Research Article

Ginger Extract Attenuates Ethanol - Induced Oxidative Stress in Rat Small Intestine

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Accepted on: 25-07-2013; **Finalized on:** 30-09-2013.

ABSTRACT

Chronic alcohol consumption can exert deleterious effects on the structures and functions of all parts of the Gastro intestinal tract (GIT). This study evaluates the protective effect of ethanol extract of ginger on ethanol-induced oxidative stress in the small intestine of rats. Oxidative stress in the intestine tissue was evaluated by estimation of the activities of superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), glutathione reductase (GR) and glutathione peroxidation (Se-GSH-Px). In the present study GST activity was significantly ($p<0.001$) increased whereas GR, Se-GSH-Px, SOD and CAT activities were significantly ($p<0.001$) decreased in the intestine of rats treated with ethanol alone (6g/kg). Ethanol extract of ginger (200mg/kg) administration exerts a significant ($p<0.001$) increase in GR, Se-GSH-Px, SOD and CAT activities, a marked reduction in the GST. However, ginger administration ameliorated the effects of ethanol, suggesting that ginger is a potential antioxidant against ethanol-induced oxidative stress.

Keywords: Gastro Intestinal tract, oxidative stress, intestine, antioxidants.

INTRODUCTION

Chronic consumption of alcohol can result in spectrum of abnormalities in many organ systems[1]. Among the many organ systems that mediate alcohol's effects on the human body and its health, the Gastro Intestinal tract (GIT) and the liver play crucial roles[2]. The oxidative stress caused by reactive oxygen species (ROS) produced during extensive ethanol metabolism is suggested as one of the main important mechanisms underlying the toxicity[3]. Acute ethanol administration is reported to convert rat heart xanthine dehydrogenase to xanthine oxidase which uses acetaldehyde as substrate and contribute to the generation of ROS [4]. Excessive ROS generation has been shown to trigger lipid peroxidation, protein oxidation and derangement of antioxidant system elements leading to eventual loss of structure and function of cells[5]. Srivastava and Shivanandappa[6] previously reported that administration of alcohol decreases the antiperoxidative enzymes. Numerous studies have proved that free radical mediated mechanisms are involved in ethanol-induced liver injury [7] .

Chronic alcohol consumption cause the gut leakiness and endotoxima can result alcohol liver diseases. The GIT is the primary site of alcohol absorption into the systemic circulation while the liver is the primary site of alcohol metabolism in the mammalian body [8]. The direct contact of alcohol with the GIT mucosa may elicit several metabolic changes among which is the induction of ethanol-inducible cytochrome P_{450} (CYP2E1) with an attendant generation of toxic acetaldehyde and reactive oxygen species[9]. Exposure of the intestinal mucosa to ethanol causes morphological injuries impairs the absorption of solutes and alters lipid metabolism and enzyme activities[10].

Z. officinale Rosc. is a well-liked spice used Worldwide as a food seasoning and has been used as a folk medicine in Korea, China and Japan for the treatment of gastrointestinal disorders, dyspepsia, nausea, vomiting, pain, common cold, diarrhea and cough[11]. It is generally considered safe and it possesses various pharmacological activities including cardiovascular protection, antioxidant, anti-inflammatory, glucose lowering, anti-cancer activities etc [12,13]. Recently, it has been shown that [6]-gingerol is endowed with strong anti-oxidant action both *in vivo* and *in vitro*, in addition to strong anti-inflammatory and anti-apoptotic actions[14]. Ginger, owing to its functional ingreadients like [6]-gingerol, [6]-shogaol, [6]- paradol and zerumbone, exhibits anti-inflammatory and antitumorigenic activities[15,16]. Ginger and its bioactive molecules are effective in controlling the extent of gastric, liver, cancers [17,15]

So far, limited information exists concerning the beneficial effects of ginger against alcohol induced oxidative injuries in intestine tissues. Hence, the present study has been undertaken to evaluate the possible ameliorative effect of ethanolic ginger extract in alcohol-treated rats.

MATERIALS AND METHODS

Preparation of ginger ethanol extract

Ginger (*Z. officinale* Rosc.) rhizomes were purchased from the local market at Tirupati, A.P. India. Four kilograms fresh ginger rhizome was cleaned, washed under running tap water, cut into small pieces, air dried and powdered. 200g of this powder were macerated in 1000 ml of 99.9% ethanol for 48 hrs at room temperature and were then filtered**.** The filtrate was concentrated to dryness under reduced pressure in a rotary evaporator. In the present

study the concentration of the extract 200 mg/kg was orally given to the animals.

