ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 3, pp: (159-168), Month: July - September 2019, Available at: www.researchpublish.com

Effect of grape seed extract as a function of cold stress and ageing: A study on the redox state in the right ventricle of rat heart

B. Jyothi, S. Asha Devi

Laboratory of Gerontology, Department of Zoology, Bangalore University, Bengaluru 560 056, Ksrnataka, India

E-mail: sambeashadevi@bub.ernet.com

Abstract: **Cold stress is known to disrupt mitochondrial metabolism of the right ventricle (RV) in rats. This study was designed to investigate the generation of reactive oxygen species (ROS) and antioxidant defense in cold stress in the presence of a natural polyphenol, grape seed proanthocyanidin extract (GSPE). Adult, late-adult and old male Wistar rats were categorized into six groups : 1) control (CON), 2) GSPE -treated (CON+PA), 3) Induced chronic cold stress (CCS), 4) GSPE-treated CCS (CCS+PA), 5) induced acute cold stress (ACS) and 6) GSPE-treated ACS (ACS+PA). Superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) activities along with glutathione (GSH) showed decreased activities in the RV of CCS group and extent was higher extent in the ACS group compared to their controls. These decreases were more effective in the old compared to adults and late-adults. On the contrary, thioredoxin reductase (TrxR) and catalase (CAT) activities were significantly increased under stress. The observed reduction in SOD, GPx and GR activities were concomitant with increased levels of hydrogen peroxide, superoxide, and nitric oxide levels in the RV of stressed rats. Total thiols (P-SH) significantly decreased as a function of age and chronic stress. The levels of HSP*72* increased in the adult and old CCS group. Therefore, cold stress induces right ventricular oxidative stress as a function of age and notably with a greater magnitude during acute compared to chronic stress. However, these changes were alleviated in the GSPE-treated groups regardless of age.**

Keywords: **Ageing, Cold stress, Free Radicals, Grape seed proanthocyanidin, Right Ventricle.**

I. INTRODUCTION

In normal aging of both humans and animals, post-mitotic heart tissue has decreased number of cells [1, 2]. Animal studies have shown that exposure to intermittent cold (IC) can be a potent environmental stressor impacting major organs such as the brain and its redox state at different levels of cell functions [3, 4]. Studies by Wei et al [5] on the heart of broilers exposed to acute cold stress (CS) have demonstrated histopathological changes, altered antioxidant status and excessive expression of heat shock proteins (HSPs). The heart muscle in rats exposed to short-term IC has increased heart mass and protein synthesis [6]. Notably, the cardiac cells from the right ventricle (RV) vary in size, number and shortening in rodent hearts [7].

Oxidative stress (OS) in cardiac cells is a result of chronic pressure or overload of the heart, cardiac ischemia or aging [8]. Despite the fact that the mitochondrial proteins of the RV and LV are similar at rest, studies indicate region-specific response in situations of stress and afterload. It is possible that the responses of the RV may vary from that of the left ventricle (LV) under stress.

HSPs are molecular chaperones that aid in the folding/unfolding of proteins in order to regulate normal physiological functions of cells. The expression levels of HSPs that are usually low under normal physiological states, are reported to increase in response to heat [9] and cold stress [10,11] and oxidative stress [12] thereby evoking stress tolerance to some extent.

Several natural and plant-derived products such as grapes are sources of flavanols containing proanthocyanidins (PACs) are are the most abundant phenolic compounds made of polymers of dimers or trimers (+) catechin (C) and (-) epicatechin (EC)[13]. In fact, phenolic compounds are known for their health benefits [14] and can reduce reactive oxygen species (ROS) [15] in the ageing myocardium. Studies from our laboratory have shown that grape seed proanthocyanidin extract (GSPE) is can alleviate stress-induced hyperlipidemia in old rats exposed to acute and chronic cold stress [16].

Studies on human and animals on low temperature effects have focused largely on the left ventricle (LV) but seldom on the right ventricle (RV). Also it is unclear whether chronic cold stress (CCS) to low temperatures can evoke differential responses compared to acute stress (ACS) and whether the responses are age-related. To this end, the present study, while addressing age and severity of cold stress in the RV, has attempted to evaluate (i) the levels of ROS; (ii) antioxidant defenses; (iii) HSP*72* expression; and (iv) the influence of GSPE in modulating the above parameters.

II. MATERIALS AND METHODS

Animal care and maintenance

All procedures on animals were approved by the Institutional Animal Ethics Committee (IAEC) of Bangalore University, Bangalore, India.

Male Wistar albino rats of 3 months of age were obtained from the Central Animal Facility of the Indian Institute of Science (Bangalore) and maintained until they reached 4 months (adult), 12 months (late-adult) and 24 months (old) of age. They were housed at 25 ± 1 °C with 12-h light/dark cycle. Rats were fed with standard laboratory chow (Amrut Feeds, Bangalore) and tap water *ad libitum*. All three age groups were further categorized into six groups : 1) control (CON), 2) GSPE -treated (CON+PA), 3) Induced chronic cold stress (CCS), 4) GSPE-treated CCS (CCS+PA), 5) induced acute cold stress (ACS) and 6) GSPE-treated ACS (ACS+PA). Supplementation was a daily oral gavage of 200 mg of GSPE/kg body weight.

