Original Article

Comparative Effect of Grape Seed Extract (*Vitis Vinifera*) and Ascorbic Acid in Oxidative Stress Induced by On-pump Coronary Artery Bypass Surgery

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Abstract

Background: This study aimed to test the beneficial effect of grape seed extract (GSE) (*Vitis vinifera*) and Vitamin C in oxidative stress and reperfusion injury induced by cardiopulmonary bypass (CPB) in coronary artery bypass surgery. **Patients and Methods:** In this randomized trial, 87 patients undergoing elective and isolated coronary bypass surgery included. The patients were randomly assigned into three groups (n = 29 each): (1) Control group with no treatment, (2) GSE group who received the extract 24 h before operation, 100 mg every 6 h, orally, (3) Vitamin C group who received 25 mg/kg Vitamin C through CPB during surgery. Blood samples were taken from coronary sinus at (T1) just before aortic cross clamp; (T2) just before starting controlled aortic root reperfusion; and (T3) 10 min after root reperfusion. Some clinical parameters and biochemical markers were compared among the groups. **Results:** There were significant differences in tracheal intubation times, sinus rhythm return, and left ventricular function between treatment groups compared with control ($P < 0.05$). Total antioxidant capacity was higher ($P < 0.05$) in both grape seed and Vitamin C groups at T2 and T3 times. In reperfusion period, malondialdehyde level was increased in control group; however, it was significantly lower for the grape seed group ($P = 0.04$). The differences in the mean levels of superoxide dismutase and glutathione peroxidase among the three groups were not significant ($P > 0.05$ in all cases). **Conclusions:** In our patients, GSE and Vitamin C had antioxidative effects and reduced deleterious effects of CPB during coronary artery bypass grafting surgery.

Keywords: *Antioxidants, cardiopulmonary bypass, coronary bypass grafting*

Introduction

Coronary artery bypass graft (CABG) generally involves cardiopulmonary bypass (CPB) with cardioplegic arrest and elective global ischemia of the heart. This operation is one of the most established procedures for coronary artery revascularization in a large number of patients with severe congenital heart disease. However, the profile of patients undergoing CABG surgery is changing with increasingly higher-risk patients being operated upon, resulting in significant morbidity and mortality in this group of patients. It has been estimated that the predicted operative risk has been increased by 30% over the past decade.[1]

The nature of the myocardial injury is likely to be multifactorial and atheroembolization, direct injury due to handling and retraction of the heart, systemic inflammation, and use of intracoronary shunts.[2] Ischemia–reperfusion (I/R) injury is probably the most important mode of injury, which is associated with worse short- and long-term clinical outcomes. It is now well established that oxidative stress is one of the major initiators of myocardial injury during ischemia and reperfusion[3] and may increase the level of reactive oxygen species (ROS) several-fold which can lead to apoptosis.[4]

Vitamin C (ascorbic acid) is one of the major dietary antioxidants that ameliorate oxidative stress.[5,6] Grape seed extract (GSE) is a natural powerful antioxidant over Vitamins E and C.[7] It has been reported that GSE is safe without any toxicity. Several studies have shown beneficial cardioprotective effects of GSE in different animal studies,[8-13] but there is no report of GSE on patients undergoing CABG surgery. We believe that in spite of modern techniques of myocardial

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protection, still new treatments are required to protect the heart during cardiac surgery in terms of reducing myocardial injury and preserving left ventricular systolic function, such that clinical outcomes can be improved after CABG surgery. This study for the first time designed to test the beneficial effect of GSE and Vitamin C to reduce the incidence of reperfusion-induced damage in CABG surgery.

