ANTIOXIDANT, ANTI-INFLAMMATORY AND ANTINOCICEPTIVE ACTIVITIES OF ESSENTIAL OIL FROM GINGER

KOTTARAPAT JEENA*, VIJAYASTELTER B. LIJU* AND RAMADASAN KUTTAN**

Amala Cancer Research Centre,
Amala Nagar, Thrissur – 680 555, Kerala, India

Abstract : Chemical compositions of ginger oil as well as its antioxidant, anti-inflammatory and antinociceptive potential were evaluated in the present study. The main constituents as detected by GC/MS analysis was ά-zingiberene which constituted 31% of the total area, ar-curcumene (15.4%) and a -sesquiphellandrene (14.02%). Ginger oil scavenged superoxide, DPPH, hydroxyl radicals and inhibited tissue lipid peroxidation *in vitro*. Intraperitoneal administration of ginger oil was found to inhibit phorbol-12-myristate-13-acetate induced superoxide radicals elicited by macrophages. Oral administration of ginger oil for one month, significantly increased superoxide dismutase, glutathione and glutathione reductase enzymes level ($P<0.001$) in blood of mice and glutathione-S-transferase, glutathione peroxidase and superoxide dismutase enzymes in liver. Ginger oil produced significant reduction in acute inflammation produced by carrageenan and dextran and formalin induced chronic inflammation ($P<0.001$). It also exhibited significant reduction in acetic acid induced writhing movements ($P<0.001$). The present report revealed that ginger oil possesses antioxidant activity as well as significant anti-inflammatory and antinociceptive property.

Key words : antioxidant activity   anti-inflammatory
              antinociceptive   essential oil GC/MS

INTRODUCTION

Ginger, the rhizome of *Zingiber officinale* Roscoe, is one of the most widely used spices and a traditional remedy in Indian, Chinese and Oriental medicine against pain, inflammation and gastrointestinal disorders. Ginger oil is produced from fresh rhizomes of *Zingiber officinale.* It possesses the aroma and flavor of the spice but lacks the pungency. The essential oil of ginger has been found to possess antibacterial, antiviral and antifungal properties (1, 2). The present study reports a detailed pharmacological screening of ginger oil evaluating its antioxidant, anti-inflammatory and antinociceptive properties. It is anticipated that this study will throw further light on the pharmacological and medicinal properties of ginger oil.

*Corresponding author : Dr. Ramadasan Kuttan, Ph.D, Research Director, Amala Cancer Research Centre, Amala Nagar, Thrissur, Kerala – 680 555, India. Tel: 91-487-2307968; Fax: 91-487-2307968; Email: amalacancerresearch@gmail.com, amalaresearch@hotmail.com

MATERIALS AND METHODS

Drugs

The essential oil isolated by steam distillation from the fresh rhizomes of ginger was supplied by Kancore Ingredients Limited, Angamali, Kerala. In order to get a uniform suspension of ginger oil for *in vitro* studies, the oil was dissolved in hexane (100 mg/10 ml) and 10 µl of Triton X 100 (Sigma-Aldrich) was added and further evaporated to dryness and finally made up to 10 ml with distilled water. The oil was dissolved in paraffin oil for all *in vivo* studies.

Animals

Balb/c mice (20–25 g) were used in the study. They were purchased from Small Animal Breeding Station, Mannuthy, Kerala, India and were housed in well ventilated cages under controlled conditions of light and humidity and provided with normal mouse chow (Sai Durga Food and Feeds, Bangalore, India) and water *ad libitum*. All the animal experiments were done as per the instructions prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India, and implemented through the Institutional Animal Ethical Committee of the Research Centre.

Chemicals and reagents

Nitroblue tetrazolium (NBT), glutathione, glutathione oxidized (GSSG), nicotinamide adenine dinucleotide phosphosphate reduced (NADPH) and 5-5'dithiobis 2-niotrobenzoic acid (DTNB) were purchased from Sisco Research Laboratories Pvt. Ltd., (Mumbai, India), 2,2-Diphenyl-1-picryl hydrazyl (DPPH) and 2,2-azobis-3-ethylbenzthiozoline-6-sulphonic acid (ABTS) were purchased from Sigma Aldrich (St.Louis, USA) and phorbol-12-myristate-13-acetate (PMA) was a gift from Dr. Allen Conney, USA. All other chemicals and reagents used were of analytical reagent grade. Carrageenan was purchased from Sigma Aldrich, USA.

