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Research Article

Evaluation of Anti Anorectic Activity of *Zingiber officinale* R. (Zingiberaceae)

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ABSTRACT

A rhizome of *Zingiber officinale* R. commonly known as Ginger is one of the most popular anti-inflammatory drug in Indian traditional medicine. 6-gingerol is one of the constituent contributing in anti-inflammatory activity of Ginger. Ginger extract and 6-gingerol act as an anti inflammatory via inhibition of proinflammatory cytokines. Increase in proinflammatory cytokines is also responsible for the reduction in food intake resulting anorexia. The aim of the present study was thus to determine antianorectic activity of standardized hydroalcoholic extract of ginger and similarly the biomarker 6- gingerol was also studied to evaluate its contribution in antianorectic activity. Anorexia was induced by intraperitoneal administration of *E.coli* lipopolysaccharide (100µg/kg) and Fluoxetine (FLU 8mg/kg) in rats. The effect of same doses of the extract was also tested in freely feeding rats. Effect of ginger and 6- gingerol both were studied The results showed that at 200 and 400 mg/kg, ginger reversed the anorectic effect while 6- gingerol at 5mg/ kg require for the effect. Moreover the same doses did not modify the food intake in freely feeding rats. These findings provide strong evidence that ginger is able to attenuate anorexia induced by proinflammatory cytokines mediators.

Keywords: *Zingiber officinale*, Anorexia, 6- gingerol, LPS induced anorexia, Fluoxetine induced anorexia.

INTRODUCTION

Zingiber officinale Roscoe (family Zingiberaceae), naturalizing in south East Asia, introduced in many parts of globe and has been cultivated for thousands of years as a spice and for medicinal purposes. The rhizome of this plant is heavily consumed dietary condiments throughout the world and most frequently used ingredients in Unani, Ayurvedic and Chinese traditional medicine for treatment of various ailments that include arthritis, rheumatism, sprains, pains, muscular aches, sore throats, indigestion, vomiting, fever, hypertension, cramps, constipation and infectious disease[1]. Phytochemistry of ginger has been explored widely and describes that 6-gingerol is one of the marker substance of ginger[2]. 6-gingerol is responsible for variety of pharmacological effects particularly analgesic, anti-inflammatory, antipyretic, cardiotonic effects and inhibition of spontaneous motor activity and prostaglandin biosynthesis[3-5]. Furthermore, it found to inhibit metastasis in human breast cancer[6]. Alcoholic extract of ginger and 6- gingerol were found to inhibit production of proinflammatory cytokines particularly TNF-α and interleukins in LPS stimulated macrophages[7].

In ayurveda ginger is recommended in *agnimandya* (anorexia) as an antianorectic agent[8]. Though the various activities of the ginger have been studied, there is no literature available about its effect on the anorexia, so the present study was undertaken to explore the claim of ginger as an antianorectic agent and most widely described anti-inflammatory marker 6- gingerol was also evaluated for its activity against anorexia.

Anorexia describes loss of appetite and concomitant reduction in food intake in the presence of readily accessible food[9]. The complex neuronal mechanisms are involved in feeding inhibition. Proinflammatory cytokines play an important role in feeding inhibition by stimulation of Tumor necrosis factor ((TNF-α) and Interleukin-1 (IL-1). Release of this cytokines can be stimulated by ingestion of Lipopolysaccharide (LPS) of bacterial cell wall[10]. Similarly anorexia can be induced by enhancing 5- HT release or inhibiting reuptake of 5-HT, as serotonergic pathway is also involved in the feeding inhibition. Fluoxetine is a selective 5-HT reuptake inhibitor which can induce anorexia[11]. Therefore, the purpose of the present study was to determine whether the hydroalcoholic extract of *Z. officinale* containing 1.05 % of 6- gingerol can prevent anorexia induced by proinflammatory cytokine mediators, and to propose the possible biomarker responsible for the activity by evaluating effect of 6- gingerol.

MATERIAL AND METHODS

Procurement, authentication and HPLC analysis of Z. officinale

Dried rhizomes of *Zingiber officinale* (Zingiberaceae) (ZO) were purchased from local market of Maharashtra, India. The raw material was authenticated and voucher specimen was deposited at Agharkar Research Institute, Pune, Maharashtra, India; with following voucher specimen number - *Zingiber officinale* (Zingiberaceae) R-106.

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The rhizomes were powdered and extracted with methanol (95%) for HPLC analysis. The sample was analyzed by using chromatographic system Agilent liquid chromatography system series 1200 with quaternary pump, Rheodyne injector with 20 µl fixed loop and photodiode array detector. The separation was achieved on Waters symmetry C-18 Column (250 X 4.6 mm, particle size 5 µ) preceded by an ODS guard column (10 µm, 10mm x 5 mm ID) at an ambient temperature. The analysis was isocratic with mixture of acetonitrile: potassium hydrogen phosphate buffer (10mM, pH 7.5): methanol (65:20:15) with flow rate of 1ml /min. The absorbance was recorded at 280nm and the from peak area the percentage amount of 6- gingerol was calculated and found to be 1.05 %w/w

Animals

Male Wistar rats weighing 250-300 g were employed for anti-anorectic tests. The animals were individually housed in a cage, maintained at 22 ± 2°C in a room with a 12 h light/dark cycle (lights on at 6AM) and had free access to feed and water *ad libitum* during quarantine period. Each group contains 10 animals. The study is complied with current ethical regulations on animal research and related rules of our Institute and all animals used in the experiment received human care. All the pharmacological experimental protocols were approved by Institutional animal ethics committee (Resolution No. RCPIPER/IAEC/2009-10/11)

Drugs

A dry hydroalcoholic extract from rhizomes of ZO was used. The HPLC analysis report showed the presence of 1.05% of 6- gingerol. The extract was dissolved in ethanol absolute and diluted in water in order to maintain final ethanol concentration 2% v/v in all treatment conditions. Then it was administered by intragastric administration at doses of 100,200 and 400 mg/kg. The same vehicle was administered to control group.

Standard 6- gingerol (97% purity) was purchased from Sigma Aldrich Pvt. Ltd. (USA). The 6 gingerol was administered in the doses of 5, 10 and 15 mg/kg by intragastric route.

Lipopolysaccharide from E. coli (Sigma-Aldrich Germany) was dissolved in pyrogen free isotonic saline and given by intraperitoneal injection (IP) at the dose of 100µg/kg.

Fluoxetine Hcl (Sigma-Aldrich Germany) was dissolved in sterile physiological saline and administered IP at the dose of 8 mg/ kg/ ml.

Experimental procedure

Effects of ZO and 6-gingerol on freely feeding rats

To evaluate the general effect of ZO and 6- gingerol on food intake, the effects were examined in freely feeding rats. The animals were divided into seven groups involving eight animals in each group. ZO is administered in the dose of 100, 200 and 400 mg/kg while 6- gingerol in the dose of 5, 10 and 15 mg/kg. Control group received vehicle. Their food was removed temporarily for 1 h and offered again later. Food consumption was determined at 30, 60, 90, and 120 min and 4, 6, and 24 h after test extract administration, by weighing the food cups and by subtracting the spillage from total food intake.

Effects of ZO and 6- gingerol on LPS induced anorexia

LPS is a pathogenic agent which induces a moderate infection which is associated with reduction in food consumption[12]. The rats (n=70) were food deprived for 20 h and were injected with 100µg/kg LPS and 4 h later they received intra gastric administration of vehicle/ ZO/ 6-gingerol in above mentioned doses. Control group rats (n =10) were food deprived and received the respective vehicles. Sixty minutes after test extracts administration, rats were provided food and food consumption of individual rat was determined 30, 60, 90 and 120 min, and 4, 6, and 24 h later.

Effects of ZO and 6- gingerol on fluoxetine induced anorexia

Fluoxetine (FLU) is a selective 5 HT reuptake inhibitor which is responsible for the reduction in food intake. It can be beneficial to evaluate the selectivity of the antianorectic effect of ZO. Rats (n=70) were food deprived for 20 h. They received intragastric administration of vehicle/ ZO/ 6- gingerol, and after 60 min injected intraperitonealy with 8mg/kg FLU [13]. 30 min later all rats were given free access to food and their food consumption was recorded 30, 60, 90 and 120 min, and 4, 6, and 24 h later. Control group animals were food deprived and received IG administration of vehicle and followed by FLU injection IP to observe the effect of FLU on food consumptions.

