Indian Journal of Experimental Biology
Vol. 44, November 2006, pp. 892-897

Ethanolic *Zingiber officinale* R. extract pretreatment alleviates isoproterenol-induced oxidative myocardial necrosis in rats

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Received 28 November 2005; revised 24 August 2006

Ethanolic *Z. officinale* (ZO) extract (200 mg/kg) pretreatment for 20 days in isoproterenol (ISO)-treated rats significantly increased the levels of endogenous myocardial antioxidants (catalase, superoxide dismutase and tissue glutathione), decreased the levels of serum marker enzymes (lactate dehydrogenase, creatine kinase, aspartate transaminase and alanine transaminase) and increased myocardial lipid peroxides. Histological examination of rat's heart section confirmed myocardial injury with ISO administration and near normal pattern with ethanolic ZO extract pretreatment. The results of the present study, for the first time, provide clear evidence that the ethanolic ZO extract pretreatment enhances the antioxidant defense against ISO-induced oxidative myocardial injury in rats and exhibit cardioprotective property.

Keywords: Creatine kinase, Isoproterenol, Myocardial necrosis, *Zingiber officinale*

Myocardial infarction (MI), the most dreaded sequel among ischemic heart diseases is invariably followed by several biochemical alterations such as lipid peroxidation, free radical damage, hyperglycemia, hyperlipidemia etc., leading to qualitative and quantitative alterations of myocardium[1]. Oxygen free radicals (OFR) are implicated as mediators of tissue injury in cardiovascular pathology[2]. Free radical generation and lipid peroxidation could be involved in isoproterenol (ISO)-induced cardiac damage[3]. ISO-induced myocardial infarction (MI) increases lysosomal hydrolase activities, which may be responsible for tissue damage and infarcted heart[4] and also causes alterations in the fragility of lysosomal membrane of heart[5].

Despite considerable progress in the management of myocardial infarction by synthetic drugs, the search for indigenous cardioprotective agents still continue. Some plant products have also been demonstrated to cause augmentation of myocardial antioxidants[6, 7].

Zingiber officinale R., commonly known as ginger (Zingiberaceae) is cultivated commercially in India, China, South East Asia, West Indies, Mexico and other parts of the world. It is consumed worldwide as a spice and flavoring agent and is attributed to have many medicinal properties.

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The *British Herbal Compendium* reported its actions as carminative, anti-emetic, spasmolytic, peripheral circulatory stimulant, and anti-inflammatory[8]. Limited *in vitro* studies have shown that water and organic solvent extract of *Z. officinale* possess antioxidant properties[9-12]. A combination of ginger and garlic has been reported to produce hypoglycemic and hypolipidemic effects[13]. In another study, dietary ginger protected the tissue from oxidative stress induced by organophosphate pesticide (malathion) in rats[14,15]. Ginger also has significant cholesterol lowering activity and shown to inhibit platelet aggregation[16,17]. Experimental evidence on biochemical role of ginger extract in myocardial damage is lacking but protective effect on atherosclerosis has been reported[16,18].

The present study has been designed to find out whether oral pretreatment of ethanolic *Z. officinale* (ZO) extract could exert any protective action against ISO-induced myocardial injury. In this context, an attempt has been made to elucidate the maintenance of myocardial integrity in presence and absence of ZO on ISO-induced cardiac damage with reference to biochemical cardiac markers and histology.

Materials and Methods

Drug—Fresh *Zingiber officinale* R. rhizomes were purchased locally during January 2005 and botanical authentification was carried out by the Division of Pharmacognosy, Faculty of Pharmacy, Hamdard

University, New Delhi, India (voucher specimen No. 2). Rhizomes were cut into thin slices and soxhlet extracted. The filtrate was evaporated under vacuum drier and brown mass residue obtained was stored at 4°C for further use. The dried extract contained 3g/100g of the starting crude material. For experimental study, the weighed amount of residue was dissolved in 1% Tween 80 in normal saline.

Chemicals and reagents—Isoproterenol (ISO) was obtained from Sigma Chemicals (St Louis, MO, USA). It was administered by subcutaneous route below skin of neck in 85 mg/kg body wt dose, in two divided doses at 24 hr intervals to induce myocardial necrosis. All other chemicals used were of analytical grade. Double distilled water was used for all biochemical assays.

Animals—The study was approved by Institutional Animal Ethics Committee (IAEC) [Reg No and Date of Reg: 173/ CPCSEA, 28th JAN-2000]. Wistar rats of either sex, weighing between 200-250 g, maintained under standard laboratory conditions at 25° ± 2°C, 50 ± 15 % RH and normal photoperiod (12 hr light : dark cycle) were used. Commercial pellet diet (Nav Maharastra Chakan Oil Mills Ltd, Delhi, India) and water were provided *ad libitum*. After acclimatization, 32 animals were divided into four groups of 8 animals each and treated as follows: Group I: normal control; received only 1% Tween 80 in normal saline. Group II: Pathogenic control; ISO administered rats. Group III: ZO *per se*; rats received ethanolic ZO extract (200 mg/kg body wt) orally for 20 days. Group IV: ZO pretreatment; rats received ethanolic ZO extract (200 mg/kg body wt) orally for 20 days followed by ISO administration on 21 and 22 day.

Blood samples were collected 24 hr after the last dose of treatment as mentioned in treatment schedule from the rat's tail vein of all the groups and serum was separated for biochemical estimation. After blood collection, all animals were sacrificed by cervical dislocation and hearts were dissected out. Heart tissues were washed with ice-cold saline for biochemical estimation. Heart tissue was weighed and minced. Homogenate (10%) was prepared; in 0.15 *M* ice cold KCl for thiobarbituric acid reactive substances (TBARS) and protein estimation; in 0.02 *M* EDTA for glutathione estimation and in phosphate buffer (*p*H 7.4) for superoxide dismutase and catalase estimation by using a Teflon tissue homogenizer[19].

The specific marker enzymes for MI *viz.* lactate dehydrogenase (LDH)[20], creatine kinase (CK)[21], aspartate transaminase (AST)[22] and alanine transaminase (ALT)[22] were measured in serum. Myocardial TBARS[23], a marker of lipid peroxidation and myocardial endogenous antioxidants e.g. SOD[24], catalase[25] and tissue glutathione[26] were estimated. Myocardial tissues were fixed in 10% formalin, routinely processed and embedded in paraffin wax. Paraffin section (5 μm) were cut on glass slides and stained with hematoxylin and eosin (H & E), and examined under a light microscope by a pathologist blinded to the groups studied.

Statistical analysis Statistical—analysis was carried out using Graphpad Prism 3.0 (Graphpad software; San Diego, CA). All data were expressed as mean ± SE. Groups of data were compared with an analysis of variance followed by Dunnett '*t*'-test. Values were considered statistically significant at $P<0.01$

Results

There was a significant ($P<0.01$) elevation in serum marker enzymes (LDH, CK, AST and ALT) levels in the pathogenic control group i.e. Group II, when compared with those of the normal healthy control group i.e. Group I, while ZO (200 mg/kg body wt, po) pretreatment significantly ($P<0.01$) reversed these elevated levels (Table 1).

Table 1—Effect of ethanolic *Z. officinale* extract pretreatment on isoproterenol induced changes in the activities of serum enzymes

[Values are mean ± SE from 8 animals in each group]

Treatment	LDH (IU/L)	CK (IU/L)	AST (U/ml)	ALT (U/ml)
Control	282.570±12.351	59.163±2.215	39.166±0.749	18.33±0.843
Isoproterenol (85 mg/kg, sc)	492.827±15.910*	191.283±11.984*	64.500±2.217*	41.5±0.500*
Z. officinale (200 mg/kg, po)	239.076±05.654	46.588±3.230	27.0±0.683	11.166±0.542
Z. officinale (200 mg/kg, po) + isoproterenol (85 mg/kg, sc)	343.811±19.426**	94.595±05.160**	20.6±1.833**	14.4±1.030**

LDH= lactate dehydrogenase; CK= creatine kinase; AST= aspartate transaminase; ALT= alanine transaminase
P values <0.01; when compared with *normal control group, **pathogenic control group.

Myocardial TBARS, an index of lipid peroxidation, was found to be significantly higher in the pathogenic control group i.e. Group II, when compared with those of the normal healthy control group (Group I), while pretreatment with ethanolic *Z. officinale* extract decreased the elevated level of TBARS significantly ($P<0.01$).

The levels of endogenous antioxidants (SOD, catalase and tissue GSH) were decreased significantly ($P<0.01$) in the pathogenic control group, as compared with the control group and this reduction was significantly reversed by ethanolic *Z. officinale* extract pretreatment (Table 2).

The increase in wet weight of myocardium after ISO administration has been observed in Group II, when compared with those of the normal healthy control group (Group I), while ZO (200 mg/kg body wt, po) pretreatment significantly ($P<0.01$) decreased the water content of the myocardium (Table 3).

Histopathological studies—The results of bio-chemical observations in serum and tissue were supplemented by histopathological examination of rat's heart sections. Heart sections of vehicle control group and ZO *per se* group depicted clear integrity of myocardial cell membrane (Fig. 1a and d) However, the heart sections of ISO-treated pathogenic rats showed fatty changes, inflammatory infiltrate, edema and congestion in myocardium, leading to impairment of membrane structural and functional integrity (Fig. 1b and c). In animals, treated with ethanolic ZO extract pretreatment, the morphology of the myocardium was essentially within normal limits. No area of necrosis and cellular infiltration was seen (Fig. 1e) indicating that ethanolic ZO extract has significant cardioprotective effect and it also, maintained myocardial membrane integrity.

Discussion

The serum marker enzymes viz LDH, CPK, AST and ALT serve as sensitive index to assess the severity of MI[27]. In ISO treated rats, the increased activities of the serum marker enzymes accompanied by their concomitant increase in wet weight of myocardium confirms the onset of myocardial necrosis. The increase in wet weight of myocardium after ISO administration may be due to the increased water content, edematous intermuscular space and extensive necrosis of cardiac muscle fibre followed by invasion of the damaged tissue by inflammatory cells. Ethanolic ZO extract pretreatment is found to protect the myocardium against infiltration and also to decrease the water content of the myocardium. This could be the reason for the observed reduction in the wet weight of the myocardium in ZO pretreatment group.

Free radicals generated by ISO, initiate lipid peroxidation of the membrane bound polyunsaturated fatty acids, leading to impairment of the membrane structural and functional integrity. This concurs with the present findings wherein the levels of lipid peroxidation were found to be significantly ($P<0.01$) increased in animals subjected to ISO exposure.

Table 3—Effect of ethanolic *Z. officinale* extract pretreatment on isoproterenol induced increased in the wet weight of myocardium [Values expressed in mg are mean ± SE from 8 animals in each group]

Treatment	Wet weight of myocardium (mg)
Control	668.56 ± 14.199
Isoproterenol (85 mg/kg, sc)	852.64 ± 28.558*
Z. officinale (200 mg/kg, po)	675.23 ± 18.811
Z. officinale (200 mg/kg, po) + Isoproterenol (85 mg/kg, sc)	773.56 ± 14.133**

P values: *<0.001, when compared with normal control group, **<0.05, when compared with pathogenic control group.

Table 2—Effect of ethanolic *Z. officinale* extract pretreatment on isoproterenol induced changes in the TBARS, tissue GSH, SOD and catalase levels in heart [Values are mean ± SE from 8 animals in each group]

Treatment	TBARS (nmol of MDA/ mg protein)	Tissue GSH (μmol/mg protein)	SOD (Unit/min/mg protein)	Catalase (nmol of H_2O_2 consumed/min/ mg protein)
Control	2.465 ± 0.064	32.739 ± 1.192	2.387 ± 0.056	10.111 ± 0.900
Isoproterenol (85 mg/kg, sc)	6.441 ± 0.225[a]	25.304 ± 1.312[b]	1.736 ± 0.028[a]	7.966 ± 0.544[b]
Z. officinale (200 mg/kg, po)	1.859 ± 0.051	33.205 ± 2.013	2.543 ± 0.038	12.133 ± 0.582
Z. officinale (200 mg/kg, po) + isoproterenol (85 mg/kg, sc)	3.657 ± 0.086[c]	44.292 ± 1.492[c]	2.496 ± 0.046[c]	10.305 ± 0.538[d]