Animals

24 Wistar strain male albino rats, weighing 300 ± 20 g, were obtained from the Indian Institute Of Science, Bangalore. The animals were housed in plastic cages and kept in the laboratory under constant temperature (27^{0}C ± 2^{0}C) with 12 hrs dark and 12 hrs light for throughout treatment period. The rats were given a standard pellet diet (Sai Durga feed, Bangalore) and water *ad libitum*. The experiments were carried out in accordance with guidelines and protocol approved by the Institutional Animal Ethics Committee resolution number (10 /i/a/CPCSEA/IAEC/SVU/KSR-GVS/dt 15/11/2010).

Experimental Design

The animals were divided into four groups of six rats each and the treatment was given everyday via orogastric tube for 7 weeks.

Group I, Normal Control (NC): rats received 2% Tween-80 solution

Group II, Ginger treatment (Gt): rats received 200mg/kg of ethanol extract of ginger suspended with 2% Tween-80 solution orally for 7 weeks treatment

Group III, Ethanol treatment (Et): rats received 20% ethanol 6g/kg dose given orally for 7 weeks treatment

Group IV, Ethanol + Ginger treatment (Et+Gt): ethanolic rats received ginger, as described group II for a period of 7 weeks.

Isolation of tissues

After completion of 7 weeks treatment period the animals were sacrificed by cervical dislocation and isolated the small intestine tissue from the animal for biochemical analysis.

Analytical procedure

Small intestine superoxide dismutase (SOD) activities were assayed in the tissue homogenates by the method of Misra and Fridovich (1972) at 480 nm for 4 min on a Hitachi U-2000 spectrophotometer. Activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 U per milligram of protein. Catalase (CAT) activity was determined at room temperature by using the method of Aebi (1984) and absorbance of the sample was measured at 240 nm for 1 min in a UVspectrophotometer.

Activity of glutathione peroxidase (GPx) was determined by the method of Flohe and Gunzler (1984), in the presence of NADPH, and absorbance was measured at 340 nm, using cumene hydrogen peroxide. Glutathione reductase (GR) activity was determined according to the method of Carlberg and Mannervik (1985). Glutathione-S-tranferase activity was measured with its conventional substrate, 1-Chloro 2, 4-Dinitro Benzene (CDNB) at 340 nm as per the method of Habig *et al.*, (1974). All the enzyme activities were expressed as per mg of protein and the tissue protein was estimated according to the method of Lowry, Rosebrough, Farr and Randall (1951), using bovine serum albumin (BSA) as a standard.

Statistical analysis

Statistical analysis of data was represented as mean ± SD. All the statistical analyses were carried out by SPSS software. Dunnett's multiple comparison test and one-way analysis of variance (ANOVA) were used to assess the differences. P Values <0.001 were considered as highly statistically significant.

RESULTS

Significant ($p < 0.001$) decrease in SOD, CAT, GPx, GR activity and a significant (P<0.001) increase in GST activity was observed in the ethanol alone treated rats when compared with normal control rats. Ethanolic rats with ginger treatment showed significant ($P < 0.001$) increase in SOD, CAT, GR, GPx activities and GST was significantly decreased (P<0.001), which reflects restoration of antioxidant enzyme system to near-normal values (Figs. 1–2).

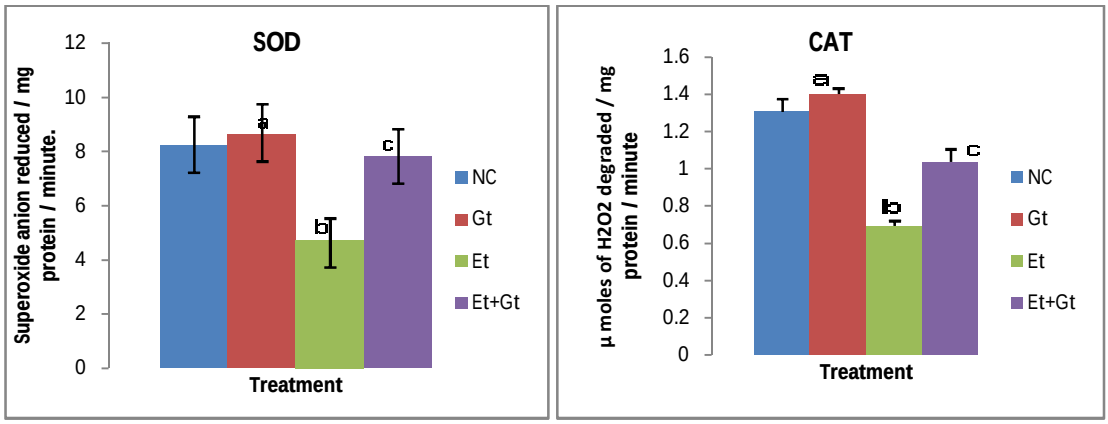

Figure 1: Effects of ginger and ethanol on SOD and CAT enzyme activities in intestine tissue of normal and experimental rats. Data was expressed as mean ± SD (n=6). The groups are not shared with same letters are significant compared to the control (a, b, c P<0.001).