Statistical analysis

The data obtained was analyzed by one way ANOVA followed Dunnett's test. Values are represented as mean ± SEM for each group of animals at indicated numbers (n). The $P \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

Effect of ginger and 6- gingerol on freely feeding rats

As shown in Fig 1, neither ZO nor 6- gingerol modify the food intake in freely feeding rats at any of the doses tested. The statistical analysis revealed no significant effects of treatment on food intake ($p > 0.05$).

Effects of ZO and 6- gingerol on LPS induced anorexia

As shown in Fig 2A ip administration of LPS produced a marked reduction in feeding as compared with the group that was administered only with vehicle. The pretreatment with ZO and 6- gingerol significantly modified the anorectic effect of LPS. Post hoc comparison showed a significant inhibition of the effects of LPS after administration of 200 and 400 mg/kg ZO ($p< 0.05$) and 15 mg/kg of 6- gingerol ($p<0.01$) for up to 24h, but not for ZO 5mg/kg and 10,15 mg/kg of 6- gingerol (Fig 2B).

Effect of ZO and 6-gingerol on Fluoxetine induced anorexia

As shown in Fig 3A, ip administration of FLU elicited a marked reduction in feeding. Pretreatment with ZO and 6- gingerol completely reversed this effect of FLU up to 24 h. Post hoc analysis reveals a statistically significant effect at higher doses of ZO (200 and 400 mg/kg) and 6-gingerol (15 mg/kg).

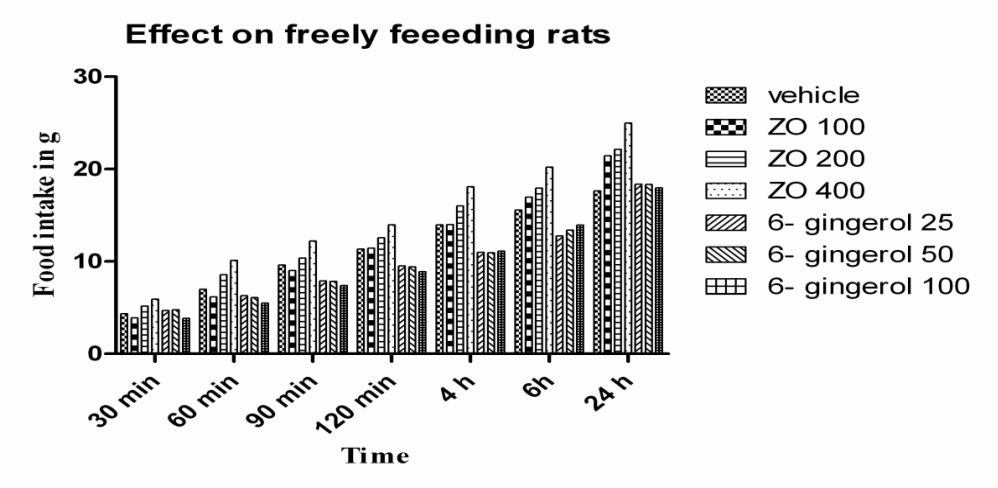

Figure 1: Effects of ZO and 6- gingerol on freely feeding rats.
Data represents mean food intake (±SEM) of 10 rats. The differences in ginger and 6- gingerol treatment from the vehicle treated group are not statistically significant.

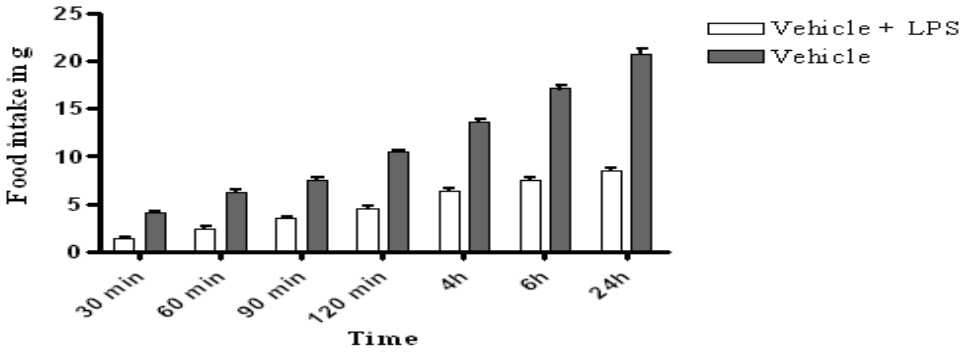

Figure 2A: Effect of LPS on food intake.

