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Efficacy of grape seed proanthocyanidins on serum and heart tissue lipids in rats subjected to isoproterenol-induced myocardial injury

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Abstract

The present study was aimed to evaluate the preventive role of grape seed proanthocyanidins (GSPs) on serum and tissue lipid enzymes in isoproterenol (ISO)-induced myocardial injury in male Wistar albino rats. GSP was administered orally to rats (150–180 g) in three different doses, by gastric gavage (50, 100 and 150 mg/kg GSP), 6 days a week for 5 weeks. At the end of this period, all the rats, except the normal untreated rats that served as the control group, were administered ISO, 85 mg/kg subcutaneously, for 2 consecutive days to induce myocardial injury. After 48 h, rats ($n=6$ per group) were anesthetized with anesthetic ether, sacrificed and the levels of biochemical observations of the serum and heart tissues were performed. Biochemical assessment of myocardial injury was done by measuring the activities of serum thiobarbituric acid reactive substances and plasma lactate, which were significantly elevated in the rats administered with ISO. Further, our results suggest that prior administration of GSPs significantly maintained the cholesterol, phospholipids, triglycerides, and free fatty acids levels in serum and heart tissue of the ISO-induced myocardial injury in rats. The experiments conclude that GSPs possess cardioprotective and hypolipidemic effect on the treatment of ISO-induced myocardial injury.

Keywords: Isoproterenol; Grape seed proanthocyanidins; Hypolipidemic; Rats; Lipids

1. Introduction

Cardiovascular diseases (CVDs) are the most prevalent cause of death and disability worldwide. CVD, a group of disorders of the heart and the vasculature, includes high blood pressure, coronary heart disease, congestive heart failure, stroke and congenital heart defects. The World Health Organization (WHO) estimates that 17 million people die of cardiovascular disease annually (MacKay and Mensah, 2004). WHO predicts that deaths due to circulatory system diseases are projected to double by 2015 (Reddy, 1993). It is well known that CVD are directly or indirectly related to oxidative damage that shares a common mechanism of molecular and cellular damage.

The rat model of ISO-induced myocardial necrosis, out of many well-known models, has often been used to evaluate several cardiac dysfunctions (Wexler, 1978). ISO causes stress in the myocardium and causes severe increase in the levels of serum and myocardial lipids, which in turn leads to coronary heart disease (Nair and Devi, 2006). The current study is an attempt made to assess the protection of the heart, through pretreatment with grape seed proanthocyanidins, by reducing the excess lipids. Proanthocyanidins are naturally occurring compounds widely available in fruits, vegetables, nuts, seeds, flowers, and bark. They are a class of phenolic compounds which take the form of oligomers or polyhydroxy flavan-3-ol units, such as (+)-catechin and (−)-epicatechin (Porter, 1998). GSPs have gained considerable attention due to their wide range of biological and pharmacological properties (Bagchi et al., 2002). There are reports of the possible use of phenolics in grapes in preventing atherosclerosis (Kovac and Pekic, 1991). Our previous studies suggested that the myocardial antioxidant enzyme activities,

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which include superoxide dismutase, catalase, and glutathione peroxidase, were modulated by consumption of GSP in ISO-induced rats (Karthikeyan et al., 2007). In view of this we have discovered that GSP possess hypolipedimic activity in ISO-induced myocardial injury in rats.

2. Materials and methods

2.1. Animals and chemicals

Male Wistar albino rats, 120–150 g, were obtained from the Tamil Nadu Veterinary and Animal Science University, Chennai, India, and were housed at 25±5 °C in a well-ventilated animal house under a 12-h light–dark cycle. The rats had adequate amounts of proteins and vitamins, and water *ad libitum*. The study was approved and carried out as per the guidelines of the Institutional Animal care Ethics Committee (IAEC No: 01/017/06). Isoproterenol hydrochloride was purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals were of analytical grade. Double distilled water was used in all biochemical assays. The GSPs of weighed dose were dissolved in saline and used for the present study as reported in our previous study (Karthikeyan et al., 2007).

2.2. Experimental induction of myocardial injury

Myocardial injury was induced in experimental rats by injection of 85 mg/kg of ISO daily for 2 consecutive days, 24 h apart and sacrificed 48 h after the first dose (Rona et al., 1959; Seth et al., 1998; Karthikeyan et al., 2003).