Patients and Methods

Study population

One hundred and twelve patients undergoing first-time elective CABG surgery without concomitant procedures were included in this study in a referral university hospital. Following cases were excluded from the study: urgent patients, complicated high-risk patients, diabetics, those who needed another heart surgery beside CABG, and if the ischemic time exceeded 120 min. None of the patients were taking vitamins, dietary supplements, or drugs with established antioxidant properties during the study. Seventeen patients withdrew from the study for the following procedure violations: undiagnosed diabetes mellitus, severe bleeding and delaying surgery, unplanned valve surgery, and one patient died during procedure. Remaining 87 patients (67 males, 20 females, mean age 57.07 ± 1.05 years) were randomly assigned to three groups (n = 29 each) using random allocation software: (1) Control group with no treatment, (2) GSE group which received GSE started 24 h before operation, 100 mg every 6 h, orally, (3) Vitamin C group which received 25 mg/kg Vitamin C through pump circulation during surgery. Study profile is shown in Figure 1. There were no statistically significant differences in demographic and preoperative variables between control, GSE, and Vitamin C groups [Table 1].

This prospective, randomized, and controlled clinical trial was in accordance with the principles outlined in the Declaration of Helsinki. Patients were informed of the procedure and written informed consent was obtained from all patients before inclusion. The Ethical Committee of the Tabriz University of Medical Sciences approved the study protocol. The study was registered in ClinicalTrials.gov with the identifier number of NCT00839085. It was also recorded in Iranian Registry of Clinical Trials (WWW.IRCT.ir) at number IRCT138708271460N1.

Coronary artery bypass graft procedures

All CABG procedures were performed using CPB. The induction of general anesthesia and coronary bypass procedure were carried out routinely to all patients. The right arterial appendage and ascending aorta were selected for two-stage venous cannula and aortic cannula, respectively. Moderate hemodilution (hematocrit [Hct] 22%–24%) and moderate systemic hypothermia (core temperature 28–32°C) were used during CPB. Cardiac arrest was achieved by

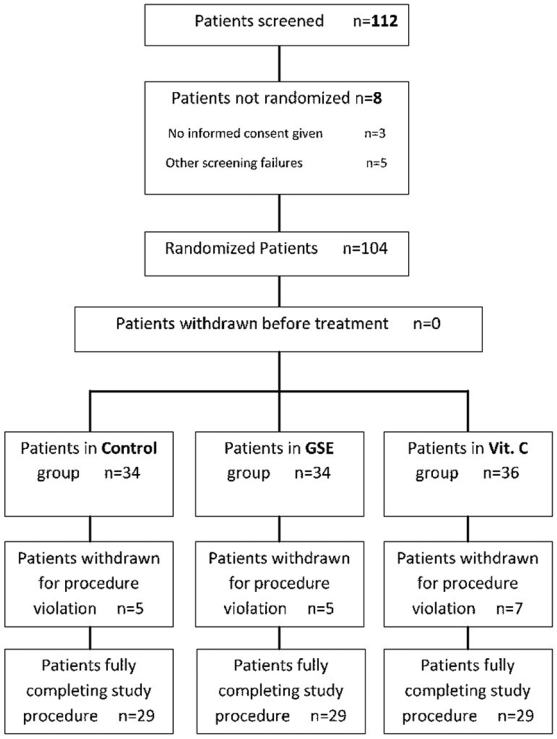

Figure 1: Flowchart depicting the different phases of the trial according to Consolidated Standards of Reporting Trials statement

delivery of high potassium-containing blood cardioplegia solution through combined antegrade and retrograde routs at onset of cross clamp and intermittently for all of the patients. Controlled aortic root reperfusion (CARR) was commenced prior cross-clamp removal. CARR-warm blood (37°C) cardioplegia (K = 10 meq/L) for 1–3 min was followed by normokalemic warm blood 10–20 min without interruption. Normal cardiac activity resumed in this time. The same surgical and anesthetic team managed all patients. Conventional therapy was allowed according to clinical judgment.

Blood sampling

Blood samples were taken from coronary sinus through retrograde cannula at the following times:

- (T1) Preischemia, just before aortic cross clamp
- (T2) Ischemic period, just before starting CARR
- (T3) Post-I/R period, 10 min after CARR.