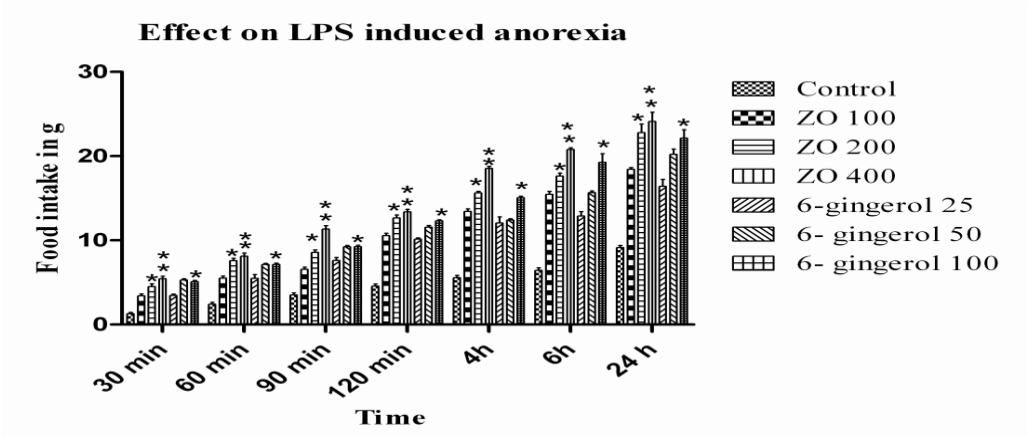

Figure 2B: Effect of ZO and 6- gingerol on LPS induced anorexia.
Figure 2A: Effect of IP injection of LPS (100µg/kg) in 20h food deprived rats. Figure 2B shows Effect of IG administration of ginger extract i.e. ZO (100, 200 and 400 mg/kg) and 6- gingerol (25, 50 and 100 mg/kg) on LPS induced anorexia. Data represent mean food intake (± SEM) of 10 rats. *p < 0.05 and **p < 0.01 considered significant differences from the vehicle treated rats; where not indicated, the differences are not statistically significant.

DISCUSSION

The present study shows that ZO extract and 6- gingerol effectively and dose dependently reduces the marked anorexia induced in rats by LPS and Fluoxetine. Pretreatment with ZO at doses 200 and 400 mg/kg while 6-gingerol at 100mg/kg significantly reversed anorectic

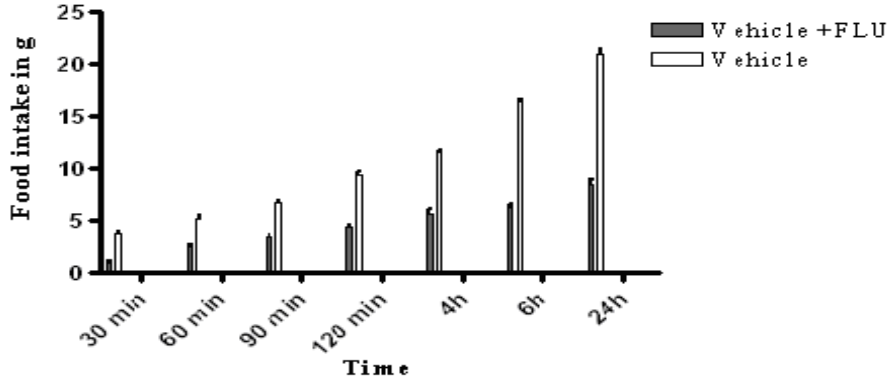

Figure 3A: Effect of FLU on food intake.

