

Effects of oral administration of an aqueous ginger extract on anxiety behavior and tryptophan and serotonin metabolism in the rat



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ABSTRACT

Background: *Zingiber officinale* (ginger) is used widely as a herb and medicine. It contains among its constituents 6-Gingerol (a phenol) and quercetin (a flavonoid) that possess anxiolytic and antidepressant properties, but the potential biochemical mechanism(s) of these effects has not been assessed, particularly in relation to serotonin synthesis and neurotransmission. **Aims and Objectives:** We investigated the anxiolytic-like activity of an aqueous ginger extract by evaluating its influence on behavior, and its effects on serotonin metabolism and on metabolism and disposition of the serotonin precursor tryptophan (Trp) in rats. **Materials and Methods:** An aqueous ginger extract was given orally in a single daily dose equivalent to 500 mg of ginger material per kg body weight for 3 weeks. The elevated plus maze test of anxiety and Trp metabolism and disposition and brain serotonin synthesis and turnover were assessed in Ginger-treated and control rats. **Results:** When compared with controls, ginger-treated rats showed a significant increase in the time spent in the open arm, indicative of decreased anxiety. However there was no effect on locomotor activity in open field test. The extract caused significant decreases in activities of liver Trp 2,3-dioxygenase and significant increases in concentrations of serum Trp and corticosterone and brain Trp, serotonin and the major serotonin metabolite 5-hydroxyindoleacetic acid. **Conclusion:** An aqueous ginger extract exerts an anxiolytic effect in a behavioral model of anxiety, which may be caused by increased serotonin synthesis, and influences tryptophan metabolism and disposition in a manner analogous to antidepressant drugs.

Key words: Anxiety; depression; ginger; serotonin; tryptophan; tryptophan 2,3-dioxygenase

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INTRODUCTION

Ginger (*Zingiber officinale*) has been in use as a herb and medicine since early times. Ginger was highly valued for its therapeutic properties and played a significant role in primary health maintenance in Asia.¹ *Zingiber officinale* also exhibits anxiolytic-like properties in rats.² Detailed studies of the effects of ginger and its active constituent 6-gingerol on biological processes have been performed. 6-Gingerol also exerts antidepressant effect in the tail suspension test in mice.³ Ginger extract was as effective as

diazepam in reducing anxiety.⁴ and its pattern of behavior in the forced swimming test resemble that of serotonergic antidepressants.⁵

The bioflavonoid quercetin is abundant in Ginger.⁶ and is known to exert a wide range of behavioral effects, such as anxiolytic and antidepressant. Quercetin administration in rats has been found to attenuate stress-induced behavioral and biochemical effects.⁷ Recently, it has been reported that administration of quercetin (10, 50, and 100 mg/kg) daily for 3 weeks improved anxiety-like behaviors.⁸ However,

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studies related to mechanisms of affect and cognition have received less attention.

That serotonin (5-hydroxytryptamine or 5-HT) metabolism and function play an important role in anxiety, depression and some other central nervous system (CNS) abnormalities is an established fact.⁹ Ginger and its active constituents may influence CNS 5-HT metabolism and function by various actions, e.g., by enhancing its synthesis, decreasing its degradation or release and/or blocking its receptors.³

In the present paper, we investigated the effects of an aqueous ginger extract on serotonin synthesis in rat brain and likely mechanisms of potential changes and demonstrate for the first-time enhancement of 5-HT synthesis by inhibition of liver tryptophan (Trp) 2,3-dioxygenase (TDO) activity leading to increased availability of the serotonin precursor Trp to the brain. We also confirm the anxiolytic-like behavior of the ginger extract in the elevated plus maze test and suggest the potential involvement of the TDO inhibition.

MATERIALS AND METHODS

Ginger material

Ginger rhizomes were purchased from a local supermarket. The ginger used is the same variety widely used as a spice and medicine, even if not validated. Validation requires identification of the material as ginger (*Zingiber officinale*) and possible variety, and, additionally, potential analysis of its active constituents. As the beneficial effects of ginger in the World population at large are derived from use of local, rather than validated, supplies, we consider our choice of the ginger source in the present preliminary study to be appropriate.

Preparation of ginger aqueous extract

An aqueous ginger extract was prepared from locally available ginger rhizomes essentially as described by.¹⁰ Ginger rhizome was peeled on ice and a 50 g portion was cut into small pieces and homogenized in 75ml of cold saline. The homogenate was then filtered (with cheese cloth) and centrifuged for 10 min. The supernatant was made up to 100 ml with physiological saline and stored in small portions at -20°C until use. The concentration of this ginger preparation was considered to be 500 mg/ml.

Animals and treatments

Locally bred male Albino-Wistar rats (150-200 g body weight) were purchased from the Aga Khan University Animal House and were kept in plastic cages. Rats were placed in a quiet room at a maintained temperature (25±2°C) under a 12-h light-dark cycle and lab chow was given to rats with tap water *ad libitum*. Rats were acclimatized to the new environment

for 3-4 days prior to treatment. The study was ethically approved by institutional animal ethics committee, University of Karachi. All measures were taken to reduce the number of animals and any likely pain or suffering. The animals were divided into two equal groups (n=6 each), with the control group receiving saline and the test group receiving the ginger extract orally at a single daily dose of 500 mg/kg/ml for 3 weeks. Ginger treated groups looked in good health as evidenced from their normal food intake and body weight gain during treatment. On the last day of treatment, the animals were subjected to the Open Field and Elevated plus Maze tests. Rats were then killed by decapitation and whole blood was collected, from which serum was isolated and stored at -4°C. Livers were perfused with ice-cold saline and were stored along with whole brains at -70°C until analysis.

Open field Test (OFT)

The behavior of rats was assessed by the OFT after 3 weeks of ginger extract administration. The open-field apparatus (76 x57x35 cm) was divided into central and peripheral areas. Each rat was placed in the center of the open field and the test session was videotaped. In the test session (5 minutes), the number of square crossings, rearing, and time spent moving were monitored to calculate the movement of the rodent.¹¹

Elevated plus maze (EPM) test

The EPM consisted of two open arms (30 × 5 × 0.2 cm) and two closed arms (30 × 5 × 15 cm) that directly opposed each other. Rats were placed individually in the center of the maze facing an open arm, and the times spent in open and closed arms were recorded during a 5- min test period.¹²

Ginger material and chemicals

The ginger was purchased from a local market. All chemicals were of analytical grade either from BDH laboratories UK or Merck, Germany.

Biochemical determinations

Tryptophan 2,3-dioxygenase (TDO) activity was determined in homogenates prepared from frozen perfused livers, either in the absence (holoenzyme activity) or presence (total enzyme activity) of added (2 μM) hematin (dissolved in 0.1 M NaOH) as described earlier.¹³ The apoenzyme activity was obtained by difference. Serum and liver [Trp] were measured fluorimetrically as described earlier.¹⁴ Brain tryptophan, 5-HT and 5-HIAA levels were determined by high-performance liquid chromatography (HPLC) with fluorimetric detection.¹⁵ Serum corticosterone was determined fluorimetrically.¹⁶

Statistical analysis

Data are presented as means ± SEM and were analyzed by Student's *t*-test. A probability *P* value of < 0.05 was considered indicative of significance.

RESULTS

Effects of ginger extract on rat liver tryptophan 2,3-dioxygenase (TDO) activity and the haem saturation of the enzyme

Table 1 shows that the ginger extract inhibited TDO activity significantly. The three forms of the enzyme were inhibited to similar extents: by 42%, 41% and 39% respectively. The haem saturation of TDO, an expression of haem availability, was not significantly altered by the ginger extract irrespective of whether saturation was expressed as a ratio or a percentage.

Effects of ginger extract on concentrations of tryptophan and/or 5-hydroxyindoles in brain, liver and serum

These effects are shown in Table 2. Serum total (free + albumin-bound) [Trp] was increased significantly by the ginger extract by 72%. Liver [Trp] was also increased by 28%. The ginger extract caused significant elevations of brain [Trp], [5-HT] and [5-HIAA] of 59%, 27% and 47% respectively. These brain indoles are also expressed in Table 2 in molar units to facilitate calculations of approximate values for 5-HT synthesis and turnover. Using group means, 5-HT synthesis and turnover were increased by 32% (mean sums of 5-HT + 5-HIAA in μM were: control 2.06; ginger 2.72). 5-HT turnover, expressed as the ratio of [5-HIAA]/[5-HT], was significantly increased by 17%.

Effects of ginger extract on serum corticosterone concentration

The extract increased the concentration of serum corticosterone, the major glucocorticoid in rats, by 39% ($P < 0.05$). Values (in $\mu\text{g}/\text{dl}$: means \pm SEM for each group of 6 rats) were as follows: controls 31 ± 2 ; ginger 43 ± 4 .

Effects of ginger extract on anxiety and exploratory behaviour

Table 3 shows the effects of ginger extract on anxiety and general exploratory behavior using the elevated plus maze and open-field tests. There was a significant 50% increase in time spent in the open arm and a significant, if small (4%) decrease in time spent in the closed arm by rats treated with the ginger extract, compared to controls. By

contrast, exploratory behavior through locomotor activity of rats in the open field test was not significantly altered by the ginger extract.