Samples were delivered to the laboratory for analysis within 3 h after surgery. Biochemical markers included Hct, blood urea nitrogen, creatinine, total antioxidant capacity (TAC), malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPX). All data were collected by an independent research nurse assigned to this research study and were blinded to the groups.

Total antioxidant capacity

TAC was assessed using a standard kit, in which 2,2′-azino-di-(3-ethylbenzthiazoline sulfonic acid) or ABTS is incubated

Table 1: Baseline demographic and preoperative characteristics of the patients in trial groups

Parameters	Groups (*n*=29 each)			*P*
	Control	GSE	Vitamin C	
Sex, male/female ratio	24/5	22/7	21/8	0.63
Age (years)	58.2±1.6	56.3±1.9	56.7±1.7	0.73
Height (cm)	166.2±1.8	165.4±1.6	164.2±1.5	0.68
Weight (kg)	74.0±2.6	75.4±2.3	73.6±2.0	0.84
Hematocrit (%)	42.5±1.0	39.9±0.9	39.9±1.3	0.17
BUN (mg/dl)	18.7±1.4	17.6±1.1	22.1±3.2	0.30
Creatinine (mg/dl)	0.92±0.3	0.89±0.03	1.02±0.06	0.31
Preoperative medication (*n*)				
Beta-blockers	22	21	23	0.60
Acetylsalicylic acid	16	22	18	0.24
Nitrates	24	21	20	0.45
ACEI/ARBs	17	13	17	0.47
Diuretics	1	1	2	0.74
CCBs	7	10	8	0.68
Statins	21	20	20	0.94
Ventricular function				
Preoperative LVEF (%)	49.1±1.2	50.3±1.4	48.4±1.3	0.59
Preoperative LVEF<50% (*n*)	11	11	13	
Occluded vessels (*n*)	3.14±0.18	2.90±0.08	2.93±0.05	0.29
NYHA Class IV/III/II/I	0/3/18/8	0/4/18/7	0/4/15/10	0.89

Values are mean±SEM. No significant differences were observed between groups (P>0.05). SEM: Standard error of mean, ACEI: Angiotensin-converting enzyme inhibitor, ARBs: Angiotensin receptor blockers, CCBs: Calcium-channel blockers, LVEF: Left ventricular ejection fraction, NYHA: New York Heart Association, BUN: Blood urea nitrogen

with a peroxidase and H_2O_2 to produce the radical cation $ABTS^+$. This compound has a stable blue-green color, which is measurable at 600 nm. Antioxidants in the added sample cause suppression of this color production to a degree which is proportional to their concentration (9). TAC was expressed as mmol/L.

Malondialdehyde assay

MDA is a naturally occurring product of lipid peroxidation and can have hazardous effects on nearby and remote tissues. MDA formation was estimated using the modified thiobarbituric acid method as described previously. The MDA level was expressed as nmol/ml.

Superoxide dismutase

The SODs are a family of enzymes that very efficiently accelerate the dismutation of the toxic superoxide radical anion ($O_2^{\bullet -}$) to hydrogen peroxide and molecular oxygen,

$O2^{\bullet -} + O2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$, which is then detoxified to H_2O by catalase (Mukherjee *et al*., 2003). SOD activity was assessed in erythrocyte samples using commercially available Randox kit and expressed as units per gram of hemoglobin.

Glutathione peroxidase

GPX catalyzes the reduction of hydrogen peroxide and organic hydroperoxide by glutathione and functions in the protection of cells against oxidative damage (GPX 2002) kit. Erythrocyte GPX activity was determined using the ultraviolet-visible spectrophotometric technique by employing a commercial kit (Ransel; Randox) and expressed as units per gram of hemoglobin. This method is based on Paglia and Valentine.[8]

Clinical variables

In addition, the following clinical outcomes were recorded in all groups: number of grafts, left ventricular ejection fraction (LVEF), arrhythmias needing treatment (atrial fibrillation, atrial flutter, ventricular tachycardia, conduction defects), need for inotropic support in operating room and Intensive Care Unit (ICU), operation time, CPB time, cross-clamp time, time for mechanical ventilatory support, normal sinus rhythm recovery time after CARR, time to heart beat start after CARR, need to pacemaker for separation from CPB, postoperative ICU duration of stay. All clinical data were collected by an independent end-point assessor team including a cardiologist and a nurse who were assigned to this clinical trial and were blinded to group assignment.