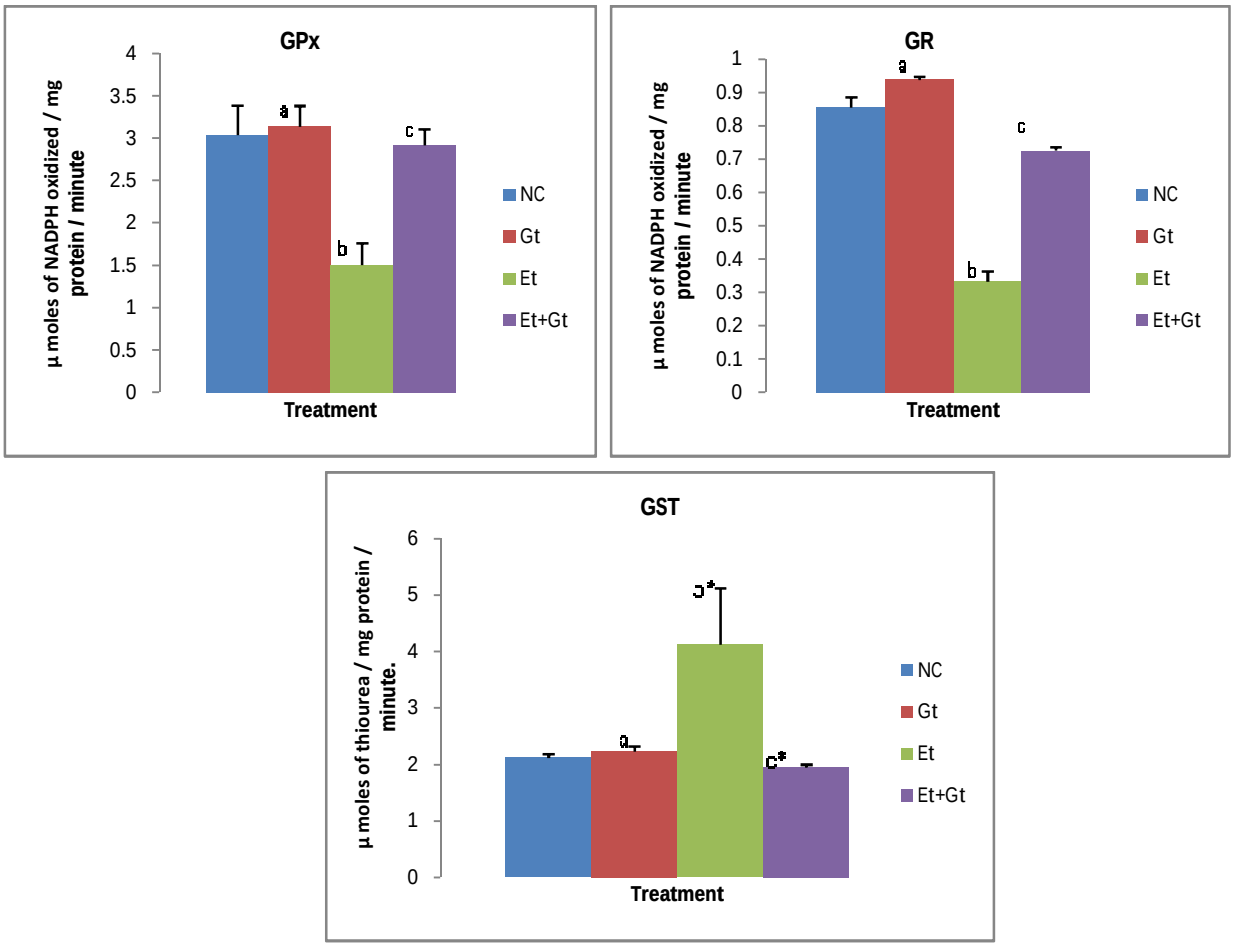

Figure 2: Effect of ginger and ethanol on GPx, GR and GST enzyme activities in small intestine tissue of normal and experimental rats. Data was expressed as mean ± SD (n=6). The groups are not shared with same letters are significant compared to the control (a P<0.05 & b*, c* P<0.001).

DISCUSSION

Alcohol plays an important role in the daily life of many healthy as well as unhealthy individuals, being a nutrient, psychoactive drug and a toxin [18]. Alcohol consumption was linked to more than 60 disease conditions in a series of meta-analyses [19]. Free radical-generated from alcohol metabolism to cause the reperfusion injury when oxygen is reintroduced to ischemic tissue. This oxidative stress mediated reperfusion injury has been reported in heart, kidney, liver, lung and intestine [20]. The GIT is the primary site of alcohol absorption into the systemic circulation it may elicit several metabolic changes with an attendant generation of toxic acetaldehyde and reactive oxygen species [9]. The oxidative stress caused by reactive oxygen species (ROS) produced during extensive ethanol metabolism is suggested as one of the main important mechanisms underlying the toxicity [3].

In the current study, SOD activity was decreased in ethanol alone treated rat intestine. Reduced SOD activity has also been observed in liver, kidney and heart under ethanol intoxication [21,22]. This decrease could be due to a feedback inhibition or oxidative inactivation of enzyme protein because of excess ROS generation. The generation of α-hydroxyethyl radical may lead to inactivation of these enzymes [23] and accumulation of highly reactive free radicals also lead to deleterious effects such as loss of cell membrane integrity and membrane function [24].The earlier reports have also been demonstrated SOD activity was decreased during ethanol intoxication in alcoholic patients [25]. SOD activity was elevated in rats dosed with ginger extract alone and also in ginger plus alcohol treated rats intestine. This elevation may be due to the presence of antioxidant bioactive compounds in ginger. The antioxidant compounds like gingerols, shogals, ketone compounds and the phenolic compounds of ginger were responsible for scavenging the superoxide anion radicals [26].

In the present study, CAT, GPx activities were decreased in the intestine of ethanol treated group. CAT, GPx antioxidant enzymes were important to convert the hydrogen peroxides into water which are toxic to the cells. [27,28]. However, alcohol consumption can decrease the CAT, GPx activity in liver underlying ethanol toxicity [29]. This may be due to ethanol can cause the inactivation of the enzymes irreversible by over production of hydrogen peroxides during ethanol metabolism [30]. The earlier reports have also been demonstrated the excessive production of reactive oxygen species (ROS) were mainly caused due to decrease in the antioxidant activity [31]. Treatment of the ethanol intoxication animals with ginger renovated the amended antioxidant enzyme activities

significantly in the intestine (CAT and GPx. $p < 0.001$). This may be due to the presence of many antioxidant compounds, such as gingerols, shogaols, phenolic ketone derivatives, volatile oils and flavonoids of ginger. These antioxidant compounds may modulate the antioxidant enzymes in ethanolic rats. [32].

GR activity was decreased in the ethanol intoxicated rat intestine. GR serves to regenerate reduced GSH from oxidised GSSG by the activation of GPx. The decrease in GR activity may reflect the decline of the production and availability of GSH to overcome H_2O_2 [33]. This may be due to over production of hydrogen peroxides which can inactivate the GPx activity [30] and finally it can led to disturb the GSH/GSSG ratio. The previous reports have also been demonstrated that GR activity decrease in the liver of rats exposed to 5 g/kg bodyweight of ethanol [34]. GR activity was elevated in ginger and ginger plus ethanol treated rats. This elevation may be due to Ginger bioactive compounds such as zingerone, sequterpenoids, tannins, polyphenolic, flavonoids and other phytochemicals antioxidant activity [35,31]

The increase in the GST activity in the intestine as a response to the ethanol consumption suggests its activation due to oxidative stress [36, 37]. Das and Vasudevan[3] in their dose dependent alcohol studies observed increased GST activity. However, Ahmed *et al.,*[38] reported that GST activity was decreased with ginger treatment. By improving the antioxidant status the ginger will undoubtedly protect against the oxidative stress involved in the etiology of many chronic diseases [13].

CONCLUSION

To conclude, the results confirmed that alcohol-induced intestine toxic effect may be due to free radical mechanism and provide evidence that ginger significantly protects the intestine and reduces the severity of damage caused by alcohol intoxication. However, further detailed studies are required to establish its clinical application.

Acknowledgment: We thank the University Grants Commission (UGC), New Delhi for financial support in the form of BSR-Fellowship**.** (Rector order No.UGCl (6)/RFSMS/Zoology/2012 Dated: 13-06-12).

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Source of Support: Nil, **Conflict of Interest:** None.