INTERNATIONAL JOURNAL
OF
PHARMACEUTICAL SCIENCES
AND
RESEARCH

CARDIOPROTECTIVE EFFECT OF ARBUTIN ON LIPID PEROXIDATION AND ANTIOXIDANT DEFENSE SYSTEM IN ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION IN RATS

Subramanian Sivasangari, Loordhurani Asaikumar and Lakshmanan Vennila [*]

Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar - 608002, Tamil Nadu, India.

ABSTRACT: The present study was designed to investigate the cardioprotective effect of Arbutin (ARB) against isoproterenol (ISO) induced myocardial infarction (MI) in rats by studying cardiac and hepatic markers, antioxidant defense system, lipid peroxidation products and histopathological changes. Rats were pretreated with ARB (25 and 50 mg/kg BW) for 21 days. After pretreatment, ISO (60 mg/kg BW) was administered subcutaneously into the rats at an interval of 24 h for 2consecutive days to induce MI. In the present study ISO-induced myocardial damage was indicated by the increased heart weight to body weight ratio and the increased activity of marker enzymes such as CK, CK-MB, ALT, AST, LDH, and the elevated levels of troponin T and I in the serum. In addition, the levels of lipid peroxidation products such as TBARS, CDs, and LHPs were significantly increased in the plasma and heart tissue of ISO rats. Activities of enzymic antioxidants such as SOD, CAT, GPx, GST and the non-enzymic antioxidants like vitamin C, vitamin E and reduced glutathione (GSH) were decreased in the erythrocytes, plasma and heart tissues of the ISO-administered rats. Histopathological observations substantiate with the biochemical parameters. The 50 mg/kg BW of ARB was more pronounced than the 25 mg/kg BW and brought back all the above parameters to near normal. ARB ameliorates myocardial damage caused by ISO in rats and provides cardioprotective effect by scavenging the free radicals, restoring the endogenous antioxidants, preserving histopathology of the myocardium and by improving the cardiac function.

INTRODUCTION: Cardiovascular diseases (CVDs) are globally considered as the leading cause of mortality an estimated that 29 percent of deaths worldwide (17.9 million deaths) were due to CVDs. By 2030 more than 23.3 million people will die annually from CVDs (WHO, 2017) [1].

CVDs are also the leading cause of death in India and it causes 3 million deaths per year, accounting for 25% of all mortality. Myocardial infarction (MI) is one of the main causes of death from CVDs, and it is accountable for 7.9 million deaths out of 17.9 due to CVDs [2]. Among the CVDs, myocardial infarction (MI) is the main cause of mortality and morbidity in the developed world and most of the developing countries. MI is an acute condition of necrosis of myocardium that occurs as a result of an imbalance between coronary blood supplies to any part of the heart, resulting in the death of cardiac tissue (myocardial necrosis).

The consequences of MI include hyperlipidemia, peroxidation of membrane lipids, and loss of plasma membrane integrity [3, 4].

The pharmacological induction of MI by subcutaneous administration of ISO in Wistar rat is a well-known model for the experimental evaluation of cardioprotective agents. ISO is a synthetic catecholamine and β-adrenergic agonist causes severe biochemical alterations and structural changes in the heart and is comparable to those taking place in human myocardial alterations [5]. Among the various mechanisms proposed to explain the ISO induced cardiotoxicity, the generation of highly cytotoxic free radicals through auto-oxidation of catecholamines has been implicated as one of the important causative factors [6]. These free radicals may attack polyunsaturated fatty acids (PUFAs) in the membranes, forming peroxy radicals. Peroxy radicals then attack adjacent fatty acids causing lipid peroxidation [7]. These lipid peroxidative products are harmful to the tissue and organs. Therefore, antioxidants or free radical scavengers are the major therapeutic

targets against oxidative stress associated with various CVDs including myocardial infarction [1].

Since ancient times, the medicinal plants are believed to exhibit antioxidant activity and play a crucial role in the management of the various diseases. Consumption of diets with flavonoids is related to reduce the risk of CVDs. Arbutin is one of the isoflavonoids mainly found in bear-berry and it is also present in wheat, pears, and blueberry. Bear-berry is one of the few known naturally occurring anti-microbial plants and is due to the presence of ARB. ARB has a variety of pharmacological and therapeutic properties, including anti-inflammatory [8], antiviral [9], antihyperglycemic [10] and antioxidant activity [11]. ARB will be an excellent phytomedicine for the treatment of myocardial infarction due to its antioxidant and anti-inflammatory properties. The present study was designed to assess the protective effect of ARB on the myocardial damage induced by ISO by assessing cardiac and liver markers, lipid peroxides and antioxidant defense system. The structure of ARB and ISO given in **Fig. 1.**
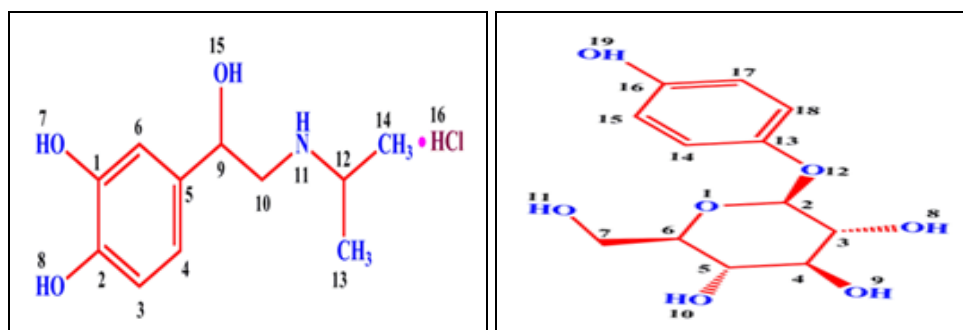
FIG. 1: CHEMICAL STRUCTURE OF ISOPROTERENOL (A) AND ARBUTIN (B)

MATERIALS AND METHODS:
Chemical and Reagents: ARB and Isoproterenol hydrochloride were purchased from Sigma–Aldrich (St. Louis, MO, USA). Kits used for the cardiac and hepatic markers assay were obtained from Agape Diagnostics (Kerala, India), Qualigens Diagnostics (Mumbai, India), and Roche Diagnostics (Risch, Switzerland). All other chemicals used in this study were of analytical grade obtained from E. Merck and HIMEDIA, India.

In vivo **cardioprotective activity of ARB**
Experimental Animals: The study was carried out using 30 male albino Wistar rats (160-180 g) obtained from Biogen Bangalore. Rats were

maintained as per the principles and guidelines of the ethical committee for animal care, Annamalai University in accordance with the Indian National Law on Animal Care (160/PO/ReBi/S/1999/ CPCSEA dated 25/11/1999 Pro.No.1127). The animals were housed in plastic cages with paddy husk for bedding at a temperature of $27 \pm 2°C$ with 12h light: dark cycles. The experiments were conducted in accordance with the "Guide for the Care and Use of Laboratory Rats".