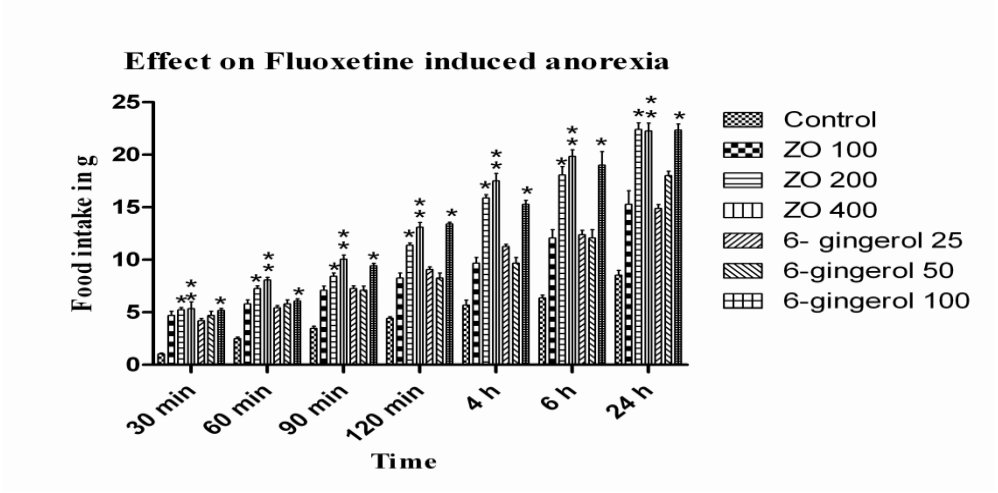

Figure 3B: Effect of ZO and 6- gingerol on FLU induced anorexia.

Figure 3A - Effect of IP injection of FLU (8mg/kg/ml) in 20h food deprived rats. Figure 3B shows Effect of IG administration of ginger extract i.e. ZO (100, 200 and 400 mg/kg) and 6- gingerol (25, 50 and 100 mg/kg) on FLU induced anorexia. Data represent mean food intake (± SEM) of 10 rats. *$p < 0.05$ and **$p < 0.01$ considered significant differences from the vehicle treated rats; where not indicated, the differences are not statistically significant.

effects induced by proinflammatory cytokine inducer and 5- HT reuptake inhibitor. These anti anorectic effects appeared within 30 min and lasts up to 24 h after single oral administration.

Conversely present study demonstrates that at the range of doses used ZO/ 6- gingerol does not increase feeding in freely feeding rats significantly. This suggests that the inhibitory effect of ZO/ 6- gingerol is specific.

LPS are purified Gram- negative bacterial cell wall constituents that are released after bacteriolysis or during periods of rapid bacterial proliferation[14]. LPS administration triggers a proinflammatory cytokines response as in systemic microbial infection[15]. Intraperitonealy administered LPS mediates anorexia by stimulating synthesis of different proinflammatory cytokines such as interleukins (IL-1, IL-6), tumor necrosis factor (TNF-α) and interferons (INF) which are responsible for reduced food intake and considered to be the major cytokines responsible for the anorexia after administration of LPS in rats[16]. Synthetic drugs like verapamil antagonizes LPS by inhibiting TNF α production and act as an antianorectic[12]. The previous reports on the ginger extract suggest that it has ability to inhibit proinflammatory cytokines[17]. The same mechanism may underlie for the antianorectic activity.

Similarly, serotonin system also plays an important role in the control of feeding inhibition. Serotonin potently inhibits the eating particularly through the 5-HT_{1b} or 5-HT_{2c} receptors[18]. Similarly specific 5 HT_{2c} receptor antagonism blocks the anorexia induced by peripheral and central injection of LPS and IL-1ß[19]. Administration of the 5-HT_{1A} agonist 8-OH-DPAT, directly into the dorsal raphe nucleus also blocked the feeding suppressive effect of peripheral LPS and IL-1ß[20]. All these data suggests that the serotonergic neurons originating in the hindbrain play a role in mediation of cytokine induced inhibition in feeding.

Both LPS and FLU reduces food intake by acting on proinflammatory cytokines, if this effect of cytokines is blocked anorexia can be attenuated. Overall literature regarding the effect of ginger extract and 6- gingerol on proinflammatory cytokines and results of present preliminary study supports the claim of Ayurveda. It can be hypothesized that the ginger extract act as an antianorectic agent by inhibiting proinflammatory cytokines. The present study was conducted with whole extract of *Z.officinale*, which comprises a range of biologically active substances. The most studied marker as an anti inflammatory compound from ginger, 6-gingerol was undertaken for the study along with ginger extract. So at present we could only conclude that 6-gingerol contributes to the anti anorectic activity of ginger extract. Along with other biological markers such as 8-gingerol, 10- gingerol, shagaols which are reported to have anti-inflammatory activity[21]. 6- gingerol may play an important role in antianorectic activity. However further studies with respect to individual marker of this extract is necessary.

In conclusion, the present study provides the first evidence that oral administration of *Z.officinale* extract containing 1.05% of 6- gingerol results in a potent inhibition of the anorectic effects induced by LPS and Fluoxetine, and provides functional evidence of claimed anti-inflammatory and antianorectic activity of ginger.

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