ($P<0.01$) في الوقت اللازم لاظهار الالتواء من الألم وهبوط معنوي ($P<0.01$) في عدد مرات الالتواء الناتجة من الألم، ان الاعطاء المنفصل لفيتامين ج عن طريق البريتون بجرعة 10 ملغم/ كغم من وزن الجسم سبب تناقصاً معنوياً ($P<0.05$) في عدد مرات الالتواء الناتج من الألم، بينما ادى الاعطاء المنفصل لفيتامين ج وبجرعة 15 ملغم/ كغم من وزن الجسم عن طريق البريتون الى اطالة معنوية في الوقت اللازم

لاظهار الالتواء من الألم ونقصان معنوي في عدد مرات الالتواء الناتج من الألم تحت مستوى احتمال (P<0.01). ان اعطاء كلاً من المستخلص الايثانولي لرايزومات نبات الزنجبيل بجرعة 25 ملغم/ كغم من وزن الجسم وفيتامين ج بجرعة 10 ملغم/ كغم من وزن الجسم معاً عن طريق البريتون نتج عنه تثبيطاً معنوياً (P<0.01) في الاستجابة للالم مقارنة مع اعطاءهما بنفس الجرع ولكن بصورة منفصلة. تم الاستنتاج بان كلاً من المستخلص الايثانولي لرايزومات نبات الزنجبيل وفيتامين ج لهما القابلية على تثبيط الألم الاحشائي، وان فيتامين ج له القابلية على تقوية التأثير المضاد للالم للمستخلص الايثانولي لرايزومات نبات الزنجبيل.

Introduction

Zingiber officinale known as ginger, belongs to the Zingiberaceae family, is a perennial herb with an aromatic pungent taste. The rhizomes of ginger are used as spice in food and beverages and in traditional medicine as carminitive, antipyretic, in the treatment of waist pain rheumatism and bronchitis 1). The rhizomes of ginger are used to relieve stomachache and (macerated in alcohol) as tonic or stimulant in china (2). Plants from this family have been reported to have antihypoglycemic (3), anti- inflammatory (4), antiulceration (5), antioxidant (6) and anti-microbial activities (7).

Vitamine C (ascorbic acid) has a wide pharmacological activity scale such as antihistaminic, anti-rheumatoid, anti- inflammatory, antioxidant (8). Also vitamine C has an important role in normal function of central nervous system and it is highly concentrated in brain (9). It has been reported that intraperitoneal injection of vitamine C inhibited pain response after intraplanter injection of formaline in mice (10).

Everyone knows from personal experience, visceral pain can be very sever, visceral pain is particularly unpleasant not only because of the effective component that it has in common with other pain types, but also because so many visceral afferents are excited by the same process that causes pain have reflex connections that initiate nausea, vomiting, and other autonomic effects and radiates to other areas (11). Therefore, visceral pain is still considered one of the most important clinical problems.

The clinical use of analgesics for alleviation of visceral pain is markedly increased at the past few years. Several trials have been performed to study the visceral pain experimentally, one of these trials was through intraperitoneal injection of irritant substance such as acetic acid in mice or rats which known as writhing test (12 and 13).

In view of the some pharmacological uses of Ginger and vitamine C, the purpose of this study was to investigate the antinociceptive activity of ethanolic extract of *Z. officinale* rhizomes and vitamine C in seperate and combined treatment against visceral pain induced by acetic acid which induce writhing in rats.

Materials and Methods

Plant material:

Sufficient amount of *Z. officinale* rhizomes was used for extraction according to method described by Somchit *et al*., (14). The rhizomes were washed with distilled water, chopped into small pieces, dried in oven (45̊ c) for about 1 to 2 days and then powdered. Ethanolic extract of the rhizomes was prepared with absolute ethanol (50 gr of powdered rhizomes/ 200 ml of absolute ethanol) in soxhlet apparatus. Extraction yield was: 5.2%. The pharmacological experiment was carried out with the dry extract dissolved in 0.9% physiological solution.

Chemical tests: The following tests were performed on sample of *Z. officinale* rhizomes extract.

1- Test for flavonoids: This test was accomplished by adding 4 ml. of ethanol 95% to 1 ml. sample then heating in water bath till boiling for 25-30 minute. Later on to 5ml of sample few

drops of 0.5 N potassium hydroxide was added. If flavonoids were present, dark color should appear (15).

2- Test for alkaloids:

By using the test known as Myer's test. Myer's reagent was prepared by dissolving 1.35g of mercuric chloride in 60ml. distilled water, then dissolving 2.5g potassium iodide in 10ml. distilled water. Subsequently both solutions were mixed and the volume was complete to 100ml. distilled water. To 5ml. of sample, few drops of the freshly prepared reagent were added. If alkaloids were present. White precipitate should appear (16).

3- Test for tannins:

By using of lead acetate 1%, where the reagent was prepared by dissolving 1g of lead acetate powder in 100ml. distilled water. To 1ml. of sample, 1ml of the freshly prepared reagent was added. If tannins were present, white Jelly precipitate should appear (17).

Animals:

Albino wistar rats weighing 200-250g of either sex were used. The animals were fed with standard rodent diet (commercial feed pellets) and watered with tap water.

Nociceptive test (Acetic acid- induced writhing in rats):

Animals were fasted for (12) hour prior to treatment. Each rat was placed in a cage for acclimation for a period of 1 hour. Rats were divided into five treatment groups and a control group (six rats per group). Rats were administered 25 mg/kg. BW. of *Z. officinale* extract, 50 mg/kg. BW. of *Z. officinale* extract, 10 mg/kg BW. of vitamin C, 15mg/kg BW. of vitamin C or 25 mg/kg BW. of *Z. officinale* extract and 10 mg/kg BW. of vitamin C. The control group received 1ml of distilled water. Treatment agents in all groups were administered intraperitonealy.