TBARS= thiobarbituric acid reactive substances; Tissue GSH= tissue glutathione; SOD= superoxide dismutase
P values: [a]<0.01, [b]<0.05 when compared with normal control group, [c]<0.01, d<0.05 when compared with pathogenic control group

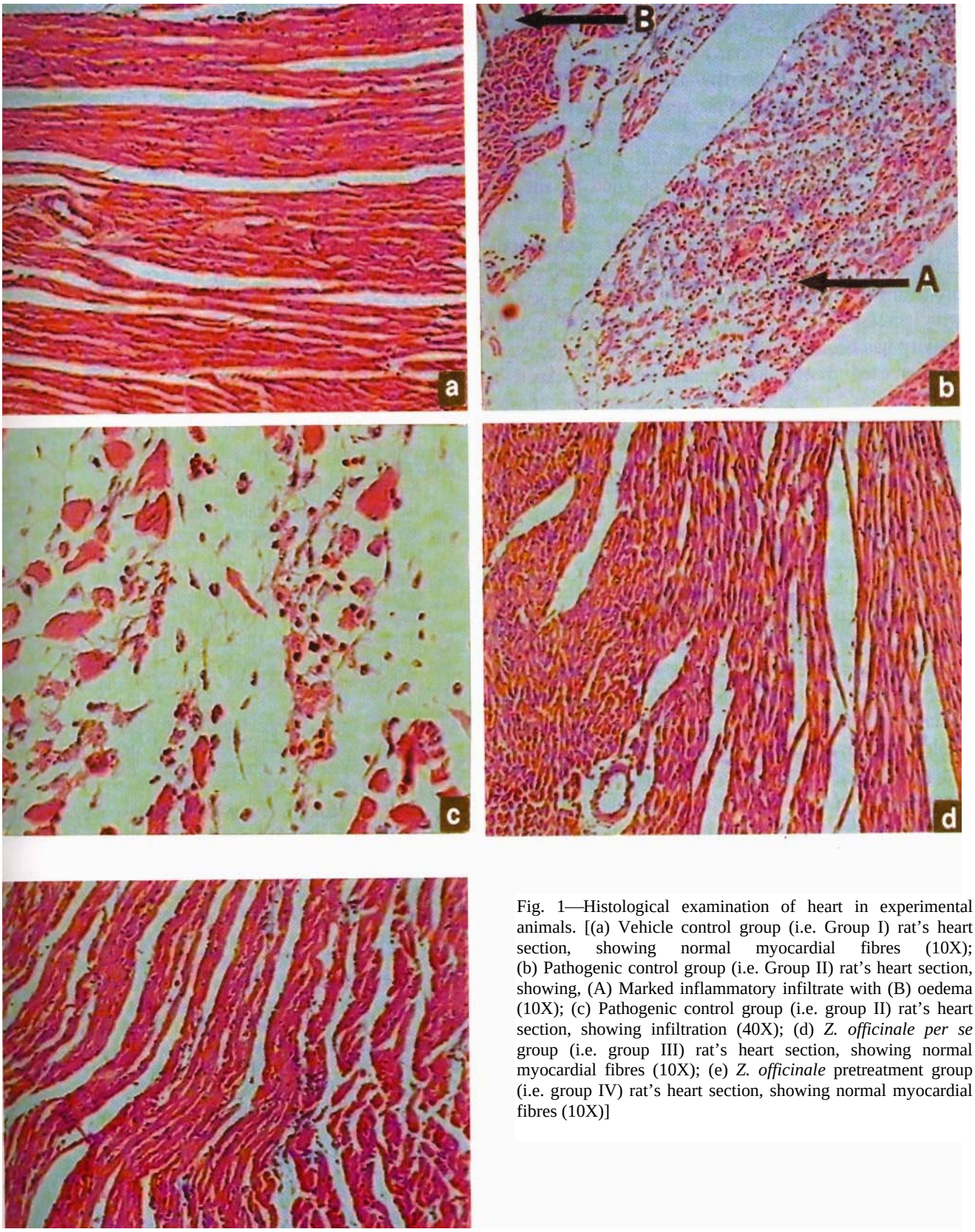

Fig. 1—Histological examination of heart in experimental animals. [(a) Vehicle control group (i.e. Group I) rat's heart section, showing normal myocardial fibres (10X); (b) Pathogenic control group (i.e. Group II) rat's heart section, showing, (A) Marked inflammatory infiltrate with (B) oedema (10X); (c) Pathogenic control group (i.e. group II) rat's heart section, showing infiltration (40X); (d) *Z. officinale per se* group (i.e. group III) rat's heart section, showing normal myocardial fibres (10X); (e) *Z. officinale* pretreatment group (i.e. group IV) rat's heart section, showing normal myocardial fibres (10X)]

Extent of cardioprotection offered by the drug is associated with significant attenuation of serum LDH, serum CK, serum AST and serum ALT levels. In the present study, near normal activity of the diagnostic marker enzymes in the serum and significant decrease (P<0.01) in levels of lipid peroxides in heart tissue of rats with ethanolic extract of ZO pretreatment is indicative of the fact that ethanolic ZO extract has significant cardioprotective effect and maintains myocardial membrane integrity.

Pharmacological augmentation of endogenous myocardial antioxidants has been identified as a promising therapeutic approach in disease associated with increased oxidative stress[28,29]. An increase in SOD activity has been reported to be beneficial in the event of increased free radical generation[30]. However, a simultaneous increase in catalase and/or tissue glutathione activity is essential for an overall beneficial effect of an increase in SOD activity[31-34].

ZO in 200 mg/kg dose offered significant protection against ISO-induced oxidative stress in terms of preservation of endogenous antioxidants. The degree of myocardial necrosis and loss of muscle fibre was also significantly less in this group. The protection may have been mediated through a ZO-induced increase in basal myocardial endogenous antioxidants activities.

ZO has beneficial effect on cardiovascular system[18]. This confirms it traditional usefulness in cardiovascular diseases. Bhandari *et al.*[16] reported the significant cholesterol lowering activity of ethanolic extract of ginger. Guh *et al.*[35] reported antiplatelet effect of gingerol. Further, gingerol (10.5-10 μ*M*) also inhibited thromboxane B_2 and prostaglandin D_2 formation caused by arachidonic acid and completely abolished phosphoinositide breakdown induced by arachidonic acid but had no effect on that of collagen, PAF or thrombin at concentrations as high as 300 μ*M*.

ZO have pronounced antioxidant activity comparable to that of synthetic antioxidant preservatives[36]. The main compounds with demonstrated activity are the pungent principles such as gingerol[37] and zingerone[38,39]. Such antioxidant activity may be expected since many inhibitors of lipoxygenase are also strong antioxidants. ZO was shown to significantly scavange superoxide and hydroxyl radicals *in vitro*[40] and to inhibit lipid peroxidation[41,42].

Combined effect of active principles present in the ethanolic extract of ZO may offer protection against cardiac damage in ZO pretreated ISO injected rats.

The histopathological observations of the heart tissue of ZO pretreated animals showed near normal pattern, supporting its role as a promising cardioprotective agent.

It can be concluded that oral administration of ethanolic ZO extract augments endogenous myocardial antioxidants and protects rat heart against ISO-induced myocardial necrosis and associated oxidative stress. The protective effect of ZO against experimental MI could be through its multiple mechanisms. Thus, it could be suggested that dietary supplementation of ZO might have significance in the prevention of cardiovascular disease.

Acknowledgement

This study was supported by a postgraduate Scholarship from the University Grants Commission (UGC), Government of India, New Delhi, India.

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