Preparation of ARB: ARB was dissolved in distilled water and administered orally by gastric intubation once a day in the morning for the first 21 days.

Induction of Experimental Myocardial Infarction: ISO (60mg/kg BW) was dissolved in physiological saline (0.9% in NaCl) and injected subcutaneously (s.c) in the right thigh of the rat for two consecutive days, at an interval of 24 h to induce myocardial infarction [12].

Experimental Design: After acclimatization, the animals were randomly divided into the following groups consisting of 6 rats each:

Group 1: Animals received standard laboratory diet and drinking water *ad libitum* for 23 days and served as a control.

Group 2: Animals received ARB (50 mg/kg BW, orally) treatment for the first 21 days.

Group 3: Animals injected with ISO (60 mg/kg BW, s.c.) at an interval of 24 h on the 22nd and 23rd day.

Group4: Animals received ARB treatment (25 mg/kg BW, orally) for the first 21 days and ISO (60 mg/kg BW, s.c.) on the 22nd and 23rd day.

Group 5: Animals received ARB treatment (50 mg/kg BW, orally) for the first 21 days and rats injected with ISO (60 mg/kg BW, s.c.) on the 22nd and 23rd day.

The total experimental duration was 23 days. After treatment, the animals were anesthetized between 8:00 am and 9:00 am using ketamine (24 mg/kg BW, intramuscular injection), and sacrificed by cervical dislocation. After sacrifice, the blood was collected and serum was separated. Immediately after blood sampling, the thoracic cavity was opened and the heart was removed from surrounding tissues, washed with normal saline and blotted dry on filter papers and weighed. Heart weight/body weight ratio was calculated according to the following formula:

$$\text{Ratio (\%)} = \text{Heart weight (g)} / \text{body weight (g)} \times 100$$

The tissue samples were then homogenized in phosphate buffer (25 mM, pH 7.4) using a tissue homogenizer (RQ 127A, REMI Motors Ltd Mumbai, India) to produce an approximately 10% wt/vol homogenate. The homogenate was centrifuged at 2,000 rpm for 10 min; the supernatant was collected and used for the biochemical analyses. The heart tissues were excised immediately and fixed in buffered formalin for the histopathological analysis. All histopathological changes were examined by a pathologist.

Biochemical Estimations:

Estimation of hepatic and cardiac marker enzymes in the serum: The activities of CK and CK-MB were determined by using kits obtained from Agape diagnostics (Kerala, India), according to the manufacturer's protocol. The activities of LDH, AST and ALT were assayed in serum using commercial diagnostic kits purchased from Qualigens Diagnostics (Mumbai, India), according to the manufacturer's protocol. The activities of all these marker enzymes were expressed as IU/L. The levels of cTnT and cTnI in serum were quantitatively measured using standard kits by chemiluminescence (Roche diagnostics Risch, Switzerland).

Estimation of Lipid Peroxidation Products in the Plasma and Heart Tissue: TBARS in plasma was estimated by the method of Yagi *et al*., 1987 [13]. TBARS were quantitated by their reactivity with thiobarbituric acid (TBA) in acidic conditions to generate a pink colored chromophore, which was read at 530 nm. In this method, TBARS were measured by their reactivity with TBA in acidic conditions to generate a pink colored chromophore, which was read at 535 nm. TBARS in the heart was estimated by the method of Fraga, Leibovitz, and Tappel, 1988 [14]. Estimation of plasma and cardiac tissue on conjugated dienes (CD) was estimated by the method of Rao *et al*., 1968 [15]. This method is based on the arrangements of double bonds in polyunsaturated fatty acids to form conjugated dines with an absorbance maximum at 233 nm. Estimation of lipid hydroperoxides (LHPs) in plasma, heart, and liver tissues was done by the method of Jiang *et al*., 1992 [16]. In this method, oxidation of ferrous ion (Fe^{2+}) under acidic conditions in the presence of xylenol orange led to the formation of a chromophore, which was read at 560 nm.

Estimation of Enzymatic and Non- Enzymatic Antioxidants in the Erythrocytes, Plasma, and Heart: Superoxide dismutase (SOD) activity in the myocardium was assayed by the method of Kakkar

et al., (1984) [18]. Superoxide radicals react with nitroblue tetrazolium in the presence of reduced nicotinamide adenine dinucleotide (NAD) and produce formazon blue. SOD removes the superoxide radicals and inhibits the formation of formazon blue. The intensity of the color is inversely proportional to the activity of the enzyme and read at 560 nm. The activity of catalase in the myocardium was assayed by the method of Sinha *et al.*, (1972) [19]. In this method, dichromate in acetic acid is converted to perchromic acid and then to chromic acetate when heated in the presence of hydrogen peroxide. The formed chromic acetate was measured at 620 nm. GPx activity was assayed by the method of Rotruck *et al.*, (1973) [20]. A known amount of enzyme preparation was allowed to react with hydrogen peroxide and GSH for a specified time period. The GSH content remaining after the reaction was measured by Ellman's reaction. The activity of GST was assayed in the cardiac tissue following the increase in the absorbance at 340 nm using 1-chloro-2,4-dinitrobenzene as substrate by the method of Habig *et al.*, (1974) [21].

Vitamin C in plasma and the heart tissue was estimated by the method of Omaye *et al.*, (1979) [22]. The ascorbic acid is converted into dehydro-ascorbic acid in the presence of thiourea, a mild reducing agent and then coupled with 2, 4-dinitrophenyl hydrazine (DNPH). The coupled DNPH is converted into a red-colored complex when treated with sulphuric acid, which was read at 530 nm. The level of vitamin E in plasma and the concentration in cardiac tissue was estimated by the method of Baker *et al.*, (1980). Estimation of GSH in plasma and the heart tissue was done by the method of Ellman *et al.*, (1959) [23]. This method is based on the development of yellow color when dithionitro benzoic acid is added to the compounds containing sulfhydryl groups. The color developed was read at 412 nm. Protein in the tissue homogenate was determined by the method of Lowry et al., (1951) [24]. The CO–NH group (peptide bond) present in the protein molecule reacts with copper sulfate in an alkaline medium to give a blue color, which was read at 620 nm.

Histopathological Examination: The heart tissues were qualitatively analyzed for histological alterations after fixing in 10% formalin. The tissues were then processed for dehydration and clearing of fixative and embedded in paraffin wax. Sections of the heart (3–5 mm thickness) were cut and stained with hematoxylin and eosin (H and E) dyes for morphological observation under the light microscope. The liver tissues were also excised immediately and fixed in 10% formalin, routinely processed and embedded in paraffin wax. 3-5 µm thick sections were cut, fixed on glass slides and stained with hematoxylin and eosin. The specimens were examined under a light microscope.

Statistical Analysis: Statistical analyses were performed by one-way analysis of variance (ANOVA) and groups were compared by Duncan's multiple range test (DMRT) using SPSS/17.0. Results were expressed as mean $\pm$ SD for six rats in each group. A value of P $\leq$ 0.05 was considered to be statistically significant.

RESULTS:
In vivo **Cardioprotective Activity:**
The Effect of ARB on Body Weight, Heart Weight and Body Weight Ratio: The effect of ARB on heart weight, body weight and heart weight to body weight ratio were depicted in **Table 1**.

TABLE 1: EFFECT OF ARB ON BODY WEIGHT, HEART WEIGHT AND HEART WEIGHT/BODY WEIGHT RATIO IN CONTROL AND ISO INDUCED RATS

Groups/ Treatments	Body weight Initial (g)	Body weight Final (g)	Heart weight (g)	Heart weight/Body weight ratio (%)
Control	165. 13 $\pm$ 4.65[a]	186.39 $\pm$ 1.32[a]	0.650 $\pm$ 0.01[a]	0.348 $\pm$ 0.01[a]
ARB (50 mg/kg b.w)	168. 39 $\pm$ 5.16[a]	183.36 $\pm$ 1.70[a]	0.640 $\pm$ 0.01[a]	0.348 $\pm$ 0.01[a]
ISO (60 mg/kg b.w)	175.50$\pm$ 8.57[a]	176.05 $\pm$ 3.68[b]	0.891 $\pm$ 0.02[b]	0.555 $\pm$ 0.02[b]
ARB (25 mg/kg b.w) + ISO (60 mg/kg b.w)	170.47 $\pm$ 6.62[a]	179.33 $\pm$ 2.84[c]	0.719 $\pm$.0.01[c]	0.400 $\pm$.0.01[c]
ARB (50 mg/kg b.w) + ISO (60 mg/kg b.w)	175.33 $\pm$ 5.43[a]	185.20 $\pm$ 2.01[a]	0.645 $\pm$ 0.01[a]	0.342 $\pm$ 0.01[d]

Values are means $\pm$ S.D for six rats.

Values not sharing a common superscript differ significantly at p $\leq$ 0.05 (DMRT)

There was no significant difference was observed between the initial and final bodyweight of the animals. ISO administered rats' showed a significant increase in heart weight and heart weight to bodyweight ratio. ARB (25 and 50mg/kg BW) pretreatment prevented the increased heart weight and heart weight to body weight ratio on ISO rats. There was no significant difference in the heart weight and the heart weight to body weight ratio in the ARB alone treated rats when compared to control.

Effects of ARB on Cardiac and Hepatic Marker Enzymes in the Serum: As shown in **Table 2,** a significant increase in the activities of AST, ALT, LDH, CK, CK-MB and the levels of CTnT and CTnI have observed in the ISO injected rats when compared with control rats. Pretreatment with ARB (25 and 50 mg/kg BW) significantly reduced the activities of these enzymes in the serum of the ISO-administered MI rats. There were no abnormal changes observed in the rats treated with ARB alone.

TABLE 2: EFFECT OF ARB ON THE ACTIVITIES OF AST, ALT, LDH, CK, AND CK-MB IN THE SERUM OF CONTROL AND ISO INDUCED RATS

Groups	AST (IU/L)	ALT (IU/L)	LDH (IU/L)	CK (IU/L)	CKMB (IU/L)	CTnT (ng/ml)	CTnI (ng/ml)
Control	56. 13 ±4.65[a]	28.39 ±1.32[a]	202.08 ±15.46[a]	154. 13 ±4.65[a]	97.39 ±1.32[a]	0.68 ±0.08[a]	0.41 ±0.04[a]
ARB (50 mg/kg b.w)	50. 39 ±5.16[a]	25.36 ±1.70[a]	199.11 ±15.62[a]	148. 39 ±5.16[a]	99.36 ±1.70[a]	0.64.09 ±0.05[a]	0.37 ±0.05[a]
ISO (60 mg/kg b.w)	99.50 ±8.57[b]	48.05 ±3.68[b]	280.11 ±22.09[b]	185.01 ±8.57[b]	139.05 ±3.68[b]	1.40.33 ±0.03[b]	0.70 ±0.04[b]
ARB (25 mg/kg b.w) + ISO (60 mg/kg b.w)	63.47 ±6.62[c]	37.33 ±2.84[c]	251.94 ±19.36[c]	160.47 ±6.62[c]	112.33 ±2.84[c]	0.97.73 ±0.07[c]	0.65 ±0.07[c]
ARB (50 mg/kg b.w) + ISO (60 mg/kg b.w)	53.33 ±5.43[a]	26.20 ± 2.01[a]	215.55 ±16.64[a]	152.33 ±14.13[a]	106.20 ±9.01[d]	0.65.73 ±0.07[a]	0.56 ±0.09[d]

Values are means ± S.D for six rats.

Values not sharing a common superscript differ significantly at p ≤ 0.05 (DMRT)
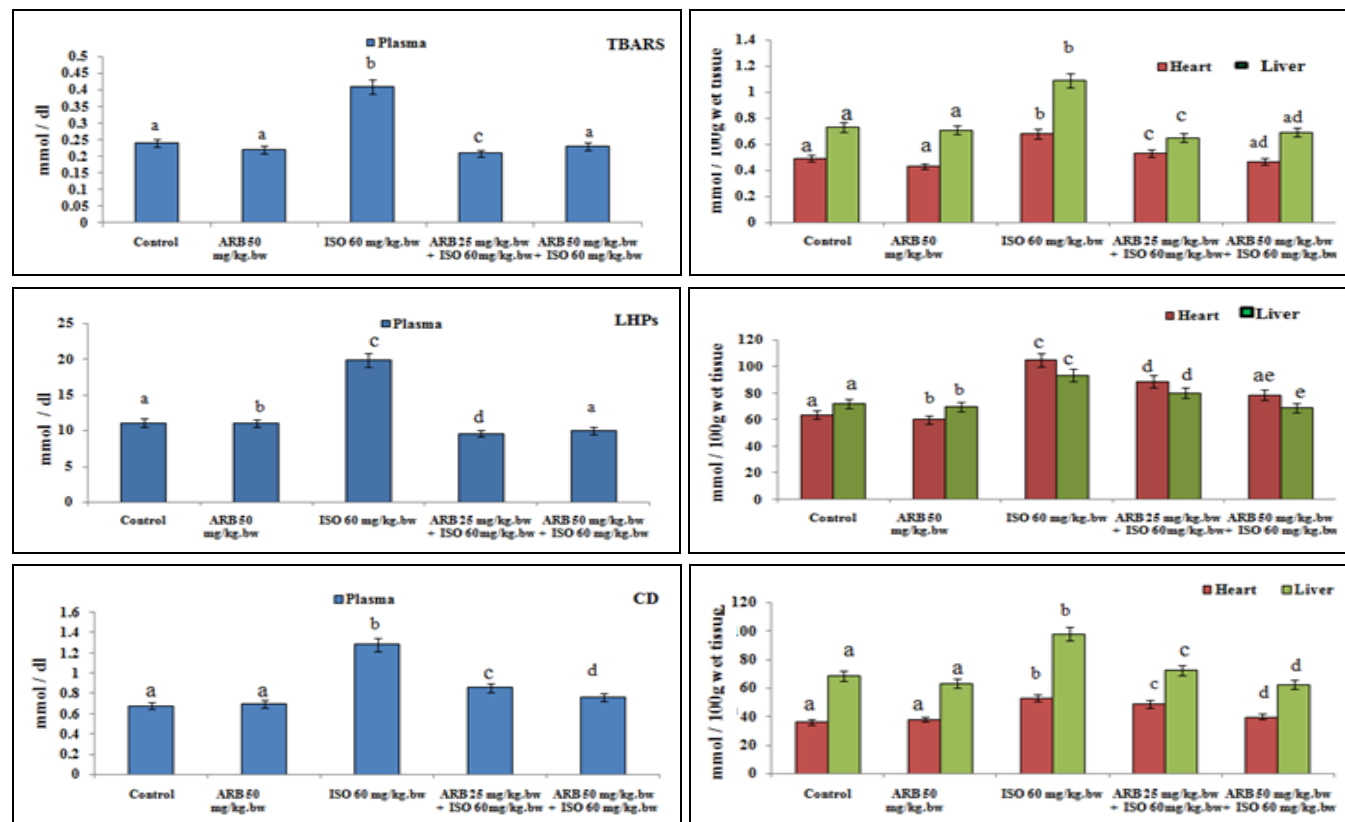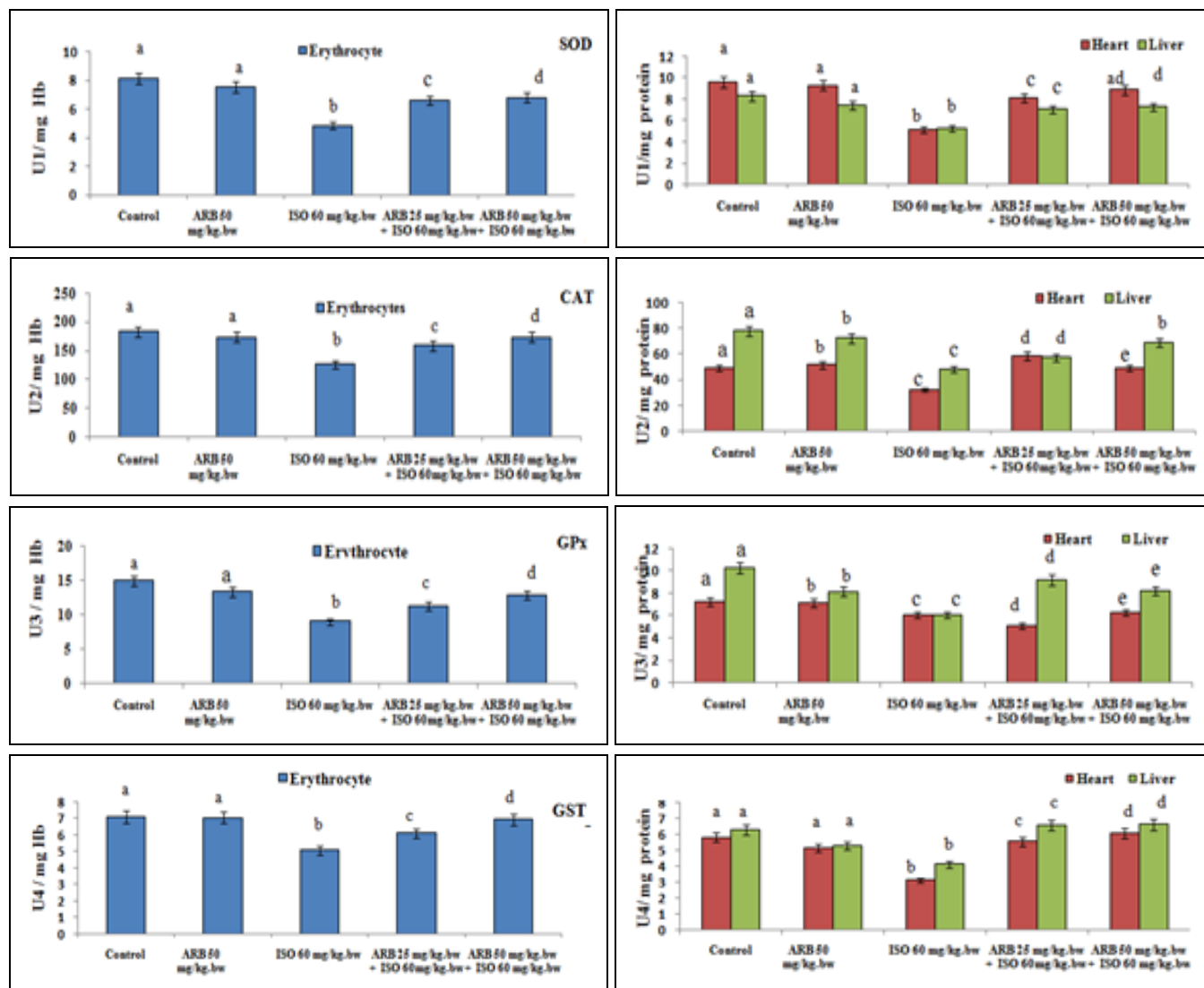

FIG. 2: EFFECT OF ARB ON TBARS, LHPS AND CD IN THE PLASMA, HEART, AND LIVER OF CONTROL AND ISO - INDUCED RATS. VALUES ARE GIVEN AS MEAN ± SD FROM SIX RATS IN EACH GROUP. A, B, C, D VALUES SHARING A COMMON SUPERSCRIPT (A, B, C, D) DO NOT DIFFER SIGNIFICANTLY AT P< 0.05 (DMRT)

Levels of Lipid Peroxidation Products in the Plasma, Heart, and Liver Homogenate: The levels of TBARS, LHPs, and CD in the plasma, heart and liver tissue homogenate of experimental rats are represented in **Fig. 2.** The levels of TBARS, LHPs and CDs were elevated significantly in the plasma, heart and liver tissues of ISO administered rats and pretreatment with ARB (25 and 50 mg/kg BW) prevented the elevation of lipid peroxidation products when compared with disease control rats. The administration of ARB alone had no significant effect on the lipid peroxidation level.

Activities of Enzymatic Antioxidant in Erythrocyte, Heart and Liver Homogenate and Non-Enzymatic Antioxidant in the Plasma, Heart and Liver Homogenate: The activities of enzymatic antioxidants such as SOD, CAT, GPx and GST are exemplified in **Fig. 3**.
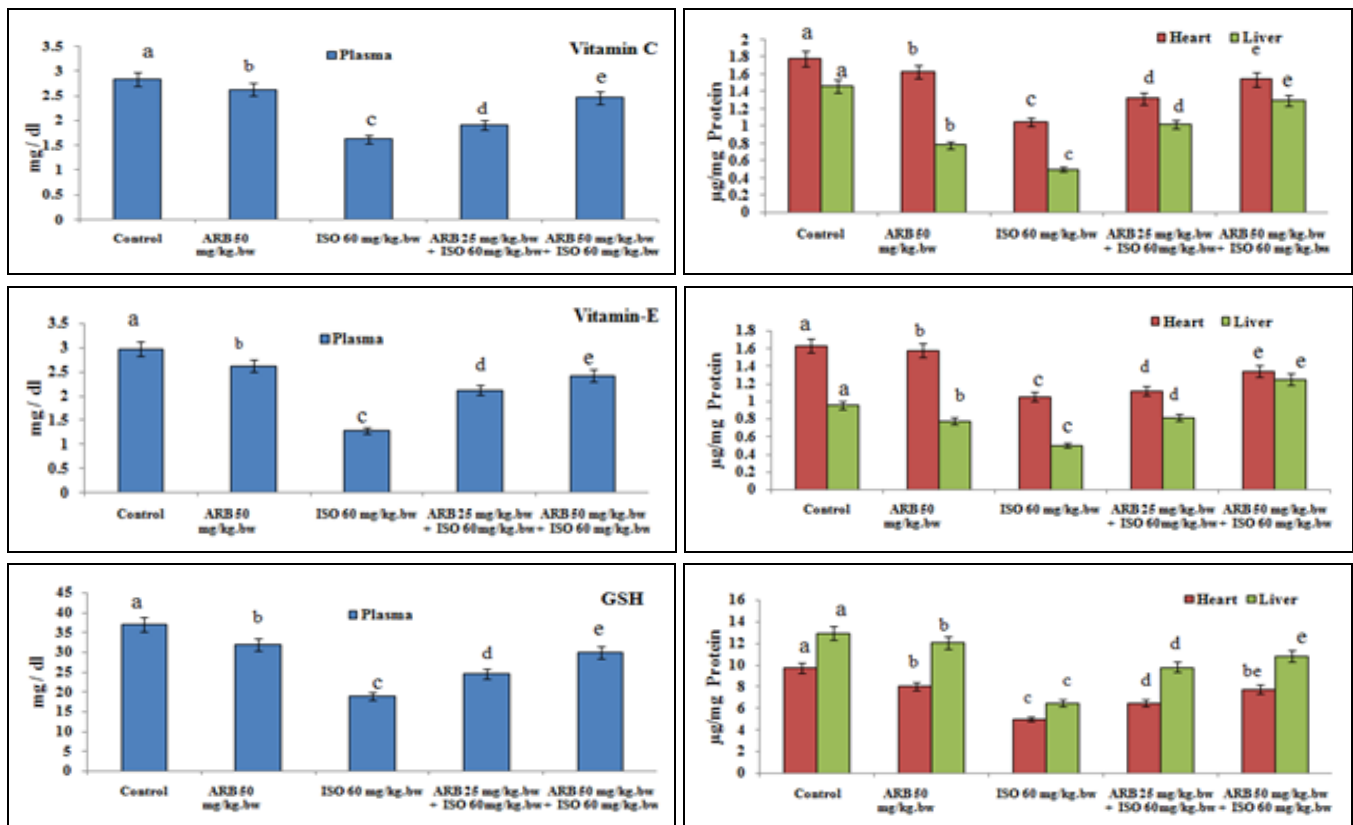

FIG. 3: EFFECT OF ARB ON THE LEVEL OF SOD, CAT, GPx, AND GST IN THE ERYTHROCYTES, HEART, AND LIVER OF CONTROL AND ISO- INDUCED RATS. VALUES ARE GIVEN AS MEAN ± SD FROM SIX RATS IN EACH GROUP. A, B, C, D VALUES SHARING A COMMON SUPERSCRIPT (A, B, C, D) DO NOT DIFFER SIGNIFICANTLY AT P < 0.05 (DMRT). U1- Enzyme concentration required to inhibit the chromogen produced by 50% in 1 min under standard condition. U2 -μmole of hydrogen peroxide decomposed/min. U3- μmole of GSH utilized/min. U4 -μg of 1-chloro-2, 4-dinitrobenzene (CDNB) formed/min.

The activities of enzymatic antioxidants (SOD, CAT, GPx, and GST) were significantly decreased in erythrocyte, heart and liver tissues of ISO-induced rats and treatment with ARB significantly restored these activities. Changes in the levels of non-enzymatic antioxidants such as vitamin C, vitamin E and GSH in the plasma, heart, and liver tissue homogenates of normal and experimental

rats are shown in **Fig. 4.** ISO caused a decrease in the levels of vitamin C, vitamin E, and GSH. ARB (25 and 50 mg/kg BW) pretreatment significantly increased these levels in ISO rats when compared to untreated ISO group rats. No significant difference observed in rats treated with ARB alone when compared to normal rats.

FIG. 4: EFFECT OF ARB ON THE ACTIVITIES OF VITAMIN C, E AND GSH IN THE PLASMA, HEART, AND LIVER OF CONTROL AND ISO -INDUCED RATS. Values are given as mean ± SD from six rats in each group. a,b,c,d Values sharing a common superscript (a, b, c, d) do not differ significantly at P< 0.05 (DMRT)
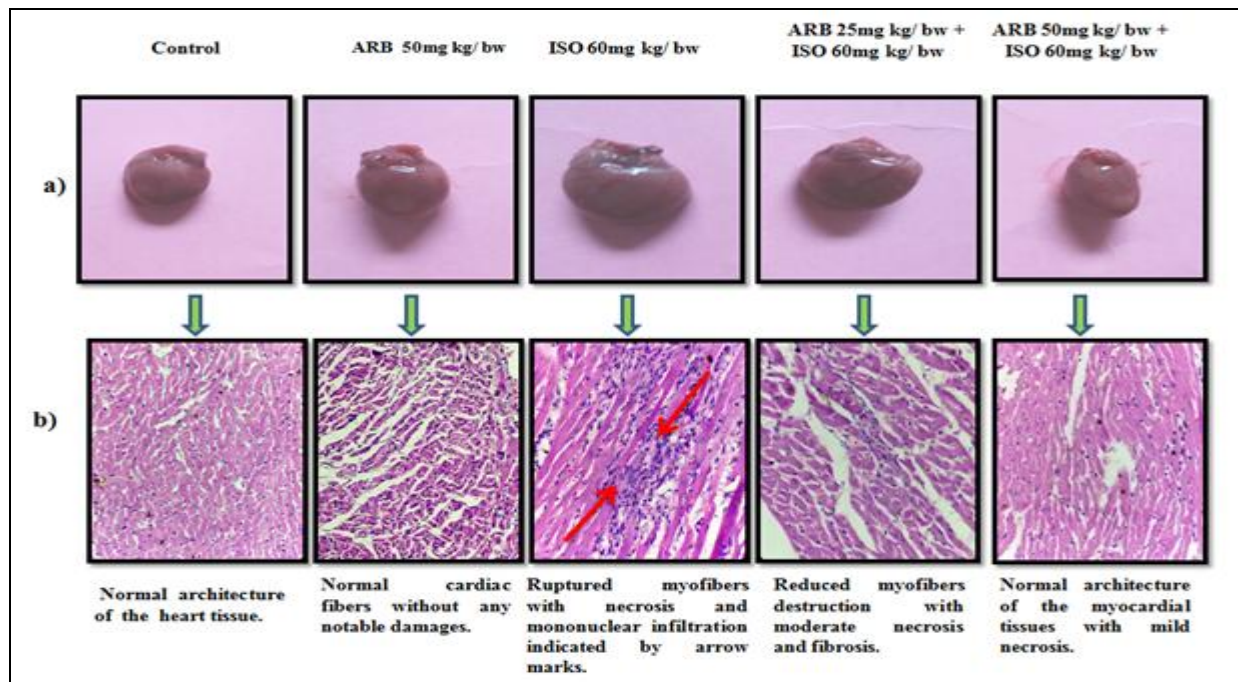