Gas chromatography/mass spectrometry analysis of ginger oil

Ginger oil was subjected to gas chromatography/mass spectrometry (GC/MS) analysis using a Hewlett–Packard gas chromatograph (Model 6890) coupled with a quadruple mass spectrometer (Model HP 5973) and a HP – 5MS capillary column (5% phenylmethylsiloxane; 30 m × 320U m × 0.25U m). The interphase, ion source and selective mass detector temperatures were maintained at 243°C, 230°C and 150°C, respectively. Helium was used as a carrier gas at a flow rate of 1.4 ml/min. For the essential oil, the oven temperature was programmed linearly at 60°C; then increased from 60°C to 243°C at the rate of 3°C/min.

Determination of antioxidant activity of ginger oil by *in vitro* method

Superoxide radical scavenging activity

Superoxide radical scavenging activity was determined by the NBT reduction method (3). Different concentrations of the oil (10-200 µg) were added to the reaction medium containing 3 µg NaCN in 6 µM EDTA, riboflavin (2 µM), NBT (50 µM) and phosphate buffer (67 mM, pH 7.8) in a final volume of 3 ml. The tubes containing

reaction mixture were uniformly illuminated with an incandescent lamp for 15 minutes. The optical density was measured at 560 nm before and after illumination. The percentage inhibition of superoxide generation was evaluated by comparing the absorbance value of the control and experimental tubes.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system (Fenton reaction). The reaction mixture (1.0 ml final volume) contained deoxyribose (2.8 mM), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O (1 mM), ascorbate (0.1 mM), KH_2PO_4 – KOH buffer (20 mM, pH 7.4) and different concentrations of the oil (10-200 µg). The reaction mixture was incubated for 1 hr at 37°C. Deoxyribose degradation was measured as TBARS and percentage inhibition calculated. (4). The percentage inhibition was calculated according to the formula: Inhibition percentage (Ip) = $[(A_B - A_A)/A_B] \cdot 100$ where A B and A are the absorbance values of the blank sample and of essential oil solution, respectively.

Determination of inhibition of lipid peroxidation

Different concentrations of the oil (10-200 µg) were added to rat liver homogenate (0.1 ml, 25% w/v), ascorbic acid (0.06 mM), 30 mM KCl, 0.16 mM $FeSO_4$ solution and final volume made up with Tris buffer (40 mM, pH-7) to 0.5 ml. Tubes were incubated for one hour at 37°C. Aliquots of the incubation mixture (0.4 ml) were treated with sodium dodecyl suphate (SDS-8%, 0.2

ml), thiobarbituric acid (TBA-0.8%, 1.5 ml), and acetic acid 20%, pH-3.5, 1.5 ml). The total volume was then made up to 4 ml with distilled water and incubated for 1 hr at 100 4°C water bath. After cooling, 1 ml of distilled water and 5 ml of mixture of n-butanol and pyridine (15:1, v/v) were added and vortexed and after centrifugation the absorbance of the organic layer was measured at 532 nm. The percentage inhibition of lipid peroxidation was determined by comparing the results of the test compound with those of the control (4).

Determination of DPPH radical scavenging activity

Ginger oil at different concentrations (0.1-1 mg) were mixed with 0.375 ml methanolic solution of DPPH (1, 1-diphenyl-2-picrylhydrazyl-0.25 g/L). Total volume was made up to 2 ml with methanol. The disappearance of DPPH was read spectrophotometrically at 515 nm after 20 min of incubation at room temperature in dark (5).

Determination of ABTS radical scavenging activity

ABTS radical scavenging activity of the extract was determined using ferryl myoglobin/ABTS protocol (6). The stock solutions and 500 µM ABTS diammonium salt, 400 µM myoglobin (MbIII), 740 µM potassium ferricyanide, and 450 µM H_2O_2 was prepared in phosphate-buffered saline (PBS; pH 7.4). Metmyoglobin was prepared by mixing equal amounts of myoglobin and potassium ferricyanide solutions. The test tubes contained ABTS (150 µM), MbIII (2.25 µM), varying concentrations of essential oil (10-200 µg) and PBS (pH-7.4) in 2 ml. The reaction was initiated by adding 75 µM H_2O_2

and lag time in seconds was recorded before absorbance of ABTS at 734 nm began to increase.

Ferric reducing antioxidant power assay (FRAP assay)

The ferric reducing ability was measured at low pH. FRAP reagent contained 25 ml 0.3 M acetate buffer, 2.5 ml 4,6-Tris-2-pyridyl-(s)-Triazine (TPTZ) and 2.5 ml ferric chloride. Different concentration of the oil (10-200 μg) was made up to one ml with freshly prepared FRAP reagent. The mixture was incubated at 37°C for 15 minutes and read against distilled water at 595 nm. Standard graphs were constructed using known concentrations of ferrous salt in water/methanol to replace $FeCl_3$ and expressed as EC_1 values. EC_1 is defined as concentration of an antioxidant having a ferric reducing ability equivalent to that of 1 mM ferrous sample (7).

Determination of the effect of extract on PMA-induced superoxide radical generation in peritoneal macrophages

Balb/c mice (4-6 weeks) weighing 20-25 g were used for the study. Animals were divided into 5 groups (3 animals/group). All the animals were injected (i.p) with sodium caseinate (5%) to elicit macrophages. Group I was kept as normal. Group II was kept as control. Group III, IV and V were treated with single dose of ginger oil at different concentration (10, 50 and 250 mg/kg body weight, respectively). On the fifth day 1 hr after oil administration, peritoneal macrophages were activated *in vivo* by injecting phorbol-12-myristate-13-acetate (PMA-100 ng/animal). After 3h, peritoneal macrophages were harvested. The effects of oil on the inhibition of superoxide generation in the macrophages were measured by inhibition in the reduction of NBT to formazan (8). The percentage inhibition was determined by comparing the absorbance values of untreated and treated animals.

Determination of effect of ginger oil on in vivo antioxidant enzyme levels

Balb/c mice (4-6 weeks) weighing 20-25 g were divided into 4 groups of five animals and they were treated orally with ginger oil dissolved in paraffin oil at different doses for 30 days. Group I was kept as normal. Group II was kept as control treated with paraffin oil only. Group III and Group IV were treated with 100 mg ginger oil/kg b.wt and 500 mg ginger oil/kg b.wt respectively.

At the end of the experiment, animals were sacrificed by ether anesthesia, and blood was collected by heart puncture, liver was excised and washed in ice-cold Tris Hcl buffer (0.1 M, pH-7.4), and cytosolic samples of liver homogenate were prepared by centrifuging at 10,000 rpm for 30 mins at 4°C.

The total protein was estimated by the method of Lowry, Rosenbroug, Farr and Randall, 1951 (9). Hemoglobin was estimated by the cyanmethemoglobin solution using Drabkin's method (10). Whole blood was used for determination of superoxide dismutase, catalase and glutathione levels. Serum was used for glutathione reductase estimation. Liver tissue homogenate was used for super oxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione –S-transferase and glutathione assessment as given below.

Superoxide dismutase activity was measured by the NBT reduction method (3). Catalase activity was estimated by the method of Aebi, 1974 (11) by measuring the rate of decomposition of hydrogen peroxide at 240 nm. Glutathione activity was assayed by the method of Moron, Depierre and Manner, 1979 (12) based on the reaction with DTNB. The assay of glutathione peroxidase was done by the method of Hafeman, Sundae and Houestra, 1974 (13) based on the degradation of H_2O_2 in the presence of GSH. Glutathione reductase activity was measured by the method of Racker, 1955 (14). The method of Habig, Pabst and Jakoby, 1974 (15) was followed to assay the activity of glutathione -S-transferase (GST) which is based on the rate of increase in conjugate formation between GSH and 1-chloro-2, 4-nitrobenzene (CDNB).

Determination of anti-inflammatory activity of ginger oil

Acute inflammation models using carrageenan and dextran induced paw edema in mice

The experimental mice were divided into 10 groups (6 animals/group). Group I was kept as control group -carrageenan (1% w/v). Group II was treated with standard drug, Diclofenac (10 mg/kg, i.p.). Group III, IV and V were treated with different concentrations of ginger oil (100, 500, 1000 mg/kg), intraperitoneally for 4 consecutive days. One hour, after the fourth dose of ginger oil administration, acute inflammation was induced by sub-plantar injection of 0.02 ml carrageenan (1% w/v, in 0.1% carboxy methyl cellulose) in the right hind paw (16). The thickness of paw was measured at 0, 1, 2, 3, 4, 6 and 24 hr using Vernier calipers after carrageenan injection to determine the formation of edema.

In the dextran model, edema was induced by injection of dextran. Group VI was kept as control goup, dextran (1% w/v). Group VII was treated with standard drug, diclofenac, (10 mg/kg.i.p). Group VIII, IX and X were treated with different concentrations of ginger oil (100, 500, 1000 mg/kg), intraperitoneally for 4 consecutive days. One hour, after the fourth dose of ginger oil administration, acute inflammation was induced by sub-plantar injection of 0.02 ml dextran (1% w/v in 0.1% carboxy methyl cellulose) in the right hind paw (17). The thickness of paw was measured using Vernier calipers at 0, 1, 2, 3, 4 and 6 hours and finally after 24 hrs. The percentages of inhibition were calculated according to the following formula :

$$\% \text{ Inhibition} = [(V_T - V_0)_{control} - (V_T - V_0)_{treated group}] \times 100/(V_T - V_0)]_{control}$$

Where V_T is paw thickness 3 hr after injection V_0 is the initial paw thickness.

Chronic inflammation model using formalin induced paw edema in mice

Balb/c mice were divided into 5 groups (6 animals/group). Group I was kept as control and group II was treated with standard drug diclofenac (10 mg/kg, i.p). Group III, IV and V were treated with different concentrations of ginger oil (100, 500, 1000 mg/kg, i.p). Chronic inflammation was induced by sub-plantar injection of freshly prepared 0.02 ml of 2% formalin on the right hind paw in all groups (18). Drug treatment was started 1 hour prior to formalin injection and continued for 6 consecutive days. The inflammation was measured using Vernier calipers before and

after injection of formalin and for 6 consecutive days. The percentage inhibition was calculated using the formula given above.

Determination of antinociceptive activity of ginger oil

Acetic acid-induced writhing in mice

Balb/c mice were divided into 5 groups (5 animals/group). Acetic acid (0.6% v/v, 10 ml/kg) was injected into the peritoneal cavities of mice, which were placed in a large observation boxes, and the intensity of nociceptive behavior was quantified by counting the total number of writhings occurring between 0 and 20 mins after stimulus injection (19). The vehicle, paraffin oil and ginger oil (100, 500 and 1000 mg/kg, i.p) were given 30 mins prior to acetic acid injection. Aspirin (100 mg/kg, i.p) was used as the standard reference drug. The writhing response consisted of a contraction of the abdominal muscle together with a stretching of the hind limbs. The antinociceptive activity was expressed as the writhing scores over a period of 20 min. Antinociceptive effect was expressed as reduction of number of writhes between control and treated groups, using the formula: [(C-D)/C] x 100 where C is the average number of writhings for control group of mice and D is the average writhings of the drug treated mice.

Statistical analysis

The values were expressed as mean± standard deviation (SD). Statistical evaluation of the data was done by one way ANOVA followed by Dunnet's test (post-hoc) using Instat 3 software package.

RESULTS

Composition of ginger oil

A number of compounds were identified by GC/MS analysis of ginger essential oil and the most prominent components are given in Table I. The principal constituent of ginger oil was zingiberene (31.08%), a sesquiterpene hydrocarbon, followed by ar-curcumene (15.4%) and α-sesquiphellandrene (14.02%). Other compounds include bisabolene (13.80%) and sabinene (8.27%). α-Zingiberene has been reported to be the major constituent by Singh, Kapoor, Singh, Heluani and Lampasona, 2008 (20) while Agarwal, Walia, Dhingra and Khambay, 2001 (21) has reported curcumene as the major constituent.

Antioxidant activities of ginger oil *in vitro*

The essential oil of ginger was found to scavenge superoxide, hydroxyl radicals and inhibit tissue lipid peroxidation (Fig. 1). Ginger oil gave an IC_{50} of 36 µg/ml for

TABLE I : Chemical composition of ginger oil.