One hour after treatment, rats were injected i.p with 1ml of 2% acetic acid solution to induce writhing (13). Immediately after injection of diluted acetic acid, the behavior of rats was monitored for one hour and the following parameters were measured.

1- Latency time to the beginning of the first writhe (where whrite was defined as a wave of contraction of the abdominal musculature followed by extension of the hind limbs (13).
2- Total number of writhes was counted.
3- Percentage of protection against writhing movement (% inhibition of writhing) was taken as an index of analgesia as it was described by Raji *et al*., (1).

% inhibition= Wr (control) – Wr(test group)/ Wr (control). Where

Wr= mean number of writhing.

Results

The preliminary chemical tests of the ethanolic extract of *Z. officinale* rhizomes showed the presence of flavanoids, alkaloids and tannins. After i. p. injection of acetic acid in control group, time to the beginning of the first writhe observed was 7.5±0.428 (latency time). After i.p. injection of the ethanolic extract of *Z. officinale* rhizomes alone at dose of 25 and 50mg/kg BW. cause significant increase ($P<0.01$) in the latency time. Whereas i.p injection of vitamin C alone caused prolongation in the latency time in dose dependent manner and a significant ($P<0.05$) effect was observed at a dose of 15mg/kg BW. (table 1).

Intraperitoneal administration of *Z. officinale* ethanolic extract alone at dose of 25 or 50 mg/kg BW. resulted in a significant ($P<0.01$) reduction in writhing number. Intraperitoneal injection of vitamin C alone caused a significant reduction in writhing number ($P<0.05$) at dose of 10 mg/kg. BW and ($P<0.01$) at dose of 15 mg/kg. BW. (table 1).

Combination of 25 mg/kg BW. of ethanolic extract of *Z. officinale* and 10 mg/kg BW. of vitamin C produced obvious increase in latency time as well as marked reduction in writhing number, latency time in this case longer than that shown by each agent when given seperatly, indicating an obvious additive effect (table1). However, the combined treatment caused marked

increase in the percentage of inhibition of writhing compared with that produced by treatment with each agent alone.

Table1: Effect of separate and combined intraperitoneal injection of ethanolic extract of *Z. officinale* rhizomes, and vitamin C on acetic- acid- induced writhing in rats.

Treatment	Dose mg/kg. BW. i. p.	Latency time(min)	Writhing number (n/h)	% inhibition of writhing
Control (distilled water)	-	7.5±0.428	42.2±1.869	-
Ethanol extract of *Z. officinale* rhizomes	25	9.0±0.365 *	25.3±1.855**	40.0
Ethanol extract of *Z. officinale* rhizomes	50	13.7±0.918**	18.5±2.320**	56.7
Vitamin C	10	8.8±0.477	37.0±0.816*	12.3
Vitamin C	15	9.7±0.714*	33.8±1.166**	19.8
Ethanol extract of *Z. officinale* rhizomes and vitamin C	25	17.8±1.108■	14.8±2.286■	64.5

Figures represent mean ± standard error. The data were analysed using one- way analysis of variance.
* mean value differs significantly ($P<0.05$) from control, ** mean value differs significantly ($P<0.01$) from control, and ■ mean value differs significantly ($P<0.01$) compared with ethanol extract of *Z. officinale* rhizomes at dose 25 mg/kg BW. or vitamin C at dose of 10 mg/kg BW.

Discussion

In the present study both the ethanolic extract of *Z. officinale* and vitamin C when given separately caused prolongation of the latency time which may, most probably, contribute to elevation of pain threshold. It is worth to mention that the preliminary chemical tests of the ethanolic extract of *Z. officinale* rhizomes, showed the presence of flavanoids, alkaloids and tannins. A number of flavanoids have been reported to produce analgesic activity (18). Alkaloid in the extract of some plants were found to produce significant analgesic effect (19 and 20). Also, there are few reports on the role of tannins in producing analgesic activity (18). So the analgesic activity of *Z. officinale* might be attributed to the presence of flavonoids, alkaloids or tannins. The present results came in agreement with those of (1) who reported that the rhizome extract of *Z. officinale* possesses anti- inflammatory and analgesic agent (s).

The antinociceptive effect of vitamin C in the present study was reported by Rose (10) who stated that vitamin C suppresses pain induced by intraplanter injections of formalin and glutamate in mice and it was suggested that glutamate receptors may be involved in vitamin C - induced antinociception.

The synergistic effect obtained after combined treatment with the extract and vitamin C may be due to the different positions situations in which each agent, in the combination, acts. for instance flavonoids, detected in *Z. officinale* ethanolic extract, had an inhibitory effect on 5-lipooxygenase pathway (which is responsible for formation of chemical mediators (Leukotrienes) that play important role in initiation and persistency of pain (21), and vitamin C activity in inhibition of cyclooxygenase products (Prostaglandins) (22) which may exert a more

profound antinociceptive activity compared with the effect of treatment with each component alone.

Intraperonteal administration of ethanolic extract of *Z. officinale* rhizomes and vitamin C each alone showed antinociceptive activity against visceral pain induced by intraperitoneal injection of acetic acid. In combined treatment an additive effect of them was observed as alleviation of visceral pain. Further work is needed to determine the exact mechanism (s) by which the *Z. officinale* and vitamin C act as antinociceptive agent.

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