FIG. 5: A). HEART TISSUES APPEARANCE OF CONTROL AND ISO-INDUCED RATS. B). HISTOLOGICAL FEATURES OF HEART TISSUES BY HEMATOXYLIN AND EOSIN STAINING (200X)

Histological Examination: Histopathological examination of the heart tissues of normal and experimental rats are depicted in **Fig. 5.** The heart tissues of the normal and ARB alone treated rats showed normal architecture without necrosis, edema, and inflammation. Heart of ISO group rats showed ruptured cardiac myofibers with necrosis and mononuclear infiltration. The rats treated with ARB 25 and 50 m/kg BW followed by ISO administration showed protection from myocardial infarction evidenced by reduced rupture of myofibers with moderate and mild necrosis and mononuclear infiltration.

The histopathological examinations of the liver tissues from the normal and experimental animals are presented in **Fig. 6.** Liver tissues from the control group rats showed normal architecture. ISO administered group of rats showed the inflammatory cells infiltration with necrosis and fibrosis. ARB (25 mg/kg BW) pretreatment significantly prevented the liver damage induced by ISO. ARB (50 mg/kg BW) treatment followed by ISO administration prevented the liver damage caused by ISO evidenced by reduced mononuclear infiltration with the almost normal architecture of the liver tissue.
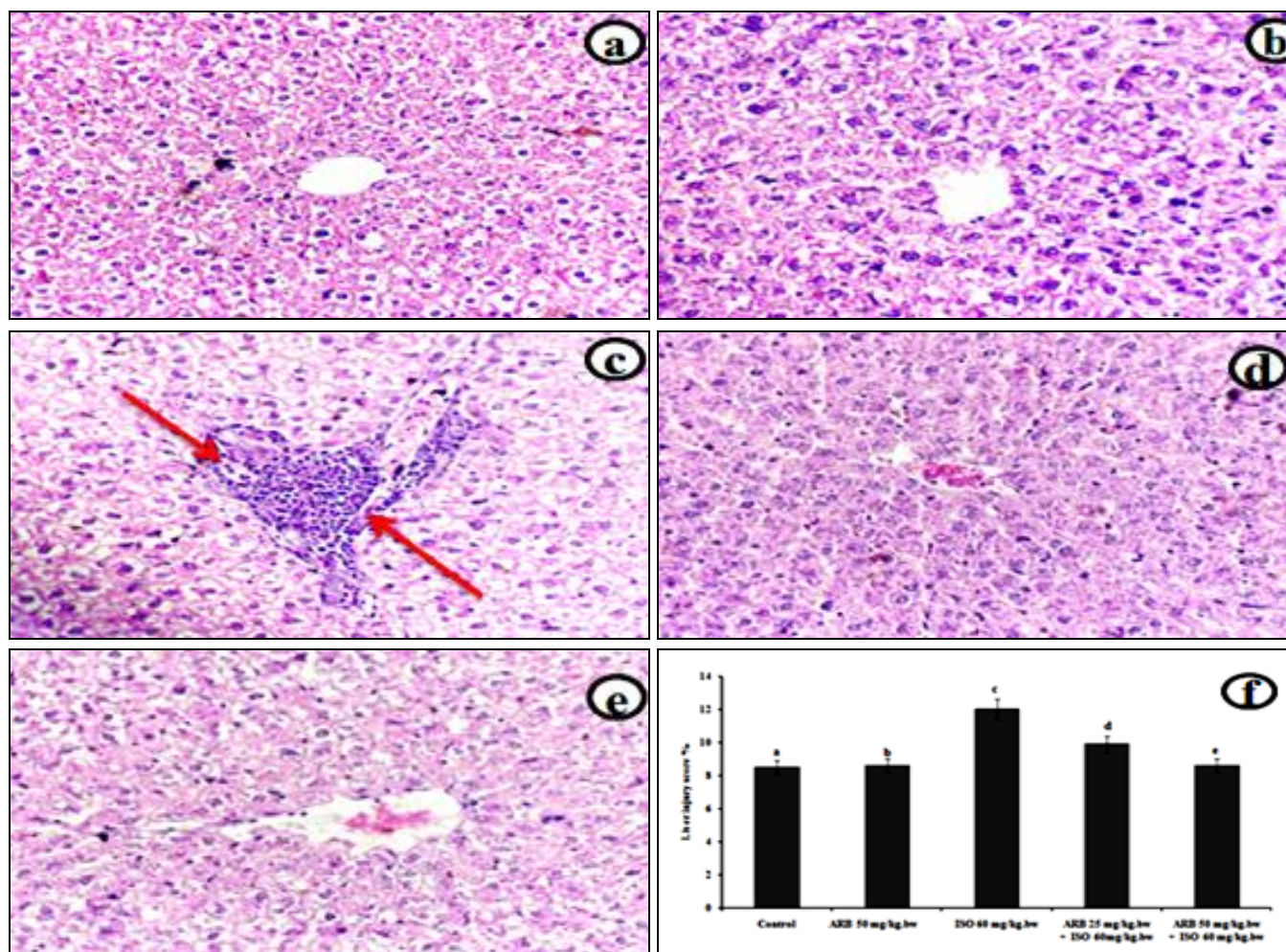
FIG. 6: HISTOPATHOLOGICAL CHANGES OF LIVER SECTIONS USING HEMATOXYLIN AND EOSIN 200X. A) CONTROL RAT TISSUE SHOWED NORMAL ARCHITECTURE B) RATS TREATED WITH ARB 50 mg/kg b.w SHOWED NORMAL ARCHITECTURE C) ARROW INDICATED RATS ADMINISTERED WITH ISO 60 mg/kg b.w SHOWED INFLAMMATORY CELLS INFILTRATION WITH NECROSIS AND FIBROSIS D) ARB 25 mg/kg b.w FOLLOWED BY ISO ADMINISTRATION SHOWED LESS CELL NECROSIS AND SINUSOID DILATION, WITH MILD INFILTRATION OF MONONUCLEAR CELLS E) ARB 50 mg/kg b.w FOLLOWED BY ISO ADMINISTRATION SHOWED MILD NECROSIS WITH ALMOST NORMAL HEPATIC ARCHITECTURE F). THE DEGREE OF LUNG INJURY IN TISSUE SECTIONS WAS ASSESSED BASED ON AN ISO SCORE.

