Zingerone regulates intestinal transit, attenuates behavioral and oxidative perturbations in irritable bowel disorder in rats

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ABSTRACT

Stress can lead to the manifestation of functional gastrointestinal disorders, the most prominent being irritable bowel disorder. The present study investigated the impact zingerone in ameliorating chronic water stress induced irritable bowel disorder, brain gut axis dysfunction and dysregulation of the intestinal barrier due to oxidative stress. Rats were randomly allocated to groups and subjected to chronic water stress for a period of 21 days for 1 h and the fecal pellet output was measured. At the end of chronic stress, behavioral assessment for anxiety like behavior was recorded and plasma corticosterone levels were measured 60 min after water stress. The colonic transit was determined, levels of oxidative and antioxidant biomarkers were measured in the colon homogenate. Myeloperoxidase activity was determined as an indirect index of neutrophil infiltration. Chronic water stress increased the rate of colonic transit, fecal output, induced behavioral changes, and decreased antioxidant levels. An increase in lipid peroxide levels, catalase and corticosterone was observed. Mast cell infiltration was evident in the stressed group. Zingerone significantly reduced colonic transit, fecal output, neutrophil infiltration, and lipid peroxide formation. The levels of catalase were not altered; however, a marginal increase in the levels of glutathione peroxidase was observed. Zingerone significantly enhanced the levels of superoxide dismutase, glutathione and decreased the levels of corticosterone. Zingerone produced marked improvement in stress induced irritable bowel disorder which could be attributed to the powerful antioxidant nature, direct effect on the intestinal smooth muscle and adaptogenic nature.

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Introduction

In an era of globalization, man is constantly confronted with stressful situations. Prolonged exposure to physical, chemical and biological stressors activate the autonomic and endocrine system leading to altered homeostasis. Epidemiological data reflects that stress in an important factor in the development of gastro-intestinal, neuroendocrine, and cardiovascular disorders. Gastrointestinal tract is most vulnerable to chronic stress, augmenting changes in the transit and visceral hypersensitivity. Irritable bowel syndrome (IBS) is a gastro-intestinal disturbance predominated by diarrhea (IBS-D) or constipation (IBS-C) or displaying both types of symptoms. IBS affects 8–22% of the population; however, the proportion could far exceed the projected

number as many endure the disease often hesitating to seek medical help. Irregular bowel habits and embarrassment associated with it create significant distress. Further, IBS is associated with comorbidities; the most prevalent being psychiatric disorders (Guthrie et al., 2003).

Stress contributes to the manifestation of IBS by deranging normal biorhythms. Activation of the hypothalamic-pituitary-adrenal (HPA) axis (Ferrier, 2008) can produce intestinal inflammation (Miampamba et al., 2002; Larauche et al., 2009; Caso et al., 2008); however, variables such as the time line of exposure, type of stressor, and the extent of exposure might affect its activation. The role of oxidative stress in IBS cannot be undermined, as stress induces formation of reactive oxygen species which impinge the integrity of the intestinal lining leading to the symptoms of IBS.

Persistent IBS can trigger organic disorders such as cancer and inflammatory bowel disease (Rey and Talley, 2009). Furthermore, the distress induced by IBS is intense forcing 38% of the affected population to contemplate suicide as a means of relief (Miller et al., 2004). Therefore, there is need to identify remedies which can minimize the trauma and distress associated with IBS.
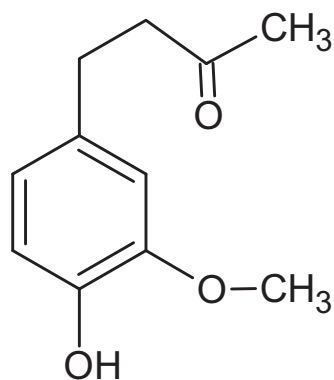
Fig. 1. Chemical structure of zingerone.

Devising modes of eluding the impact of stress on the gastrointestinal tract could improve the quality of life. Keeping this in perspective, we have selected zingerone with an aim of elucidating its role in attenuating oxidative burden and colonic dysfunction in IBS. Zingerone (Fig. 1) is a phenolic alkanone which is chemically [4-(4-hydroxy-3-methoxyphenyl)-2-butanone] obtained from the rhizomes of *Zingiber officinalis* commonly referred to as ginger. Zingerone has been found to circumvent radiation induced genetic damage and apoptosis in human lymphocytes (Rao et al., 2011); exert a lipolytic action in adipocytes (Pulbutr et al., 2011); suppress activation of pro-inflammatory enzymes and age related inflammation (Kim et al., 2010); exhibit strong antioxidant action, and inhibit contractile movements of isolated colonic segments (Iwami et al., 2011).

