LIST OF PUBLICATIONS
Antilipoperoxidative and membrane stabilizing effect of diosgenin, in experimentally induced myocardial infarction

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Abstract Altered membrane integrity has been suggested as a major factor in the development of cellular injury during myocardial necrosis. The present study was designed to investigate the effect of diosgenin on lysosomal hydrolases, membrane-bound enzymes, and electrolytes during isoproterenol (ISO)-induced myocardial necrosis in rats. Animals were pretreated with DIOS (80 mg/kg) for a period of 35 days. Myocardial infarction was experimentally induced with ISO (85 mg/kg) twice at 24 h interval. Experimental myocardial infarction was evidenced with marked elevation of creatine kinase-MB (CK-MB) in serum with concomitant increase in lipid peroxidation (plasma thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP)). Activity of lysosomal hydrolases (β-glucuronidase, β-N-acetyl glucosaminidase, β-D-galactosidase, cathepsin D, and acid phosphatase) was found to be increased in serum and heart tissue of ISO-alone treated animals. DIOS (80 mg/kg) pretreated groups showed significant decrease in CK-MB, lipid peroxidation, and lysosomal hydrolases activity. The membrane-bound enzymes such as Ca$^{2+}$-ATPase and Mg$^{2+}$-ATPase activity was increased and Na$^+$/K$^+$-ATPase activity was decreased in the heart tissues of ISO-alone treated animals. These enzyme alterations lead to the change in the electrolytes content such as sodium, potassium, and calcium in the heart tissue. However, DIOS (80 mg/kg) pretreatment reversed the membrane-bound enzymes activity and thereby maintained the normal electrolyte concentration. These results suggest the protective action of diosgenin in ISO-induced myocardial infarction. The salubrious effect observed in this study might be due to the antioxidant and membrane stabilizing potential of diosgenin.

Keywords Isoproterenol · Diosgenin · Lysosomal hydrolases · Membrane-bound enzymes · Antioxidant · Electrolyte

Introduction

Ischemic heart disease, the leading cause of mortality and morbidity, is a condition resulting in myocardial hypoxia and accumulation of waste metabolites is most often due to atherosclerotic disease of the coronary arteries. Myocardial ischemia may directly or through activation of the complement pathway result in cell injury and death and releases the intracellular lysosomal enzymes [1]. The damage caused by the enzymes of lysosomal and mitochondrial origin and the modification of tissue constituents by these enzymes play an important role in myocardial ischemia [2]. Lysosomal enzymes are important mediators of acute myocardial infarction and their release into the cytoplasm stimulates the formation of inflammatory mediators such as oxygen radicals, prostoglandins, etc. [3]. It has been postulated that the intracellular release of lysosomal enzymes and their subsequent extra-lysosomal activity may exercise a pivotal role in the progressive modifications that lead from reversible myocardial ischemia to irreversible infarction [4].
Flavonoid Rich Fraction of *Dioscorea bulbifera* Linn. (Yam) Enhances Mitochondrial Enzymes and Antioxidant Status, Thereby Protects Heart from Isoproterenol Induced Myocardial Infarction

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Abstract: With recent advances in nutrition sciences, natural products and health-promoting foods have received extensive attention from both health professionals and the common population. The flavonoid rich fraction (FRF) of *Dioscorea bulbifera* Linn. has a strong free radical scavenging activity. FRF (150 mg/kg) when intervened for a period of 35 days prior to isoproterenol (ISO) challenge to rats maintained the creatine kinase – MB (CK-MB) activity in serum without elevation. Alterations in the antioxidant status in the mitochondria were recognized in the heart tissue of ISO induced rats. ISO induced rats pretreated with FRF (150 mg/kg) ameliorated the lipid peroxidation and thereby enhanced the antioxidant status as evidenced by the increase in the reduced glutathione (GSH) content and the activity of antioxidant enzymes. Moreover, the tricarboxylic acid cycle enzymes such isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH), malate dehydrogenase (MDH) and a-ketoglutarate dehydrogenase (a-KGDH), which were found decreased in the ISO induced rats showed an enhanced activity in FRF (150 mg/kg) pretreated rats. The activity of NADH dehydrogenase and cytochrome-C-oxidase, the enzymes which transfer the electron in the electron transport chain (ETC) was also increased significantly \((p<0.05)\) in FRF (150 mg/kg) pretreated rats, when compared with ISO induced rats. These results suggest the cardioprotective effect of FRF of *Dioscorea bulbifera* Linn. in ISO induced MI by attenuating the lipid peroxidation by scavenging free radicals and modulating the energy producing mitochondrial enzymes.

Keywords: Isoproterenol, mitochondrial enzymes, antioxidant, free radical, lipid peroxidation, flavonoids.

INTRODUCTION

Consumption of plant based foods is helpful in health promotion and reduction of disease risk. Growing interest in research, development and commercialization of functional food ingredients, nutraceuticals and dietary supplements has focused in better processing of the foods so that the active ingredients is not left out but concentrated [1]. *Dioscorea bulbifera* Linn. tuber is a variety of yam which is described in Ayurvedic medicine as Varhikanda, Charmakaraluka, Varahavadana, Grishti and Varada. It is used as a main ingredient in Ayurvedic preparations such as Narashimachurna and Shivagutika. The tuber is used in the treatment of anti-helmentic, anti-diabetic and hematological disorders [2]. It is also used as a food by tribal populations in India. These have attracted scientist to study its nutrition [3], anti-nutritional [4] and pharmacological properties such as anorexiant [5], anti-fungal [6] and anti-tumor [7,8] properties. In this context, ethylacetate fraction of *Dioscorea bulbifera* was found to contain a group of flavonoids exhibiting anti-tumor promoting property in JB6 cells induced by 12-O-Tetradecanoylphorbol-13-acetate (TPA) [2].