DISCUSSION: The present investigation revealed the cardioprotective effects of ARB against ISO-induced myocardial damage in albino Wistar rats. ISO is widely used to evaluate the cardioprotective

effect of many drugs. Numbers of studies have reported the pathophysiological, morphological and metabolic changes that occur in the heart of experimental animals following ISO administration are similar to those observed in humans [25]. The autooxidation of hydroxyl groups in ISO leading to the conversion into quinones and the subsequent formation of adrenochromes [26]. Adrenochrome and other oxidation metabolites of ISO can cause cell necrosis and contractile failure in the myocardium of the rats [27]. Furthermore, during this reaction highly toxic free radicals are generated, and it could initiate the peroxidation of membrane-bound polyunsaturated fatty acids, leading to myocardial injury [28].

In this study, animal body weight, heart weight and heart weight to body weight ratio were analyzed, there was no significant difference observed in the body weights between the initial and final bodyweight of the animals and significant increase was observed in the heart weight and heart/body weight ratio in the ISO treated rats, which may be due to the ventricular stiffness, increased water content, edematous intermuscular space and extensive necrosis of cardiac muscle followed by invasion of the damaged tissue by inflammatory cells [29]. ARB pretreatment significantly prevented the increase in both the heart weight and heart weight to body weight ratio as compared to the ISO alone treated group.

Enzymes released from the damaged tissue are the best markers of tissue damage due to their tissue specificity and catalytic activities. Oxidative stress in myocardium induces cell membranes to rupture, which results in leakage of cardiac enzymes into the circulation. AST, ALT, CK, CK-MB, LDH, and cTns normally exist in the cellular compartment and leak out into circulation during myocardial and liver injury due to the disintegration of contractile elements. cTns have been shown to be specific markers of myocardial cell injury and the elevated levels of cTnT and cTnI predicted the risk of cardiac damage [30]. These are contractile proteins that normally not found in the blood and only found in heart tissue. In our study, we noticed the elevated levels of cTnT and cTnI and the increased activities of the AST, ALT, CK, CK-MB, and LDH in serum of ISO-induced rats; it might be due to the cardiac damage induced by ISO. Our results are consistent with a previous report by Vennila et al., (2010) [31]. ARB pretreatment prevented theses increase in ISO administered rats.

The ISO-induced myocardial damage in rats can also generate lipid peroxidation products such as TBARS, LHPs and CD due to the membrane lipid peroxidation [32]. In the present study, these parameters were increased in the plasma, heart and liver tissues of ISO-induced rats and this might be due to free radical-mediated membrane damage. Administration of ARB resulted in a significant reduction in the levels of TBARS, LHPs, and CD in the plasma, heart and liver tissues of ISO-induced rats.

ISO decreased the activities of enzymatic antioxidants SOD, CAT, GPx and GST in the myocardium of the rats [33]. SOD is usually found in the plasma membrane, which protects the cells from oxidative stress. CAT is a tetrameric hemoprotein which acts as a catalyst for the removal of hydrogen peroxide. GPx acts as a catalyst in the reaction of hydroperoxides with reduced glutathione (GSH) to form glutathione disulfide (GSSG) and the reduction product of the hydroperoxides. GST detoxifies ROS and other xenobiotic molecules by the utilization of GSH [34]. GPx and GST activities were decreased in ISO-administered rats and which might be due to decreased availability of GSH. Suppression of all these enzymes leads to the accumulation of ROS and makes the cardiomyocytes more susceptible to oxidative injury. In our study, ISO rats showed a significant decrease in the activities of SOD, CAT, GPx and GST and the administration of ARB brought back the activities of all the above enzymes to near normal, which is attributed to the therapeutic nature of ARB against peroxidative injury.

The levels of non-enzymatic antioxidants such as vitamin C, vitamin E, and GSH were significantly declined in ISO-administered rats, which may be due to the enhanced utilization for the ROS scavenging function. Vitamin C is a potent free radical scavenger that prevents radical-mediated lipid peroxidation. GSH is a powerful cellular antioxidant, which is directly involved in the removal of superoxide radicals, peroxyl radicals and singlet oxygen [35, 36].

In this study, we have observed a significant decrease in the levels of vitamin C, vitamin E, and GSH in ISO rats. Pretreatment with ARB significantly increased the levels of all the above non-enzymatic antioxidants in ISO rats. This might be due to the ROS scavenging property of ARB.

Histopathological observations of heart tissues from the control and ARB alone administered rats showed a well preserved normal morphology of cardiac muscle with no evidence of necrosis, which showed the non-toxic nature of the ARB. Myocardial tissues of the ISO rats showed the ruptured myofibers with necrosis and fibrosis, which showed the toxicity of the ISO. The better morphological protection was observed with ARB at 50 mg/kg BW than the 25 mg/kg BW evidence by reduced muscle fibers destruction with mild necrosis in 50 mg/kg BW. These data's further confirmed the cardioprotective effect of ARB. The administration of ISO resulted in histopathological changes in the liver of rats manifested by inflammation with necrosis and fibrosis. Treatment with ARB significantly reduced the histological changes of the liver induced by ISO and normalized the architecture of the liver tissues.

Overall, the results of this study offer. Information that will be useful in developing a safe and effective therapy for the treatment of patients with coronary occlusion. Nevertheless, more molecular level studies are needed for further validation.

CONCLUSION: The present study demonstrated that the ARB has the potential to protect the myocardium against ISO induced cardiac injury. ARB 50 mg/kg BW had a more cardioprotective effect than 25 mg/kg BW of ARB, which is evidenced by lowering the cardiac and hepatic markers, lipid peroxidation products and increasing the antioxidant status in the ISO rats by scavenging the free radicals produced by ISO.

Histopathological observations are also a correlation with the biochemical parameters. The cardioprotective effect of ARB could be due to its antioxidant and membrane protection potential. However, further studies are warranted for use in humans.

CONFLICTS OF INTEREST: The authors declare that they have no conflict of interest.

REFERENCES:

1. Ahmed R, Tanvir EM, Hossen MS, Afroz R, Ahmme I and Rumpa NE: Antioxidant properties and cardioprotective mechanism of malaysian propolis in rats. Evid Based Complement Alternat Med. 2017; 5370545.
2. Ayala A, Munoz MF and Arguelles S: Lipid peroxidation: Production, metabolism, and signalling mechanisms of malondialdehyde and 4- hydroxy-2-nonenal. Oxid Med Cell Longev 2014; 360431-38.
3. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R and Muntner P: Heart disease and stroke statistics. Circulation, 2017; 135(10): e146–e603.
4. Chapman AR: Adamson PD & Mills NL. Assessment and classification of patients with MIand infarction in clinical practice. Heart 2016; 103(1); 10-18.
5. Panda, S, Kar A and Biswas, S: Preventive effect of Agnucastoside C against isoproterenol-induced myocardial injury.Scientific Reports 2017; 7: 16146.
6. Sushil, KM, Zheng H, Mahata S, Liu X and Kaushik P: Effect of heart failure on catecholamine granule morphology and storage in chromaff in cells. The Journal of Endocrinology 2016; 230(3): 309-23.
7. Khadir AF, Pouramir M, Joorsaraee SG, Feizi F, Sorkhi H and Yousefi F: The effect of arbutin on lipid peroxidation and antioxidant capacity in the serum of cyclosporine-treated rats. Caspian J Intern Med 2015; 6(4): 196-00.
8. Taha MM, Salga MS and Ali HM: Gastroprotective activities of *Turnera diffusa* Willd. Ex Schult. Revisited: Role of arbutin. J Ethnopharmacol 2012; 141: 273-81.
9. Yousefi F, Mahjoub S and Pouramir M: Hypoglycemic activity of *Pyrus biossieriana* Buhse leaf extract and arbutin: Inhibitory effects on alpha-amylase and alpha glucosidase. Casp J Intern Med 2013; 4: 763-7.
10. Shahaboddin ME, Pouramir M and Moghadamnia AA: *Pyrus biossieriana* Buhse leaf extract: An antioxidant, antihyperglycaemic and antihyperlipidemic agent. Food Chem 2011; 126: 1730-3.
11. Padma VV, Devi CS and Kalaiselvi P. Protective effect of fish oil on changes in the activities of membrane-bound ATP ases and mineral status in experimentally induced MIin wistar rats. Biological Trace Element Research 2010; 137(3): 344-52.
12. Yagi K: Lipid peroxides and human disease. Chem Phys Lip 1978; 45: 337-51.
13. Fraga CG, Leibovitz BE and Tappel AL: Lipid peroxidation measured as thiobarbituric acid-reactive substances in tissue slices: Characterization and comparison with homogenates and microsomes. Free Radical Biology and Medicine 1988; 4(3): 155-61.
14. Rao K.S and Recknagel RO: Early onset of lipid peroxidation in rat liver after carbon tetrachloride administration, Exp Mol Pathol 1968; 9: 271-78.

15. Jiang ZY, Hunt JV and Wolff SP: Detection of lipid hydroperoxides using the 'fox method'. Anal Biochem 1992; 202: 384-89.

16. Kakkar P, Das B and Viswanathan PN: A modified spectrophotometric assay of superoxide dismutase, Ind. J. Biochem. Biophys 1984; 21: 130-32.

17. Sinha AK: Colorimetric assay of catalase, Anal Biochem 1972; 47: 389-94.

18. Rotruck JT, Pope AL and Ganther HE: Selenium: biochemical role as a component of glutathione peroxidase, Science 1973; 179: 588-90.

19. Habig WH, Pabst MJ and Jakoby WBC: Glutathione-S-transferases: the first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249: 7130-39.

20. Omaye ST, Turnbull TD and Sauberlich HE: Selected method for the determination of ascorbic acid in animal cells, tissues and fluid. Enzymol 1979; 62: 3-11.

21. Ellman GL: Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82: 70-77.

22. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 1951; 193: 265-75.

23. Houston M: The role of nutrition and nutraceutical supplements in the treatment of hypertension, World J Cardiol 2014; 26(6): 38-66.

24. Wang Q, Yu X, Xu H, Jiang Y, Zhao X and Sui D: Ginsenoside Re Attenuates Isoproterenol-Induced Myocardial infarctionin Rats. Evidence-Based Complementary and Alternative Medicine 2018; 1–8.

25. Li H, Xie YH, Yang Q, Wang SW, Zhang BL, Wang: Cardioprotective effect of Paeonol and Danshensu combination on isoproterenol-induced myocardial infarction in rats. PLoS One 2012; 7: e48872.

26. Mythili S and Malathi N: Diagnostic markers of acute myocardial infarction. Biomed Reports 2015; 3(6): 743-48.

27. Phaniendra A, Jestadi DB and Periyasamy L: Free radicals: Properties, sources, targets, and their implication in various diseases. Indian Journal of Clinical Biochemistry 2015; 30(1): 11–26.

28. Upaganlawar A, Gandhi H and Balaraman R: Isoproterenol induced myocardial infarction: Protective role of natural products. Journal of Pharmacology and Toxicology 2011; 6(1): 1–17.

29. Akila P, Asaikumar L and Vennila L: Chlorogenic acid ameliorates isoproterenol- induced myocardial injury in rats by stabilizing mitochondrial and lysosomal enzymes. Biomedicine and Pharmacotherapy 2017; 85: 582-91.

30. Vennila L and Pugalendi KV: Protective effect of sesamol against MI caused by isoproterenol in Wistar rats. Redox Report. 2010; 15: 36-42.

31. Asaikumar L, Vennila L and Akila P: Preventive effect of nerolidol on isoproterenol induced myocardial damage in Wistar rats: Evidences from biochemical and histopathological studies. Drug Dev Res 2019; 1–10.

32. Wang S, Tian S, Yang F, Yang HG, Yang XY and Du GH: Cardioprotective effect of salvianolic acid A on iso-proterenol-induced MIin rats. Eur J Pharmacol 2009; 615: 125-32.

33. Karthikeyan K, Bai BR and Devaraj SN: Cardioprotective effect of grape seed proanthocyanidins on isoproterenol-induced MIin rats. Int J Cardiol 2007; 115: 326-33.

34. Panda S, Kar A and Biswas S: Preventive effect of Agnucastoside C against isoproterenol-induced myocardial injury. Scientific Reports 2017; 7: 16146.

35. Sharma M, Kishore K, Gupta SK, Joshi S and Arya D: Cardioprotective potential of *Ocimum sanctum* in isoproterenol induced MIin rats, Mol Cell Biochem 2001; 225(1): 75-83.