Owing to the powerful antioxidant and anti-inflammatory nature of zingerone, we speculated that it might be beneficial in IBS. Furthermore, as it is beneficial in hypermotility, it might be effective in minimizing stress induced IBS-D. Therefore, the goals of the present study were to explore the impact of zingerone in alleviating chronic water stress induced alterations in the structure and function of the rat intestine. Secondly, the impact of zingerone in reversing behavioral changes and oxidative damage were also elucidated.

Materials and methods

Drugs and chemicals

Gingerone was purchased from Sigma–Aldrich, USA. Thiobarbituric acid was purchased from S.D. Fine Chemicals, Hyderabad, India. 5, 5′ dithiobis 2-nitrobenzoic acid, reduced glutathione, hexadecyltrimethyl ammonium bromide and o-dianisidine were procured from Sisco Research Laboratories, Mumbai, India. All the other reagents and chemicals used were of analytical grade.

Animals and experimental design

Male Wistar rats weighing 180–250 g were selected and kept in 12:12 light:dark cycle under controlled temperature of $22 \pm 0.5\,°C$. Rats were fed with standard pellet diet and had free access to food and water ad libitum. Animal welfare and experimental procedures were carried out in accordance with the guidelines provided by the Council for the Purpose of Control and Safety of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. This experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC). Rats were divided into 5 groups each containing 6 animals. The first group was designated as the control group which was subjected to sham water stress and administered saline daily for 21 days by the oral route;

group 2 animals were treated with zingerone (10 mg/kg) alone by the oral route for 21 days; group 3 was subjected to water stress for a duration of 1 h for 21 days; group 4 and 5 animals were subjected to chronic stress and treated with zingerone in doses of 10 mg/kg and 20 mg/kg respectively.

Dose selection

The dose of zingerone selected was 10 and 20 mg/kg approximately corresponding to 1/100th and 1/50th the LD $_{50}$ (Rao et al., 2009).

Induction of chronic stress

The test apparatus consisted of a plexiglas tank (45 cm length × 25 cm width × 25 cm height) with a block (10 × 8 × 8 cm) affixed to the center of the floor. The tank was filled with fresh water (25 °C) to 1 cm below the surface of the platform. The animals were placed on the platform mounted in the center of the tank for a period of 1 h daily for 21 consecutive days corresponding to the chronic stress protocol. Sham water stress (WS) consisted of placing the rats similarly for 1 h daily for 21 days on the same platform in a waterless container (Venkova et al., 2010). Rats were weighed every day before exposure to WS or sham WS to assess weight change from baseline.

Fecal pellet output

At the end of 1 h water stress or sham WS, fecal pellets present in the tank were scored with 0 as normal, 1 for soft formed stools, 2 for soft stools with no pellet formation, and 3 for watery diarrhea. The stool scores were averaged for each group.

Behavioral studies

Elevated plus maze (EPM)

The apparatus consisted of an elevated, plus-shaped runway elevated 50 cm above the floor. Two open arms and two closed arms, measuring 50 × 15 cm, emerged from a central open platform of 15 cm per side. The height of the walls of the closed arms was 20 cm. Testing was conducted in a room lighted only with dim red light. The animals were placed in the center of the EPM, where the four arms intersect each other, facing a closed arm. The number of entries in the open arms was recorded by a blind observer. An entry was scored when both front paws were placed in an arm. The test duration was 5 min (Pellow et al., 1985).

Open field test

The open field comprised of a square wooden arena measuring 90 × 90 × 25 cm with the floor divided by black lines into 36 small squares (15 × 15 cm). All testing was conducted between 09:00 and 15:00 h. All treatments groups were tested on the same day in a random order. Rats were gently placed in a corner of the arena and exploratory measures were recorded (Shoji and Mizoguchi, 2010). The number of central area entries and time spent in the central arena was recorded. After the 3 min test session, the rat was returned to its home cage.

Determination of corticosterone levels in plasma

At the end of the study, animals were anesthetized with pentobarbital (60 mg/kg, i.p. injection) and blood was collected by retro orbital puncture into tubes containing heparin. The tubes were immediately centrifuged at 3000 rpm for 10 min and plasma samples were used for assay. Plasma (0.5 ml) was taken in a stoppered glass tube and extracted with 10 ml dichloromethane for