2.3. Experimental design

The rats were divided into eight groups of six rats each: Group C, vehicle (saline); Group ISO, vehicle and 85 mg/kg ISO; Group GSP-50-BL, 50 mg/kg of GSP; Group GSP-50-ISO, 50 mg/kg of GSP and 85 mg/kg ISO; Group GSP-100-BL, 100 mg/kg of GSP; Group GSP-100-ISO, 100 mg/kg of GSP and 85 mg/kg ISO; Group GSP-150-BL, 150 mg/kg of GSP; Group GSP-150-ISO, 150 mg/kg of GSP and 85 mg/kg ISO. There were no significant differences in the body weights of the treated rats when compared with control, either at the beginning or at the end of the study period. The treated rats did not offer any abnormal resistance to drug administration. The treatment schedule did not cause any change in food and water intake pattern.

All drugs and saline were given once a day at a fixed time, by oral gavage, 6 days a week for 5 weeks. At the end of the experimental period, *i.e.*, 24 h after the last injection of ISO, all the rats were anesthetized with sodium pentobarbitone (60 mg/kg i.p). Blood was drawn from the external jugular vein of the rat and serum separated by centrifugation was used for the determination of serum thiobarbituric acid reactive substances (TBARS) (Yagi, 1982), LDH isoenzymes and lipids; plasma was separated and used for the estimation of lactate (Barker and Summerson, 1941). Heart tissue was excised immediately and rinsed in ice-chilled normal saline. A known weight of the heart tissue was homogenized in 5.0 ml of 0.1 M Tris–HCl buffer (pH 7.4) solution. The homogenate was centrifuged and the supernatant was used for the of biochemical estimations.

2.4. Extraction and estimation of serum and heart tissue lipids

From the samples of serum and heart tissue homogenate the lipids were extracted by the method of Folch et al. (1957). To a known volume of serum or tissue homogenate, 10 ml of chloroform–methanol (2:1 v/v) mixture was added and mixed well for 30 min and was filtered through Whatman filter paper (No. 42) into a separating funnel. The filtrate was mixed with 0.2 ml of physiological saline and the mixture was kept overnight undisturbed. The lower phase containing the lipid was drained off

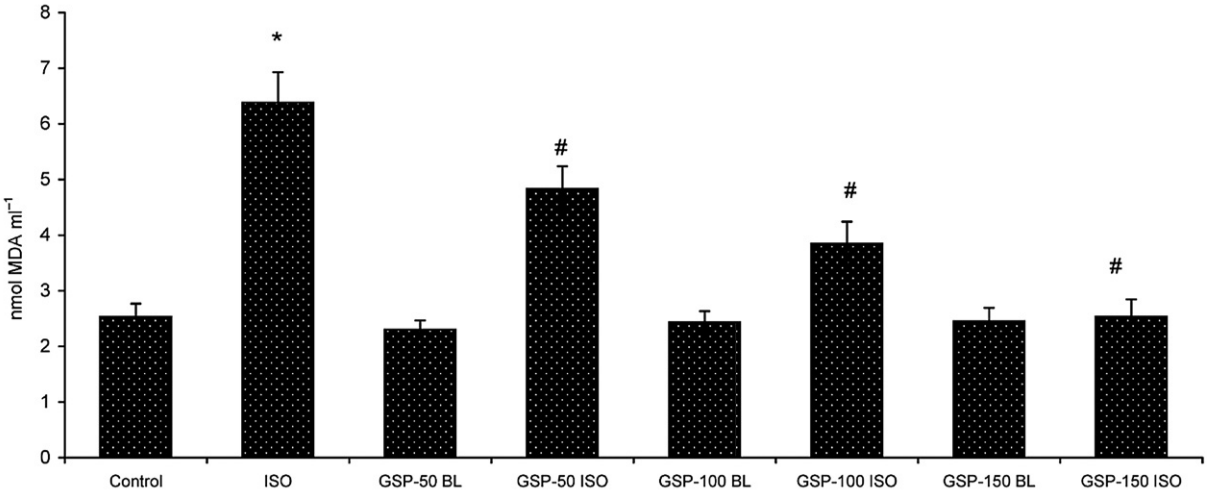

Fig. 1. Effect of GSP on serum TBARS of control and experimental groups of rats. All values are expressed as mean±SD ($n=6$). *$p<0.001$ *vs* Control; #$p<0.001$ *vs* ISO (one-way ANOVA). Rats received the following treatments: Control: vehicle (saline); ISO: vehicle and 85 mg/kg ISO; GSP-50-BL: 50 mg/kg of GSP; GSP-50-ISO: 50 mg/kg of GSP and 85 mg/kg ISO; GSP-100-BL: 100 mg/kg of GSP; GSP-100-ISO: 100 mg/kg of GSP and 85 mg/kg ISO; GSP-150-BL: 150 mg/kg of GSP; GSP-150-ISO: 150 mg/kg of GSP and 85 mg/kg ISO. Units: nmol MDA/ml for TBARS.