Statistical analysis

All continuous variables are expressed as mean ± standard error of mean. Descriptive statistics were obtained for all studied variables for each study group. One-way analysis of variance (ANOVA), Tukey's posttest, and Pearson Chi-square were applied as appropriate for comparing groups for each variable. Pearson Chi-square analyses were

performed for all categorical variables. In all statistical analyses, a value of $P < 0.05$ was considered statistically significant.

Results

Patients preoperative data

The patients included in this study were admitted for elective coronary revascularization of the heart. The preoperative clinical and demographic data are presented in Table 1 which shows no significant differences with respect to gender, age, weight, preoperative oral medications (β-blockers, aspirin, nitrates, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, calcium channel blockers, diuretics, and statins), LVEF, number of occluded vessels, New York Heart Association functional class, and preoperative laboratory analysis.

Patients surgical data

There were significant differences in tracheal intubation times, sinus rhythm return, temporary pacemaker assistance, and left ventricular function [Figure 2] between treatment groups comparing with control ($P < 0.05$). For other surgical data, the groups were similar without statistically significant difference ($P > 0.05$) with respect to the number of anastomoses, aortic cross-clamp time, surgical time, CPB time, beating time, postoperative ICU length, need for inotropic support in operating room or ICU, and arrhythmias needing treatment [Table 2]. We did not observe any adverse effects attributable to GSE or Vitamin C. No hospital mortality, neurological accidents, myocardial infarction, or acute renal failure occurred in study groups. The summary results for each study group are displayed in Table 2.

Total antioxidant capacity

The TAC in control group was significantly decreased in ischemic (T2) and post-I/R (T3) periods ($P < 0.01$) comparing with baseline preischemic period (T1). TAC was significantly higher ($P < 0.05$) in both GSE and Vitamin C groups comparing control group at T2 and T3 times [Figure 3].

Malondialdehyde assay

The MDA levels in both GSE and Vitamin C groups were always lower compared to control. In post-I/R period, MDA level was increased in control group; however, it was significantly lower for the GSE group compared to the control group [$P = 0.04$, Figure 4]. Besides this, the Vitamin C group also had lower MDA levels, but it was not statistically different compared to the control group after reperfusion ($P > 0.05$).

Superoxide dismutase and glutathione peroxidase

Statistical analysis (ANOVA) showed that differences in the mean levels of activities of SOD and GPX, two antioxidant enzymes, among three groups of the study were not significant ($P > 0.05$ in all cases) [Figures 5 and 6].

Discussion

Ours is the first study to assess GSE in patients undergoing elective CABG surgery. In this study, GSE and Vitamin C had antioxidative effects. In control group, TAC decreased in post-I/R in the end of CPB, while TAC levels remained constant and even slightly increased in GSE and Vitamin C groups. Post-I/R period MDA level was increased in control group, but it was significantly lower in the GSE group compared to the control group. However, SOD and GPX levels did not change during CPB among groups.