Isoproterenol (ISO) is a synthetic catecholamine which induces myocardial infarction on administration to rats by its action on the sarcolemmal membrane, stimulation of adenylylate cyclase, activation of Na\(^+\) and Ca\(^{2+}\) channels, exaggerated Ca\(^{2+}\) inflow and energy consumption, leading to cellular death [9]. Generation of highly cytotoxic free radicals through the auto-oxidation of catecholamine has also been one of the causative factor for cardiac damage [10]. Hence, in this work we aimed to study the free radical scavenging potential and cardioprotective property of the flavonoid rich fraction of *Dioscorea bulbifera* (FRF) in ISO-induced myocardial infarction (MI).

MATERIALS AND METHODS

Chemicals

Isoproterenol hydrochloride, 1,1-Diphenyl-2-picyril hydrazyl (DPPH) and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) were purchased from Sigma Chemical Company, St. Louis, MO, USA. All the other solvents and reagents used in this study are of analytical grade.

Collection of Plant material and Processing

*Dioscorea bulbifera* Linn. tuber was purchased from Ayurmed Biotech Pvt. Ltd, Mumbai and it was authenticated at the Centre for Advanced Research in Indian System of Medicine (CARISM) by qualified botanists. The tubers were cut into pieces of 5 to 8 mm thickness and it was air dried. The air-dried tubers were coarsely pulverized.

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Protective role of air potato (Dioscorea bulbifera) of yam family in myocardial ischemic reperfusion injury

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Hydroalcoholic extract of Dioscorea bulbifera (DB), a yam variety called air potato, was tested for its protective effect on myocardial ischemic/reperfusion (I/R) injury in rats due to apoptosis and necrosis. Myocardial I/R injury was induced by 30 min ischemia followed by 2 h reperfusion by perfusing isolated rat hearts with Krebs Henselet bicarbonate (KHB) buffer in a Langendorff set up. Pretreatment of DB (150 mg kg⁻¹ body weight) for 30 days significantly reduced myocardial infarct size and improved the ventricular function (aortic flow and coronary flow, LVDP, LVMmax dp/dt). Role of DB on apoptosis was also evaluated by determining caspase 3 as well as by examining pro-apoptotic and anti-apoptotic proteins Bax and Bcl2 by Western blot analysis followed by TUNEL assay. DB also prevented I/R-mediated down regulation of survival protein Akt and HO-1. Our results indicated that Dioscorea bulbifera could ameliorate myocardial ischemia and reperfusion injury by improving ventricular function and inhibition of cardiomyocyte necrosis and apoptosis.

Introduction

Ischemia and reperfusion (I/R) causes myocardial infarction potentiated by both necrosis and apoptosis.1 Moreover, accumulating evidence indicates that, apart from necrosis, apoptosis contributes significantly to post ischemic cardiomyocyte death, suggesting that therapeutic intervention that inhibits apoptotic cell death may attenuate I/R-induced cardiomyocyte injury.2,3 Recently, there have been many scientific claims that botanicals provide cardioprotection against (I/R) injury in a variety of experimental models via multiple mechanisms including inhibition of apoptotic cell death.4–6 Dioscorea bulbifera (DB), commonly known as aerial yam or air potato, belonging to the Dioscoreaceae family, is widely distributed in India, Ceylon, the Malay Peninsula, Australia, East Africa and Brazil.7 It is one of the important medicinal plants used in indigenous systems of medicine in Asia.8 Dioscorea species are most noted for the abundance of diosgenin, a steroidal saponin used as a precursor for the synthesis of corticosteroids, estrogen, contraceptives, and spironolactones.9 Recently, diosgenin has been found to ameliorate myocardial infarction by its anti-liperoxidative activity.10 Hence, this study aimed to investigate whether the extract of Dioscorea bulbifera could reduce myocardial I/R injury-induced apoptosis in the rat heart.

Materials and methods

Chemicals and antibodies

The solvents used for plant extraction were of analytical grade and obtained from Sisco Research Laboratory (SRL), India. All other chemicals used were of analytical grade and were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO), unless otherwise specified. Primary antibodies such as Akt, pAkt, Bax, Bcl2, HO-1, pro and cleaved caspase - 3 and glyceraldehyde-6-phosphate dehydrogenase (GAPDH) were obtained from Santa Cruz Biotechnology, Santa Cruz, CA.

Preparation of plant extract

Fresh Dioscorea bulbifera (DB) tubers were purchased from Ayurved Biotech Pvt. Ltd, Mumbai, India, and it was taxonomically identified and authenticated by a taxonomist and a voucher specimen has been preserved for further reference. The tubers were cleaned, dried under shade at room temperature and coarsely pulverized using a mechanical grinder. The dried powder (1 kg) was extracted using a Soxhlet-extractor in 70% ethanol. Hydroalcoholic extracts were evaporated (free of solvents) using a rotary evaporator under reduced pressure at 40 °C. A brown concentrated hydroalcoholic extract was obtained (yield 8.52% w/w with respect to the dried starting material). The final product was then stored at room temperature in a dessicator for further use.

Animals

All animals used in this study received humane care in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving