Cardioprotective Activity of Polyherbal Extracts in Experimental Myocardial Necrosis in Rodents: An Evidence of Antioxidant Activity

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Recommended Citation:
Available at: http://www.bepress.com/jcim/vol5/iss1/35
DOI: 10.2202/1553-3840.1191
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Cardioprotective Activity of Polyherbal Extracts in Experimental Myocardial Necrosis in Rodents: An Evidence of Antioxidant Activity

Suresh R. Naik and Vandana S. Panda

Abstract

The present study investigates the antioxidant activity of A.V. Circulo (AVC), a polyherbal formulation in isoproterenol (ISO)-induced oxidative stress in rats and attempts to correlate its cardioprotective activity with antioxidant activity. Myocardial necrosis was produced in rats with ISO (85 mg/kg, s.c.), injected twice at an interval of 24 h. AVC (500 mg/kg, p.o.) was administered to rats for 21 days and 45 days and its effect was evaluated on ISO-induced cardiac injury. The marker enzymes - AST, LDH & CPK were assayed in serum and heart, and antioxidant parameters, viz., reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) & glutathione reductase (GR), and malondialdehyde (MDA), were determined in heart homogenate. Significant myocardial necrosis, depletion of endogenous antioxidants and an increase in serum levels of marker enzymes were observed in ISO-treated rats when compared with normal rats. Daily pretreatment of AVC (500 mg/kg) for 21 & 45 days to rats which were treated with ISO on the last 2 days, resulted in a significant cardioprotective and antioxidant activity, reflected by decreased levels of serum marker enzymes & MDA, and restored activities of marker enzymes, GSH, and antioxidant enzymes in the heart. AVC (500 mg/kg) administration for 45 days showed greater cardiac protection than for 21 days. It is concluded that AVC (500 mg/kg) oral treatment for 21 & 45 days to ISO-challenged rats augments endogenous antioxidant enzymes in the rat heart and prevents lipid peroxidation of the membrane, thereby salvaging the myocardium from the deleterious effects of ISO.

KEYWORDS: antioxidant activity, cardioprotective, isoproterenol, marker enzymes, polyherbal product

Author Notes: Thanks are due to Amsar Ltd., Indore, India for providing us A.V.Circulo capsules for our research. We also acknowledge Mumbai University for funding this study with its research grants.
INTRODUCTION

Myocardial infarction (MI) is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand (Boudina et al., 2003). It is well recognized that ischemic tissue generates oxygen-derived free radicals and other reactive species which bring about oxidative damage of membrane lipids, proteins, carbohydrates and DNA, leading to vascular and microvascular injury, myocyte edema, increased myocyte apoptosis, increased myocyte necrosis and cardiac contractile dysfunction (Dhalla et al., 1999; Goldhaber and Weiss, 1992).

Isoproterenol (ISO), a synthetic catecholamine and β adrenergic agonist is documented to produce myocardial infarction in large doses (Rona et al., 1959; Rona, 1985). On auto-oxidation, it generates highly cytotoxic free radicals which are known to stimulate peroxidation of membrane phospholipids and cause severe damage to the myocardial membrane (Rona et al., 1973).

It has been well established that dietary factors influence the risk of cardiovascular diseases (CVD). As human beings shift from grain based to high fat diets, the incidence of CVD escalates. Since an entire population cannot change dietary habits, it becomes imperative for the population to have remedies to counter this disease. Modern medicine, so far, does not have definite cures, and those offered by it have many side effects. Besides consuming a high fiber diet with plenty of fruits and vegetables, a safe alternative is the use of plant products. Hence, these herbal products need to be evaluated scientifically with an aim to define the role of these agents in alleviating CVD, by providing scientific data to validate their use as prophylactic approaches or as adjuncts to a standard treatment (synthetic compounds employed in conventional treatment protocols) of the cardiac disorder.

A.V. Circulo (AVC) is a polyherbal formulation containing the most well documented Asian herbs for protecting the heart and improving heart function viz., Terminalia arjuna, Crataegus oxycantha, Withania somnifera, Boerhaavia diffusa, Coleus forskohlii, and Piper longum. These herbs are known to improve blood flow to the heart and stabilize the rate of heart contraction and blood pressure, when taken regularly (Wadsworth et al., 2004; Jayalakshmi and Niranjali, 2004; Lindner et al., 1978). The phyto-constituents of AVC are documented to possess potent cardiotonic and free radical scavenging activity (Bhattacharya et al., 1987; Chatterjee et al., 2000).

Subacute and long-term treatment of AVC in our earlier studies has demonstrated significant cardioprotective activity in rats (Chauhan and Naik, 2006). The objective of the present study was to understand the molecular mechanism of cardioprotective activity of AVC, by determining biochemical
markers, antioxidant enzymes and lipid peroxidation in the etiology and pathophysiology of cardiac necrosis.

MATERIALS AND METHODS

Plant material

A.V. Circulo capsules were a gift from Amsar Ltd., Indore, India. AVC is a standardized poly-herbal formulation comprising extracts of the following herbs per 500 mg of the capsule:

- Terminalia arjuna 150mg
- Crataegus oxyacantha 100mg
- Forskolin 4% 100mg
- Withania somnifera 100mg
- Boerhaavia diffusa 100mg
- Piper longum 5mg

Drugs and chemicals

Thiobarbituric acid (TBA), reduced glutathione, oxidized glutathione and NADPH were obtained from Himedia Laboratories, Mumbai, India. 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB), isoproterenol and epinephrine were purchased from Sigma Chemical Co., St. Louis, USA. All other chemicals were obtained from local sources and were of analytical grade.

Experimental animals

Wistar albino rats (150-200g) of either sex, procured from Bharat Sera & Vaccines Ltd., India were used. They were housed in clean polypropylene cages under standard conditions of humidity (50 ± 5 %), temperature (25 ± 2°C) and light (12h light/12h dark cycle) and fed with standard diet (Amrut Laboratory Animal Feed, Nava Maharashtra Chakan Oil Mills, Pune, India) and water ad libitum. All animals were handled with humane care. Experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee and conform to the Indian National Science Academy Guidelines for the Use and Care of Experimental Animals in Research. Our Animal House Registration No. with Govt. of India is 25/1999/CPCSEA.
Preparation of AVC and ISO solutions

The contents of the AVC capsule were suspended in 1% (w/v) aqueous carboxymethyl cellulose solution and used.

Isoproterenol was dissolved in distilled water and used immediately for subcutaneous administration.

Experimental procedure

Wistar albino rats after acclimatization (6-7 days) in the animal quarters were randomly divided into 6 groups of 6 animals each and treated in the following way:

Group I - termed as Normal Control, received distilled water (0.5ml/kg, s.c) daily for 2 days at an interval of 24h.

Group II - termed as ISO Control, received 2 injections of ISO (85mg/kg, s.c) for 2 days at an interval of 24h.

Group III – termed as AVC21, received AVC (500 mg/kg, p.o) daily for 21 days and in addition received distilled water (0.5ml/kg, s.c) on the 20th and 21st day at an interval of 24h.

Group IV – termed as AVC45, received AVC (500 mg/kg, p.o) daily for 45 days and in addition received distilled water (0.5ml/kg, s.c) on the 44th and 45th day at an interval of 24h.

Group V – labeled as IAVC21, received AVC (500 mg/kg, p.o) daily for 21 days and in addition received ISO (85mg/kg, s.c) on the 20th and 21st day at an interval of 24h.

Group VI – labeled as IAVC45, received AVC (500 mg/kg, p.o) daily for 45 days and in addition received ISO (85mg/kg, s.c) on the 44th and 45th day at an interval of 24h.

Rats were weighed and sacrificed 24 h after the last subcutaneous injection of ISO. Blood was collected by cardiac puncture under light ether anesthesia and allowed to clot for 30 min at room temperature. The serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and used for the estimation of marker enzymes viz., AST, LDH & CPK.
The hearts were dissected out immediately, chilled and washed with ice cold saline. After washing with ice cold saline, the hearts were patted dry and divided into 2 equal parts. One part was used to prepare 10% (w/v) homogenate in phosphate buffer (50mM, pH 7.4). An aliquot of the homogenate was used for the determination of lipid peroxidation (LPO). The homogenates were centrifuged at 7000 × g for 10 min at 4°C and the supernatants were used for the assays of AST, LDH, CPK, GSH, SOD, CAT, GPX and GR. The remaining part of heart was fixed in 10% buffered formalin and used for histological studies.

**Marker enzyme assays**

The marker enzymes AST, LDH & CPK were assayed in serum and heart tissue using standard kits supplied from Accurex Biochemicals, Mumbai, India and Erba Mannheim, Germany. The results were expressed as IU/L for AST, LDH & CPK.

**Protein estimation**

The levels of total proteins were determined in heart homogenates of experimental animals by using the Lowry et al method using bovine serum albumin as standard (Lowry et al., 1951).

**Lipid peroxidation**

The quantitative estimation of LPO was performed by determining the concentration of Thiobarbituric Acid Reactive Substances (TBARS) in heart using the method of Ohkawa & Yagi (Ohkawa et al., 1979). The amount of malondialdehyde (MDA) formed was quantified by reaction with TBA and used as an index of lipid peroxidation. The results were expressed as nmol of MDA/g of wet tissue using molar extinction coefficient of the chromophore (1.56 × 10⁻⁵/M/cm) and 1,1,3,3-tetraethoxypropane as standard.

**Glutathione estimation**

GSH was estimated in the heart homogenate using DTNB by the method of Ellman (Ellman, 1959). The absorbance was read at 412 nm and the results were expressed as µmol of GSH/g of wet tissue.
Antioxidant enzyme assays in heart homogenate

SOD was assayed by the method of Sun et al in which the activity of SOD was inversely proportional to the concentration of its oxidation product adrenochrome, which was measured spectrophotometrically at 320 nm (Sun and Zigman, 1978). 1 unit of SOD activity is defined as enzyme concentration required to inhibit the rate of auto-oxidation of epinephrine by 50% in 1 min at pH 10.

CAT was estimated by the method of Clairborne (Clairborne et al., 1991), which is a quantitative spectroscopic method developed for following the breakdown of H₂O₂ at 240 nm in unit time for routine studies of catalase kinetics.

GPx estimation was carried out using the method of Rotruck et al which makes use of the following reaction (Rotruck et al., 1973).

\[ H_2O_2 + 2 \text{GSH} \rightarrow 2H_2O + \text{GSSG (oxidized glutathione)} \]

GPx in the tissue homogenate oxidizes glutathione and simultaneously, H₂O₂ is reduced to water. This reaction is arrested at 10 min using trichloroacetic acid and the remaining glutathione is reacted with DTNB solution to result in a colored compound, which is measured spectrophotometrically at 420 nm.

GR activity was determined by using the method of Mohandas et al in which the following reaction is implicated (Mohandas et al., 1984).

\[ \text{NADPH} + H^+ + \text{GSSG} \rightarrow \text{NADP}^+ + 2 \text{GSH} \]

In presence of GR, oxidized glutathione undergoes reduction and simultaneously, NADPH is oxidized to NADP⁺. Enzyme activity is quantified at room temperature by measuring spectrophotometrically at 340 nm the disappearance of NADPH/min.

Histological analysis

Rats were sacrificed 24h after the last treatment and blood was withdrawn from the hearts. The hearts were removed, washed immediately with ice-cold saline and divided into 2 equal parts. One part was used for biochemical studies and the other part was stored in 10% (v/v) buffered formalin, embedded in paraffin, sections cut at 5 µm and stained with hematoxylin and eosin. These sections were then examined under a light microscope for histo-architectural changes.
Statistical analysis

The results of cardioprotective and antioxidant activities are expressed as mean ± SEM from 6 animals in each group. Results were statistically analyzed using one-way ANOVA followed by Tukey-Kramer post test for individual comparisons. \( P < 0.05 \) was considered significant. GraphPad InStat version 3.00 of GraphPad Software Inc., San Diego, USA was the software used for statistical analysis.

RESULTS

ISO dose fixation

Our pilot experiments for dose finding indicated that ISO 85 mg/kg administered subcutaneously induced moderate necrosis in rat heart and a significant alteration in biochemical parameters (Panda and Naik, 2008). Therefore, 85 mg/kg was selected as the toxicant dose in the present study.

Biochemical parameters

The effect of AVC on serum marker enzymes AST, LDH & CPK for 21 days and 45 days is presented in Figure 1. Rats treated with ISO showed a significant increase (\( p<0.001 \)) in the serum activities of the marker enzymes when compared with the normal group of rats. Oral pretreatments with AVC 500 mg/kg for 21 days (IAVC21 group) as well as AVC 500 mg/kg for 45 days (IAVC45 group) to rats, followed by ISO administration on the last 2 days prevented significantly (\( p<0.001 \)) the increase in activities of the marker enzymes due to ISO.

Marker enzyme activities were also assayed in hearts. The effect of AVC treatment for 21 and 45 days on marker enzymes in heart is shown in Figure 2. ISO control group exhibited a significant decrease (\( p<0.001 \)) in AST, LDH & CPK activities in heart when compared with the normal group of rats. Treatment with AVC 500 mg/kg (IAVC21 group) for 21 days as well as 45 days (IAVC45 group), followed by ISO challenge on the last 2 days to rats increased significantly the activities of AST [(\( p<0.05 \) for IAVC21) & (\( p<0.01 \) for IAVC45)], LDH (\( p<0.001 \)) & CPK (\( p<0.001 \)) when compared with the ISO control rats.

The effect of AVC treatment for 21 days & 45 days on antioxidant enzymes, GSH and LPO in heart tissue is outlined in Table 1.

MDA, the myocardial lipid peroxidation marker was significantly elevated (\( p<0.001 \)) in the ISO control group of rats in comparison with the normal group. Pretreatment of AVC 500mg/kg for 21 days as well as 45 days (IAVC21 &
IAVC45 groups) to ISO challenged rats, depleted significantly (p<0.001) the increased levels of MDA due to ISO.

Significant decline in myocardial GSH (p<0.001) was observed in the ISO treated group as compared to the normal group of rats. IAVC21 as well as IAVC45 treatments failed to restore significantly the ISO depleted GSH levels.

ISO treatment induced a significant depletion of antioxidant enzymes SOD (p<0.001), CAT (p<0.001), GPx (p<0.001) & GR (p<0.001) in rat hearts. Oral administration of AVC for 21 days as well as 45 days to rats, followed by ISO injection, restored significantly (p < 0.001) the activities of CAT & GPx; whereas, activities of SOD and GR were significantly (p<0.01) restored only in the IAVC45 group of rats.

Per se oral treatment of AVC for 21 days as well as 45 days (AVC21 & AVC45 groups) to rats did not alter significantly the basal levels of MDA, GSH, SOD, CAT, GPx & GR in comparison with the normal rats.

**Histological analysis**

Histo-architectural examination of the myocardium of normal rats showed clear integrity of the myocardium (Figure 3). Endocardium and pericardium were seen within normal limits. No inflammatory cell infiltration was seen. 85mg/kg ISO treated rats showed moderate to marked myocytic necrosis with moderate infiltration of lymphocytes and macrophages (Figure 4). The changes were more prominent along the endocardium and in papillary muscles.

Hearts of AVC 500 mg/kg treatment for 21 days (IAVC21 group) with ISO challenge, showed mild multifocal myocytic necrosis with mild to moderate infiltration of inflammatory cells (Figure 5). The lesions were distributed mostly along the papillary muscles and endocardium.

AVC 500 mg/kg treatment for 45 days to ISO administered rats (IAVC45 group) showed minimal to mild multifocal myocytic necrosis with mild multifocal and minimal diffused inflammatory cell infiltration (Figure 6). The changes were more prominent along the papillary muscles and endocardium.

IAVC45 group (Figure 6) showed greater cardioprotection than IAVC21 group (Figure 5).

Per se treatments of AVC 500 mg/kg for 21 as well as 45 days (AVC21 & AVC45 groups) to rats showed the heart sections to have histo-architecture similar to that of normal rats.
DISCUSSION

Isoproterenol, a synthetic β adrenergic agonist by its positive inotropic and chronotropic actions increases the myocardial oxygen demand, that leads to ischemic necrosis of myocardium in rats. A number of patho-physiologic mechanisms have been outlined to explain the ISO-induced myocardial damage, viz., altered permeability, increased turnover of norepinephrine and generation of cytotoxic free radicals on auto-oxidation of catecholamine. Free radical mediated lipid peroxidation and consequent changes in membrane permeability are the primary reasons for cardiotoxicity induced by ISO (Noronha-Dutra et al., 1984). Oxidative stress increases cAMP levels by exhausting ATP, depresses sarcolemmal Ca\(^+2\) transport resulting in intracellular calcium overload, leading to ventricular dysfunction and contractile failure in rat heart (Bhagat et al., 1978; tappia et al., 2001).

The lesions produced by ISO in rat heart are similar to those found in myofibrillar degeneration in Ischemic Heart Disease (IHD) in man (Melei et al., 1978). Hence, the study of ISO induced myocardial necrosis and its underlying mechanisms might provide better insight into the pathogenesis of IHD.

The diagnostic marker enzymes AST, LDH & CPK serve as a sensitive index to assess the degree of myocardial necrosis. Rats treated with ISO exhibited increased activities of the marker enzymes in serum accompanied by their concomitant reduction in heart homogenate, indicating the onset of myocardial necrosis.

Free radicals generated by ISO are known to initiate LPO of membrane bound PUFA, leading to damage of the structural and functional integrity of the myocardium. The myocardium once metabolically impaired, releases its marker enzymes into the blood stream. Hence, the activities of AST, LDH & CPK were found to be decreased in the heart tissue of ISO treated animals when compared with normal animals, which is indicative of cellular injury, and can be attributed to excessive lipid peroxide formation.

Elevation in the activities of serum AST, LDH & CPK in ISO control animals when compared with the normal animals was observed. This was due to the leaking out of marker enzymes from a damaged myocardium into blood stream. AVC pretreatment to ISO myocardial necrotic rats showed a significant amelioration of cardiac damage. AVC pretreatment for 21 days as well as 45 days effectively attenuated the ISO elevated activities of the marker enzymes AST, LDH & CPK in serum and significantly restored their activities in the myocardium. These findings are suggestive of the cardioprotective activity of AVC by its ability to maintain myocardial integrity, possibly by preventing myocardial damage due to lipid peroxidation.
The increased levels of MDA reflect excessive formation of free radicals by auto-oxidation of ISO and greater formation of lipid peroxides, resulting in severe damage to the myocardium of animals treated with ISO. AVC treatment significantly decreased the MDA levels by preventing formation of lipid peroxides from fatty acids of the myocardium.

Reduced glutathione is one of the most abundant non-enzymatic antioxidant bio-molecules present in the tissues (Meister, 1984). Its functions are removal of free oxygen species such as H$_2$O$_2$, superoxide anions & alkoxo radicals, maintenance of membrane protein thiols and to act as a substrate for GPx and glutathione S-transferase (GST) (Townsend et al., 2003). Decreased GSH levels in ISO intoxicated rats may be due to its increased utilization for augmenting the activities of GPx & GST. GSH levels depleted by ISO were significantly restored by AVC oral administration. It may be understood that an increase in the levels of GSH could be due to its enhanced synthesis or improved GR activity in the presence of AVC.

Free radical scavenging enzymes such as SOD, CAT & GPx are known to be the first line cellular defense against oxidative damage, disposing O$_2^-$ & H$_2$O$_2$ before their interaction to form the more harmful hydroxyl (OH·) radical (Lil et al., 1988).

In the present study SOD activity decreased significantly in the ISO treated group of animals, which maybe due to an excessive formation of superoxide anions. These excessive superoxide anions might inactivate SOD and decrease the activities of the H$_2$O$_2$ scavenging enzymes CAT & GPx. In the absence of adequate SOD activity, superoxide anions are not dismutated into H$_2$O$_2$, which is the substrate for CAT & GPx. This probably results in an inactivation of the H$_2$O$_2$ scavenging enzymes. Administration of AVC to ISO challenged rats effectively prevented the depletion of SOD, CAT & GPx activities, which can be correlated to the scavenging of free radicals by AVC, resulting in the prevention of depletion of these enzymes.

GR is an antioxidant enzyme involved in the reduction of GSSG (an end product of GPx reaction) to GSH. In ISO treated rats there was a marked reduction in GPx activity, leading to reduced availability of substrate for GR, thereby, decreasing the activity of GR. Oral treatment of AVC to ISO myocardial infarcted rats restored the activity of GR, thus, accelerating the conversion of GSSG to GSH.

Summarizing the effect of AVC on marker enzymes and endogenous antioxidants, it was found that AVC 500mg/kg administration for 45 days showed greater cardiac protection than administration for 21 days.

It has been reported in experimental findings in literature that various phytoconstituents of A.V. Circulo have beneficial effects on the cardiovascular system. Our earlier findings have confirmed their traditional usefulness in
cardiovascular diseases (Chauhan and Naik, 2006). Our present research demonstrates that the cardioprotective activity of AVC is mainly due to its antioxidant activity. The exact mechanism of action of the various phytoconstituents of AVC is not clearly known, but an interplay of antioxidant activity and the mechanism(s) mentioned below, might be responsible for the cardioprotective action of AVC.

*Terminalia arjuna*, a major constituent of AVC is known to enhance aortic PGE\(_2\) like activity, which is responsible for vasodilatory properties (Singh et al., 1982). The bark of this herb also contains a large amount of Ca\(^{2+}\) which might be responsible for its + ve inotropic activity (Wadsworth et al., 2004). The flavone glycosides of *Terminalia arjuna* are potent antioxidants, which have been demonstrated to exhibit vasodilation, inhibition of platelet aggregation & hypolipidemic activity, and are known to prevent many degenerative diseases including cardiovascular diseases (Dwivedi, 2007).

Forskolin and *Crataegus oxycantha* are known to increase cyclic AMP in the myocardium. Forskolin directly activates adenylate cyclase and thereby elevates cAMP levels (Schlepper et al., 1989). *Crataegus oxycantha*’s constituents - catechin, vitexin and kaempferol, which are structurally similar to some known phosphodiesterase inhibitors, increase the concentration of cAMP by inhibiting its metabolism (Verma et al., 2007). Increased cAMP levels result in relaxation of arteries, increased force of contraction and decrease in hypertriglyceridemia.

Recent computer-aided modeling research reports that *Crataegus oxycantha* contains ursolic acid, which is known to act on a digitaloid binding site of Na+/K+ ATPase pump, and thereby induce cardiotonic activity akin to digitalis (Newhope.com). The plant also elicits hypotensive activity due to the presence of procyanidins which are ACE inhibitors.

*Withania somnifera* and *Boerhaavia diffusa*, the other herbs in A.V. Circulo are well documented for their cardiotonic activity. *Withania somnifera* consisting of withanolides, exerts a prolonged hypotensive action; whereas *Boerhaavia diffusa* is mainly included in the formulation for its diuretic properties (Chatterjee et al., 2007; Singh et al., 1992). Piperine, a major active component of long pepper (*Piper longum*) has been reported to enhance drug bioavailability and hence, has been added to this polyherbal formulation (Annamalai and Manavalan, 1990). Most drugs co-administered with piperine are probably more bioavailable as a result of 2 mechanisms - viz., a) increased absorption from the gut and b) the slow down of biotransformation, inactivation and elimination from the system. The latter mechanism is probably the most important one in sustaining the elevated blood levels of the drug, and making it more bioavailable to the tissue (Atal, 1985).
It can be concluded that AVC 500 mg/kg significantly alleviated ISO induced myocardial injury, most likely by its underlying antioxidant activity. The histopathological observations of the myocardium of rats pretreated with AVC and injected with ISO showed less intensity and distribution of myonecrotic lesions and also some regenerating zones, suggesting a cardioprotective effect of AVC. Decreased myocardial necrosis (as evidenced by reduced AST, LDH & CPK release in the serum, and histoarchitectural changes) and augmentation of the endogenous antioxidant enzymes, contribute to its myocardial salvaging effect. Therefore, A.V. Circulo is a potent cardioprotective agent and deserves clinical use in the treatment of IHD and associated Cardiovascular disorders.
Table 1: Effect of AVC treatment for 21 and 45 days on heart TBARS, GSH, SOD, CAT, GPx and GR in ISO induced cardiac necrosis in rats

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Normal ISO control</th>
<th>AVC21</th>
<th>IAVC21</th>
<th>AVC45</th>
<th>IAVC45</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmol MDA/g wet tissue)</td>
<td>33.51 ± 2.61</td>
<td>84.20 ± 4.93*</td>
<td>32.58 ± 0.99</td>
<td>52.08 ± 1.06*</td>
<td>35.57 ± 0.62</td>
</tr>
<tr>
<td>GSH (μmol /g wet tissue)</td>
<td>2.02 ± 0.10</td>
<td>1.34 ± 0.07*</td>
<td>2.22 ± 0.1</td>
<td>1.51 ± 0.07</td>
<td>2.01 ± 0.07</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>10.74 ± 1.01</td>
<td>5.64 ± 0.48*</td>
<td>10.32 ± 0.37</td>
<td>7.2 ± 0.24</td>
<td>10.04 ± 0.05</td>
</tr>
<tr>
<td>CAT (U/mg protein)</td>
<td>21.24 ± 1.09</td>
<td>11.21 ± 0.64*</td>
<td>22.79 ± 1.36</td>
<td>19.58 ± 0.52*</td>
<td>21.93 ± 0.39</td>
</tr>
<tr>
<td>GPx (U/mg protein)</td>
<td>0.33 ± 0.01</td>
<td>0.19 ± 0.01*</td>
<td>0.36 ± 0.01</td>
<td>0.31 ± 0.02*</td>
<td>0.33 ± 0.07</td>
</tr>
<tr>
<td>GR (U/mg protein)</td>
<td>15.90 ± 1.55</td>
<td>7.50 ± 0.72*</td>
<td>16.55 ± 1.03</td>
<td>11.31 ± 0.83</td>
<td>15.52 ± 0.5</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M.; N = 6 in each group

P values: * < 0.001 when ISO group compared with Normal group
$ < 0.01, * < 0.001 when IAVC groups compared with ISO group

1 unit of CAT = μmol H₂O₂ consumed / min / mg protein
1 unit of GPX = μg GSH utilized / min / mg protein
1 unit of GR = nmol NADPH oxidized / min / mg protein
Figure 1: Effect of AVC treatment for 21 and 45 days on serum AST, LDH & CPK in ISO induced cardiac necrosis

Values are mean ± SEM for 6 animals in each group

*p< 0.001 when ISO group compared with Normal group

*p< 0.001 when IAVC groups compared with ISO group
Figure 2: Effect of AVC treatment for 21 and 45 days on heart AST, LDH & CPK in ISO induced cardiac necrosis

Values are mean ± SEM for 6 animals in each group

# p< 0.001 when ISO group compared with Normal group

a p< 0.05, *p< 0.01, **p< 0.001 when IAVC groups compared with ISO group
Figure 3: H & E staining of heart of normal rat

Figure 4: H & E staining of rat heart treated with ISO (85 mg/kg)
Figure 5: H & E staining of rat heart treated with AVC (500 mg/kg) for 21 days & ISO (85 mg/kg)

Figure 6: H & E staining of rat heart treated with AVC (500 mg/kg) for 45 days & ISO (85 mg/kg)
REFERENCES


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