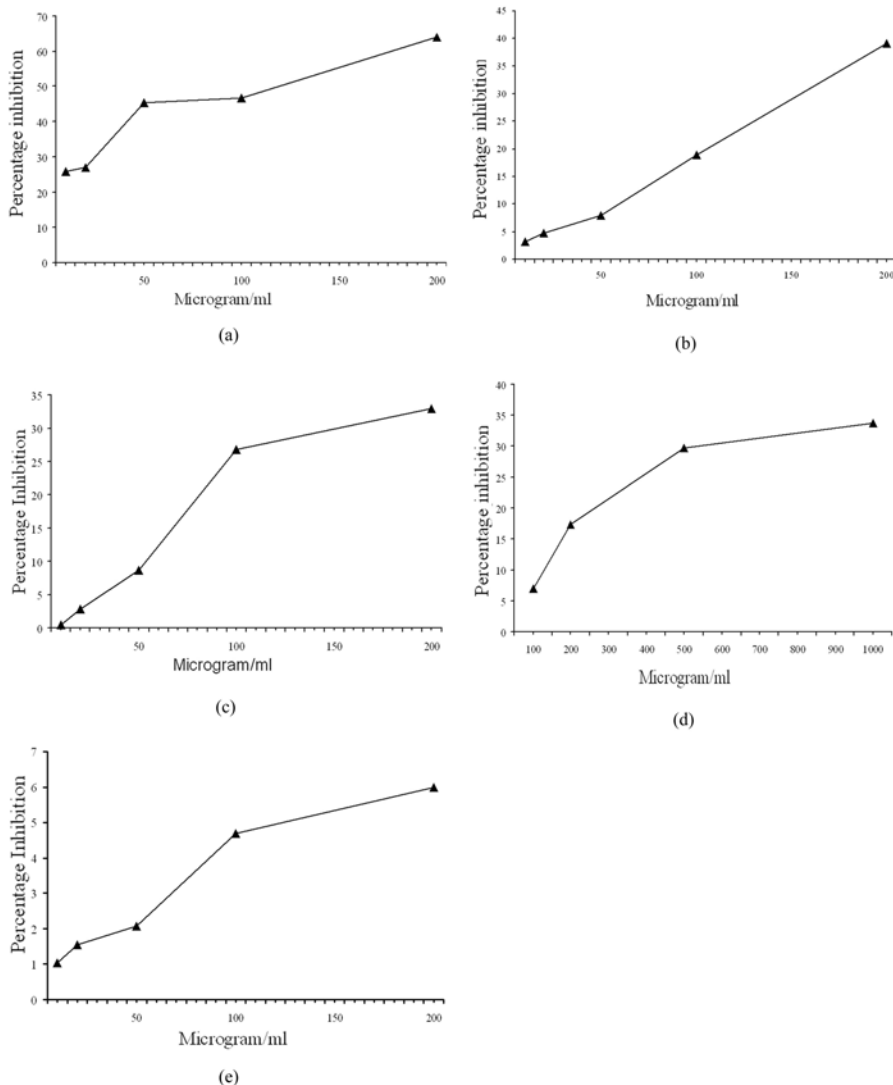| No.	Compound	(% of total area)
1.	Camphene	5.14
2.	Sabinene	8.28
3.	Isoborneol	1.14
4.	α-Cubebene	0.75
5.	α-Elemene	1.57
6.	γ-Elemene	0.58
7.	Aromadendrene	0.34
8.	Germacrene	2.40
9.	Ar-Curcumene	15.35
10.	beta-Panasinsene	1.47
11.	Zingiberene	31.08
12.	Beta Bisabolene	13.81
13.	α-Sesquiphellandrene	14.02
14.	Verbenone	0.29
15.	Elemol	0.45
16.	Thujopsene	1.04
17.	Silane	1.61
18.	Farnesol	0.70

Fig. 1 : *In vitro* free radical scavenging activity of ginger oil. (A) Superoxide radical scavenging activity. (B) Hydroxyl radical scavenging activity. (C) Inhibition of lipid peroxidation. (D) DPPH radical scavenging activity.(E) ABTS radical scavenging activity.

scavenging superoxide radicals but IC_{50} for inhibition of hydroxyl radicals and lipid peroxidation was found to be greater than 200 µg/ml and 400 µg/ml respectively and so could not be calculated. The ferric reducing activity for 50 µg of ginger oil was found to be 1.8 mM for ginger oil. Ginger oil possessed only mild DPPH radical scavenging ability as IC_{50} was more than 1000 µg/ml. It

showed only a mild capacity to scavenge ABTS radical.

Effect of ginger oil on PMA-induced superoxide radical generation

Superoxide radicals generated during the activation of PMA in sodium caesinate induced macrophages *in vivo* were found to

be scavenged by ginger oil. The percentage inhibition was 18.25% for ginger oil at 250 mg/kg bw respectively.

Effect of administration of ginger oil on antioxidant enzymes and glutathione

Antioxidant enzymes in blood and serum of mice were increased after administration of ginger oil for a period of 30 days (Table II). Catalase was found to be increased in all animals treated with 100 and 500 mg/kg bw of ginger oil (P<0.05). The essential oil administration significantly elevated superoxide dismutase (P<0.001) at both concentrations. Glutathione level was also found to be significantly increased in treated

groups at 100 mg/kg bw (P<0.01) and 500 mg/kg bw (P<0.001). Glutathione reductase was elevated by the administration of ginger oil (P<0.05) in all animals treated with 100 mg/kg bw and significant activity was shown at 500 mg/kg bw (P<0.001).

Ginger oil also showed a significant effect on some of the antioxidant enzymes in liver tissue of mice after treatment for 30 days (Table III). Catalase did not change singnificantly in any of the treated groups. Superoxide dismutase activity was elevated in the group treated with 500 mg/kg.bw (P<0.01) ginger oil. The level of glutathione peroxidase enzyme was significantly increased by ginger oil at 500 mg/kg bw

TABLE II : Effect of oral administration of ginger oil for one month on antioxidant systems in blood.

Treatment	Catalase (k/g Hb)	Superoxide dismutase (U/g Hb)	Glutathione reductase (U/g Hb)	Glutathione (nmol/ml)
Normal	34.74±8.18	478±43.97	2.88±0.56	46.5±4.9
Control	32.52±7.38	445±39.62	2.76±0.25	44.8±7.3
100 mg/kg bw	57.91±8.30*	610±31.07*** (↑ 27.12%)	6.58±.82* (↑ 58%)	68.6±6.4** (↑ 34.69%)
500 mg/kg bw	51.22±18.19*	872±24.77*** (↑ 48.95%)	8.15±1.62*** (↑ 66.14%)	78.4±5.7*** (↑ 42.85%)

Each value represents the mean±S.D (n=5). *P<0.05 compared with control, **P<0.01 compared with control, ***P<0.001 compared with control. ↑ indicates % increase.

TABLE III : Effect of oral administration of ginger oil for one month on antioxidant systems in liver.

Treatment	Catalase (U/mg protein)	Superoxide dismutase (U/mg protein)	Glutathione peroxidase (U/mg protein)	Glutathione -S-transferase (nmol/mg protein)	Glutathione reductase[a]	Glutathione (nmol/ml)
Normal	5.29±0.66	0.84±0.12	8.13±0.30	41.57±1.66	88.83±12.53	12.7±1.6
Control	4.97±0.76	0.83±0.03	8.82±1.51	39.46±3.43	79.00±20.16	11.4±1.7
100 mg/kg bw	5.48±0.98	0.90±0.16	12.98±6.09	32.00±11.61	79.79±17.49	12.2±2.3
500 mg/kg bw	7.36±0.45	1.10±0.13* (↑ 24.54%)	18.89±7.34 (↑ 53.30%)	103.09±12.6*** (↑ 61.72%)	69.78±9.53	13.8±3.4

*P<0.05 compared with control, **P<0.01 compared with control, ***P<0.001 compared with control. ↑ indicates % increase. Each value represents the mean±S.D (n=5). [a](nmol of NADPH consumed/min/mg Protein).

(P<0.01). Even though glutathione was found to be increased, it was not found significant. The level of glutathione reductase was found to be unaltered in both the treated groups. Glutathione-S-transferase (GST) level was found to be elevated in animals and significant activity was shown at 500 mg/k bw (P<0.01) treated group.

Effect of administration of ginger oil in acute inflammation models

Anti-inflammatory effect of ginger oil was evaluated in acute edema models. As observed in Fig. 2a, intraperitonieal treatment with ginger oil in mice at 100, 500 and 1000 mg/kg was capable of reducing the odema formation by carrageenan in a dose dependent manner by 27.8, 44.4 and 61.1% (P<0.001) when compared to control. Diclofenac (10 mg/kg) gave a percentage inhibition of 55.6%.

The inhibitory values of edema at 3 hr post dextran treatment were 35, 41.2 and 52.9% (P<0.001 for 100, 500 and 1000 mg/kg of ginger oil respectively (Fig. 2b) while diclofenac gave similar activity at 10 mg/kg body weight.

Effect of administration of ginger oil in chronic inflammation models

Ginger oil showed significant suppression of inflammation in chronic model of inflammation induced by formalin in a concentration dependent manner. Percentage inhibition of 54.17, 62.5 and 70.8% (P<0.001) were shown at doses of 100, 500 and 1000 mg/kg respectively (Fig. 2c) while diclofenac (10 mg/kg) showed an inhibition of 54.8%.

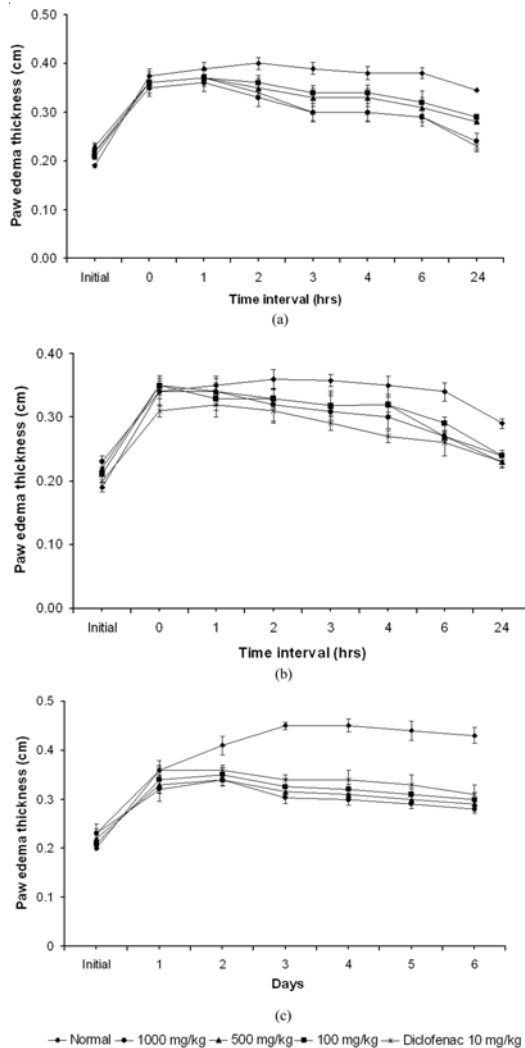Fig. 2 : Anti-inflammatory activity of ginger oil. (A) Carrageenan model. (B) Dextran model. (C) Formalin model.

Effect of ginger oil on acetic acid induced writhing model

Ginger oil showed marked and significant (P<0.001) reduction in the number of writhings induced by acetic acid. As shown in Fig. 3, the analgesic activity at all tested doses (100, 500 and 1000 mg/kg) indicated a dose dependent relationship in the inhibition
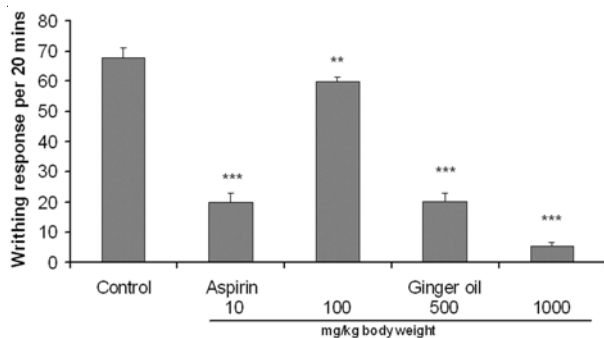
Fig. 3 : Effect of ginger oil in nociceptive behaviour of mice evaluated in acetic acid induced abdominal writhing model. Groups of animals were pretreated with aspirin (10 mg/kg bw), ginger oil (1000, 500 and 100 mg/kg bw, i.p) 30 mins before acetic acid injection. Nociception was measured by the number of writhes the animal showed 20 mins following intraperitonieal 0.6% acetic acid injection. Values are reported as mean±S.D. **$P<0.01$, ***$P<0.001$.

of writhing reflux by 13.08, 70.64, and 92.15%. The antinociceptive activity of ginger oil was compared with that of the standard drug at aspirin (10 mg/kg).

DISCUSSION

Reactive oxygen species (ROS) are formed in both physiological and pathological conditions in mammalian tissues. The uncontrolled production of free radicals is considered to be an important factor in tissue damage which can induce pathophysiological changes. Free radicals also play an important role in inflammation that can mediate tissue destruction. Cells have several antioxidant enzymes and other antioxidant mechanisms to protect themselves from the harmful effects of oxidants. The latter include glutathione (GSH) and numerous GSH dependent enzymes, metal binding proteins, and vitamins. Major antioxidant enzymes are the superoxide dismutase (SOD), catalase and peroxidases, of which glutathione peroxidases (GPx) are thought to be the most important.

The current study indicates that ginger oil could inhibit the oxygen radicals as seen from the inhibition of lipid peroxidation, scavenging of superoxide and hydroxyl radical *in vitro*. However they showed only a mild scavenging activity against stable free radicals such as DPPH and ABTS. Ginger oil also scavenged the superoxide radicals generated *in vivo* in mice peritoneal macrophages. The antioxidative potential of ginger oil may be due to the mixture of dozens of compounds of different functional groups, polarity and chemical behavior which produces either synergistic or antagonistic effect on antioxidant activity. Many reports emphasis that phenolic groups play an important role in antioxidant activity (22). In the present study, percentage of phenolic compounds was found to be low compared to previous reports (20). Administration of ginger oil was capable of protecting the cells from intracellular oxidative damage *invivo* by increasing the serum antioxidant status as clearly observed from the data.

Carrageenan and dextran are widely used as noxious agents to induce experimental inflammation to screen anti-inflammatory agents. The irritant effect induced by carrageenan is the result of activation of kinin and complement cascades and the release of anti-inflammatory mediators such as vasoactive amines (histamine, serotonin, 5-hydroxytryptamine and bradykinins) during the early phase of inflammation and kinin like substances and eicasanoids (prostaglandins, proteases and lysosomes) during the later phase of inflammation (23, 24). Dextran induces paw edema mainly by releasing histamine and 5-hydroxytryptamine to the

site of inflammation from the mast cells (25). In the present study, treatment with essential oil of ginger oil was effective in reducing the oedamatogenic response evoked by carrageenan and dextran in a dose dependant manner. In chronic inflammatory models, inhibition of formalin induced oedema is considered as one of the most suitable test procedures to screen anti inflammatory agents as it closely resembles human arthritis (26). Inflammation induced by formalin comprises an early neurogenic response mediated by substance P and bradykinin followed by a tissue mediated response where histamine, 5-hydroxytryptamine, prostaglandins and bradykinin are involved (27). Ginger oil displayed strong anti-inflammatory activity in chronic inflammation model and the mode of action may be due to the inhibition of prostaglandin release.

Acetic acid induces pain by enhancing the levels of endogenous substances like PGE_2 and PGF_2 (28) in the peritoneal cavity. This indicates that acetic acid acts indirectly in the stimulation of nociceptive neurons by the release of endogenous mediators. Thus, acetic acid is used to screen compounds for peripheral analgesic activity. In the present study, ginger oil showed dose dependent and significant inhibition of acetic acid induced writhes in mice suggesting that ginger oil has strong antinociceptive activity and the mode of action might involve the inhibition of synthesis of arachidonic acid metabolite mediated by COX inhibition.

Thus, in addition to imparting flavor to food, ginger oil possesses potential health benefits by scavenging the free radicals formed in the body. The study also indicates the efficacy of ginger oil as an efficient therapeutic agent in acute and chronic inflammatory conditions.

ACKNOWLEDGEMENTS

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