Table 2: Patients' intra- or post-operative characteristics

Parameters	Groups (n=29 each)			*P*
	Control	GSE	Vitamin C	
Number of grafts	2.90±0.12	2.86±0.11	2.97±0.10	0.72
Aortic cross clamp time (min)	53.7±2.9	59.8±2.3	58.2±3.1	0.28
Operation time (min)	344.1±8.2	335.2±8.2	322.4±10.1	0.22
Cardiopulmonary bypass time (min)	99.7±4.4	104.5±3.9	100.6±4.6	0.70
Tracheal intubation time (h)	22.9±3.8	14.5±0.9	15.1±1.0	0.019
Postoperative ICU stay length (h)	71.2±6.1	68.7±3.0	67.0±3.0	0.61
Sinus rhythm return after CARR* (min)	9.8±0.9	6.8±0.4	5.9±0.4	0.0001
Beating time after CARR (min)	9.3±0.7	9.3±0.5	8.6±0.6	0.60
Temporary pace-maker (at end of CPB) (%)	3	0	0	0.045
Ventricular function				
Postoperative LVEF (%)	41.7±1.5	48.1±1.1	49.7±1.0	0.0002
Postoperative LVEF<50% (n)	18	11	12	
Need for inotropic support in operating room and ICU (n)	7	4	1	0.074
Arrhythmias needing treatment (n)	6	4	2	0.31

*P<0.05. Values are mean±SEM. LVEF: Left ventricular ejection fraction, SEM: Standard error of mean, ICU: Intensive Care Unit, GSE: Grape seed extract, CARR: Controlled aortic root reperfusion, CPB: Cardiopulmonary bypass

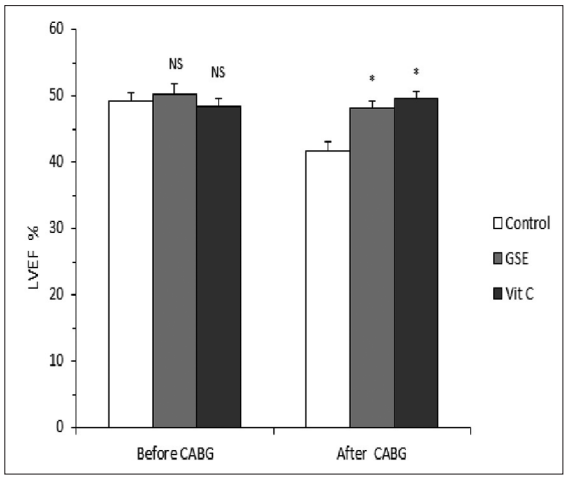

Figure 2: Effect of grape seed extract or Vitamin C administration on left ventricular ejection fraction in patients undergoing coronary artery bypass graft surgery before and after operation. Data are expressed as mean ± standard error of mean (*n* = 29 each group). NS: not significant difference with control group; **: P* <0.05 compared with control group

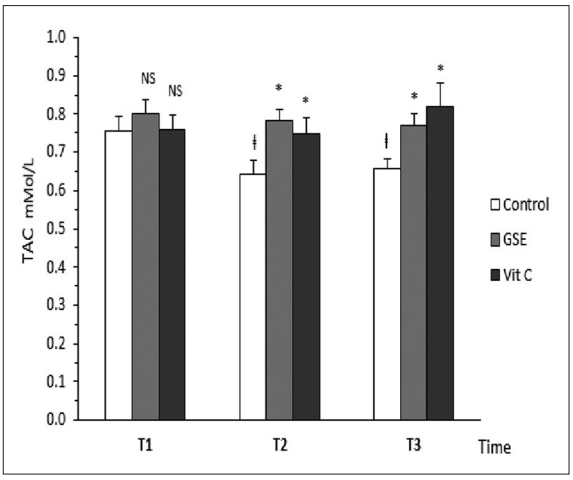

Figure 3: Effect of grape seed extract or Vitamin C administration on plasma total antioxidant capacity in patients undergoing coronary artery bypass graft surgery at the following times: T1: Preischemia, just before aortic cross clamp; T2: Ischemia, precross-clamp removal, T3: Reperfusion, 10 min after cross-clamp removal. Data are expressed as mean ± standard error of mean (*n* = 29 each group). NS: not significant difference with control group; **: P* <0.05 compared with control group; ǂ: *P* <0.01, paired *t*-test comparing with baseline (T1) control