DISCUSSION

The present behavioral findings in rats with the ginger extract demonstrating its anxiolytic-like effect in the elevated plus maze test (Table 3) confirm previous observations in mice.⁴ Our studies are concerned mainly with the role of the serotonin system in anxiety and also in depression. That serotonin plays an important role in anxiety-related behavior and in the mechanisms of action of anxiolytics is well documented,⁹ see also references cited therein, with serotonin receptors playing differential roles. The serotonergic system may be involved in the molecular mechanism of action of the anticonvulsant, antiemetic and anxiolytic effects of ginger.²

A factor determining the role of 5-HT in anxiety is its synthesis from Trp. This 2-step process involves hydroxylation of Trp to 5-hydroxytryptophan (5-HTP) by Trp hydroxylase (TPH) followed by decarboxylation of the latter to 5-HT by aromatic *L*-amino acid decarboxylase (ALAAD). Of these 2 enzymes, TPH is the rate-limiting, as its activity is determined by availability of the Trp substrate, because it exists partially (at least 50%) unsaturated with Trp, i.e., the K_m of the enzyme is higher than brain [Trp].¹⁷ Accordingly, moderate fluctuations in circulating Trp availability to the brain can have significant effects on 5-HT synthesis. In humans, however, ALAAD activity may also be rate-limiting.¹⁸ Evidence for the role of 5-HT synthesis in anxiety is based on the findings that inhibition of synthesis induces anxiety, whereas enhancement of synthesis decreases anxiety. Thus acute Trp depletion (ATD), a diagnostic test of the role of 5-HT in behavioral and other disorders,¹⁹ which induces a transient inhibition of brain 5-HT synthesis, increases anxiety under experimental conditions in normal subjects and in patients with anxiety disorders.²⁰ though this is by no means a universal effect. Factors such as gender, nutrition and type of anxiety and of comorbidity may all play a part. By contrast, increasing 5-HT synthesis

Table 1: Effects of administration of aqueous ginger extract on rat liver tryptophan 2,3-dioxygenase (TDO) activity and saturation of the enzyme with its haem cofactor

Treatment	TDO activity			TDO haem saturation	
	Holoenzyme	Total enzyme	Apoenzyme	HSR	% HS
Control	3.47 \pm 0.24	7.43 \pm 0.03	3.96 \pm 0.24	0.88 \pm 0.08	47 \pm 3
Ginger extract	2.00 \pm 0.09*	4.42 \pm 0.20*	2.42 \pm 0.05*	0.83 \pm 0.06	45 \pm 2

Rats (n=6 per group) were treated with a single daily oral dose of an aqueous ginger extract equivalent to 500 mg of ginger material/kg body weight or a saline control for 3 weeks. Activity of TDO and TDO saturation with haem were determined as described in the Materials and Methods section. Values are means \pm SEM. TDO activity is expressed in μmol of kynurenine formed/h/g wet weight of liver. The TDO haem saturation is expressed as the haem-saturation ratio (HSR: holoenzyme activity/apoenzyme activity) and as the % haem saturation (%HS: 100 X holoenzyme activity/total enzyme activity). Differences were significant at P values of $* < 0.001$.

Table 2: Effects of administration of aqueous ginger extract on concentrations of rat liver, serum and brain tryptophan and brain 5-hydroxyindoles

Parameter	Treatment	
	Control	Ginger extract
Liver [Trp] ($\mu\text{g/g}$ wet wt)	7.02 \pm 0.20	9.00 \pm 0.19***
Serum [Trp] ($\mu\text{g/ml}$)	7.40 \pm 0.50	12.70 \pm 0.70***
Brain [Trp] ($\mu\text{g/g}$ wet wt)	4.25 \pm 0.79	6.74 \pm 0.69 [¶]
Brain [Trp] (μM)	20.81 \pm 3.80	33.00 \pm 3.30 [¶]
Brain [5-HT] (μM)	1.45 \pm 0.09	1.83 \pm 0.12 [¶]
Brain [5-HIAA] (μM)	0.61 \pm 0.05	0.89 \pm 0.09*
Brain [5-HIAA]/[5-HT] ratio	0.42 \pm 0.01	0.49 \pm 0.02**

Experimental details are as described in Table 1 and analytical methods are as described in the Materials and Methods section. Values are means \pm SEM for each group of 6 rats. Significance of the differences between the test and control groups is indicated as follows: [¶] $P < 0.05$; * $P < 0.02$; ** $P < 0.01$; *** $P < 0.001$

Table 3: Effects of administration of aqueous ginger extract on anxiety and exploratory behaviors using the elevated plus maze and open-field tests

Test	Elevated plus maze		Open field (number of squares crossed in 5 min)
	Closed arm (sec)	Open arm (sec)	
Control	280 \pm 2	20 \pm 0.5*	120 \pm 20
Ginger extract	270 \pm 2*	30 \pm 0.6*	117 \pm 15

Values are means \pm SEM for each group of 6 rats. * $P < 0.001$

can have an anxiolytic effect. This is best illustrated in experimental animals. Thus, deletion of the TDO gene, which leads to remarkable increases in circulating and brain [Trp] and in brain 5-HT and its major metabolite 5-hydroxyindoleacetic acid (5-HIAA), decreases anxiety in mice subjected to the elevated plus maze and open field tests.²¹ The present results in Tables 2 and 3 demonstrate similar phenomena, namely an anxiolytic effect of a ginger extract associated with increased availability of circulating Trp to the brain and a subsequent augmentation of 5-HT synthesis and turnover. The absence of an effect of the ginger extract on locomotor activity cannot be explained without investigating potential changes in serotonin release or reuptake.

Adrenal glucocorticoids play an important role in emotionality and the protection by 5-HT_{1A} receptor agonists against stress-induced emotional changes involves adrenocortical activity.²² In our study, rat serum [corticosterone] was elevated by the ginger extract by 39%. As in a human study²³ such corticosterone elevation is unlikely to involve an effect on Trp availability to the brain, because, although corticosterone (glucocorticoids) decreases Trp availability to the brain and hence 5-HT synthesis by liver TDO induction,²⁴ the increases in serum [Trp] and brain indoles in the present study exclude this latter mechanism.

The increased cerebral 5-HT synthesis and turnover observed in the present study is caused by the ginger extract increasing Trp availability to the brain, as suggested by the elevation of serum total [Trp]. This latter effect is due to liver TDO inhibition. However, although the ginger-induced increase in liver [Trp] (29%) is broadly similar to that (24-27%) induced by TDO inhibition by chronic administration of drugs of dependence,²⁴ the elevations of serum and brain [Trp] by the ginger extract (72% and 59% respectively) are far greater than those by drugs of dependence (the latter being 21-26% and 23-31% respectively). Elevation of liver, serum and/or brain [Trp] by acute administration of antidepressant drugs is also lower than that by ginger (13-47%), with only a few exceptions.²⁵ A possible explanation of these differences is that of the ginger extract inhibiting activity of the TDO active holoenzyme, whereas the above drugs inhibit only the apoenzyme by preventing its activation by the haem cofactor. Inhibition of the active form is more likely to be effective in eliciting changes in Trp disposition than prevention of conjugation of the apoenzyme (whose half-life is \sim 2h, compared with \sim 7h for the holoenzyme²⁶ and so is dependent on haem availability. The similar levels of inhibition of the TDO forms suggest that the ginger extract does not act by preventing the haem conjugation. Also, the absence of changes in the haem saturation of the enzyme further suggests that ginger does not influence haem synthesis or degradation.

TDO inhibition leading to elevation of brain [Trp] and enhancement of 5-HT synthesis and also turnover is a common effect of a wide range of antidepressant drugs of different chemical structures and pharmacological profiles, and other agents.^{13,18,25} The ginger extract shares this effect. As stated in the Introduction, ginger extracts and 6-gingerol exert antidepressant-like effects in rat and mouse models.^{5,2}

The majority of active constituents of ginger are phenolic in nature. Several earlier studies showed that TDO activity is inhibited *in vitro* by phenols, such as catechol, dihydroxyphenylalanine, epinephrine, 5-HTP, norepinephrine, resorcinol and salicylate. Authors of these studies postulated that inhibition may involve interaction with the haem iron at the TDO haem-binding site and it is noteworthy that only the holoenzyme was the TDO form examined in all these studies. Further studies are clearly required to identify the TDO inhibitory constituents of ginger and to further develop the present findings.

CONCLUSION

We have demonstrated for the first time in this preliminary study the ability of an aqueous crude ginger extract

to enhance cerebral serotonin synthesis and turnover by increasing circulating tryptophan availability to the brain secondarily to inhibition of hepatic tryptophan 2,3-dioxygenase activity. Our results suggest that these biochemical changes are involved in the anxiolytic effects of ginger and possibly also its potential antidepressant activity, which may be due to synergistic action of phenolic and flavonoid compounds on tryptophan metabolism and disposition. Thus, the present results provide a new avenue of experimental and clinical studies with this plant and its active constituents in affective disorders.

LIMITATIONS OF THIS STUDY

These limitations include absence of information on the content of the active constituents of the ginger extract, mainly 6-gingerol and 6-shogaol, which component(s) mediates the TDO inhibition and consequent changes in Trp disposition, and on whether the extract has a direct inhibitory effect *in vitro* on TDO activity. Future studies should address these questions.

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Author's contribution:

SB – Concept, design of the study and revision of the manuscript; **HS**- Reviewed literature, statistically analyzed and prepared first draft of manuscript; **AA, BB** – interpreted the results and revision of the manuscript.

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