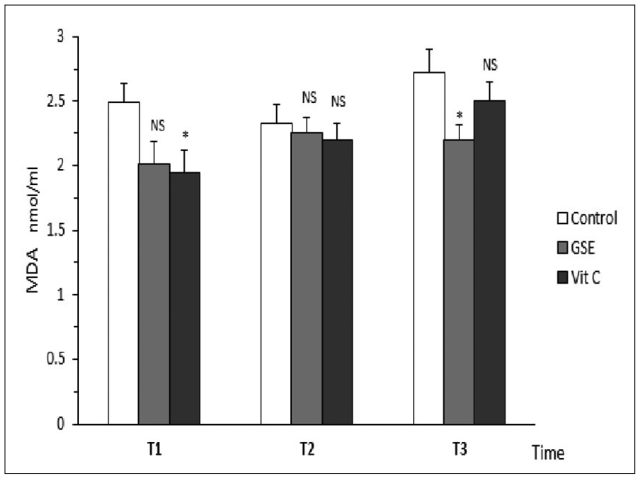

Figure 4: Effect of grape seed extract or Vitamin C administration on malondialdehyde level in patients undergoing coronary artery bypass graft surgery at the following times: T1: Preischemia, just before aortic cross clamp; T2: Ischemia, precross-clamp removal, T3: Reperfusion, 10 min after cross-clamp removal. Data are expressed as mean ± standard error of mean (*n* = 29 each group). NS: not significant difference with control group; **: P* <0.05 compared with control group

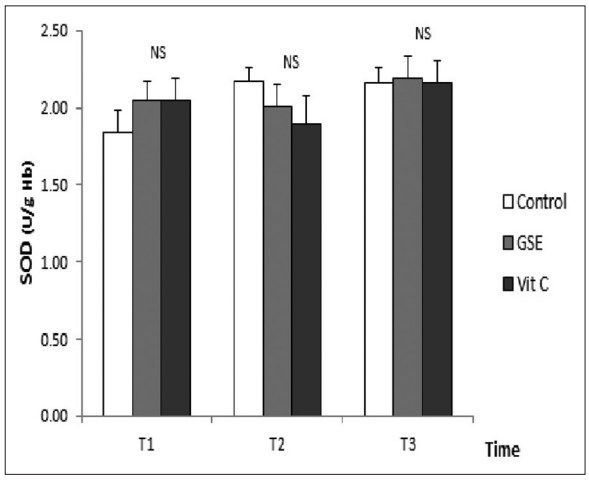

Figure 5: Effect of grape seed extract or Vitamin C administration on superoxide dismutase activity in patients undergoing coronary artery bypass graft surgery at the following times: T1: Preischemia, just before aortic cross clamp; T2: Ischemia, precross-clamp removal, T3: Reperfusion, 10 min after cross-clamp removal. Data are expressed as mean ± standard error of mean (*n* = 29 each group). No significant difference between control and treatment groups

GSE is a natural potent antioxidant which has been shown more beneficial effects in different disease including cancer, diabetes, nonalcoholic fatty liver[13] rat heart I/R injury.[10,12] The nature of the myocardial injury is not entirely clear and is likely to be multifactorial and significantly influenced by the length of cardioplegic arrest, composition of cardioplegic solutions, number of grafts, time required for each anastomosis, atheroembolization, direct injury due to handling and retraction of the heart, systemic inflammation, and use of intracoronary shunts.[2,14]

I/R injury is probably the most important mode of injury, related to the global cardiac ischemia induced by cross-clamping of the aorta during the performance of the distal coronary anastomosis, which is associated with worse short- and long-term clinical outcomes.

It is now well established that oxidative stress is one of the major initiators of myocardial injury during ischemia and reperfusion[3] and may increase the level of ROS several-fold which can lead to apoptosis.[4] Several studies indicate that antioxidant therapy before or during I/R would help the heart recover from ROS-induced damages. Adverse effects of ROS on myocardium can be blocked by antioxidant enzymes such as SOD and catalase.[14-16]

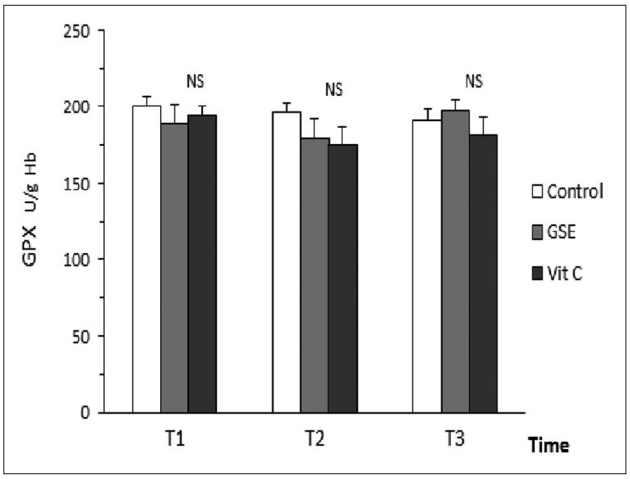

Figure 6: Effect of grape seed extract or Vitamin C administration on glutathione peroxidase activity in patients undergoing coronary artery bypass graft surgery at the following times: T1: Preischemia, just before aortic cross clamp; T2: Ischemia, precross-clamp removal, T3: Reperfusion, 10 min after cross-clamp removal. Data are expressed as mean ± standard error of mean (*n* = 29 each group). No significant difference between control and treatment groups

In experimental studies on isolated rat hearts, ischemia and reperfusion are known to generate oxygen-derived free radicals and have also shown that this production can be prevented by various antioxidants or scavenger agents. A large number of experimental studies that simulate this surgical I/R have shown that various antioxidant agents capable of scavenging ROS, including reactive oxygen free radicals such as superoxide and hydroxyl radicals, can ameliorate the detrimental effects of oxidative stress resulting from I/R and lead to improved postischemic function, either when the agents are provided as a pretreatment before the elective ischemia or as additives to the cardioplegic solution.[17]

Vitamins C (ascorbic acid) is one of the major dietary antioxidants that ameliorate oxidative stress.[5,6] As a water-soluble antioxidant, Vitamin C is in a unique position to "scavenge" aqueous peroxyl radicals before these destructive substances have a chance to damage tissues. It works along with Vitamin E, a fat-soluble antioxidant, and the enzyme GPX to stop free radical chain reactions. GSE is a natural powerful antioxidant over Vitamins E and C.[8] It has been reported that GSE is safe without any toxicity. The no-observed-adverse-effect level (NOAEL) of the GSE in the subchronic toxicity study was 2% in the diet. Thus, the NOAEL was equal to 1410 mg/kg body weight/day in males and 1501 mg/kg body weight/day in females. These results indicated a lack of toxicity and supported use of proanthocyanidin-rich extracts from grape seeds.[18] Several studies have shown beneficial cardioprotective effects of GSE in different animal studies,[9-12] but there is no report of GSE on patients undergoing CABG surgery.

Cardioprotective effect of grape seed proanthocyanidins on doxorubicin-induced cardiac toxicity in rats and humans investigated extensively.[19-24] Most studies on effects on GSE have been done in animals, usually rat hearts, and we did not find any report on the human heart. There are some studies about using antioxidant in CABG surgery (either off-pump or on-pump) in reducing reperfusion injury.[25-27] Thus, we unable to compare our findings with literature systematically.

Conclusions

In this study, GSE and Vitamin C had antioxidative effects and reduced deleterious effects of CPB during CABG surgery. Exact GSE effect observed in post-I/R period MDA level was increased in control group, but it was significantly lower in the GSE group compared to the control group. The Vitamin C group also had lower MDA levels, but it was not statistically different compared to the control group. In our patients, differences in the mean levels of activities of SOD and GPX among the three groups were not significant.

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Conflicts of interest

There are no conflicts of interest.

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