Induction of cold stress

Cold stress was induced by exposing the rats to 10 ^{o}C. Control group remained at normal temperature (25 ± 1 ^{o}C) while rats of the CCS group were initially pre-exposed to cold by reducing the temperature by 5 ^{o}C for 10 min/day until they reached 10^{o}C and time period was progressively increased by 10 mins until they reached 60 mins/day. Chronic stress was for a period of 14 days including 7 days of pre-exposure. Rats belonging to ACS group were exposed to 10 ^{o}C for 60 mins without any pre-exposure since acute stress is achieved in a relatively short period of time. Rats of CON+PA and CCS+PA and ACS+PA groups were supplementation of GSPE throughout the study period of 14 days. ACS+PA and ACS groups were subjected to single exposure to cold.

Isolation of mitochondrial pellet

Free Radicals

The isolation of mitochondrial pellet from the RV myocardium was by the method of Gellar and Winge [17]. Tissue homogenates was prepared in 3 volumes of homogenizing buffer: 0.17 M KCl, 0.01 M EDTA, 0.01 M Tris-HCl (Sigma-Aldrich, USA) and 1% BSA(Sigma-Aldrich, MO, USA), and further diluted with another 5 volumes with the buffer and centrifuged at 300 x g for 15 mins. The supernatant was saved; the resultant precipitate was washed twice by resuspending it in 5 volumes of homogenizing buffer and centrifuged at 300 x g for 15 rnins. The combined supernatants were centrifuged at 10,000 x g for 15 mins. The precipitate was resuspended in 5 volumes of the buffer and centrifuged at 10,000 x g and the resulting 10K pellet was washed twice and stored at -80 °C till the assays were performed.

Superoxide ($O_2^{\bullet-}$)

$O_2^{\bullet-}$ was estimated using the method described by Das et al.[18]. To the 10K suspension, 0.1% NBT (Sigma-Aldrich) was added and kept in boiling water bath for 45 mins until a violet color indicative of superoxide was visible. The mixture was centrifuged at 1000 x g for 10 mins. The diformazan formed was dissolved in 1ml of glacial acetic acid and the absorbance was measured at 560 nm and expressed as µmol of diformazan/mg protein.

Hydrogen peroxide (H_2O_2)

H_2O_2 generation was determined in 10K pellet by the method of Josephy et al.[19]. 100nmol of 3,3',5,5'-tetramethylbenzidine(Sigma-Aldrich), 0.05 mL of horse radish peroxide (Himedia, India) and 0.1 ml 10K pellet were taken in 0.2 M of an acetate buffer. Absorbance was recorded at 700 nm and H_2O_2 is expressed as nmol generated/mg protein.

Nitric oxide ($NO^{\bullet}$)

$NO^{\bullet}$ was estimated by the method of Yucel et al.[20]. The mixture of 10K pellet and 0.3 NaOH were taken in equal volumes and incubated for 5 mins. To this mixture, 10% $ZnSO_4$was added, vortexed, and centrifuged twice at 1000 x g for 5 mins at 4 ^{o}C. 1% zinc dust was added to the supernatant to initiate nitrate-nitrite conversion. This was followed by the addition of 2% sulphanilamide and 0.1% NED and incubated for 30 mins at room temperature. The absorbance was measured at 540 nm and $NO^{\bullet}$ is expressed in terms of µmol nitrite/mg of protein.

Protein Oxidation

Protein oxidation in terms of protein sulphydryl (P-SH) was measured in the homogenate by the method of Habeeb [21]. 1.5 ml of 0.08M of sodium phosphate buffer containing Na_2-EDTA, and SDS were added to sample tube followed by DTNB (Sigma-Aldrich). The solution was vortexed and the absorbance of developed color was recorded at 412 nm. P-SH level is expressed in terms of µmol/ mg protein.

Endogenous antioxidants

Superoxide dismutase (SOD, EC 1.15.1.1)

SOD activity was measured in the tissue homogenate as described by Misra and Fridovich [22]. To 100µl of sample, 880µl of carbonate buffer (0.5M, pH 10.2) was added followed by 20µl of 30 mM epinephrine (Sigma-Aldrich, MO, USA) in 0.05% acetic acid. The enzyme activity was recorded for 4 mins at 480 nm. SOD activity is expressed as the amount of enzyme that inhibits oxidation of epinephrine by 50% which is equal to 1 unit.

Catalase (CAT, EC 1.11.1.6)

CAT activity was determined as described by Aebi [23]. Equal volumes of sample and absolute alcohol were incubated for 30 mins in an ice-bath followed by the addition of 10µl of Triton x-100 and vortexed. To 50µl of sample mixture, 250µl of 0.06M H_2O_2 in phosphate buffer was added and decrease in absorbance was read at 240 nm at 30sec interval for 2 mins. Enzyme activity was determined using a molar extinction coefficient of 43.6 M/min/cm was used to determine enzyme activity. One unit of CAT activity is equal to mM of H_2O_2hydrolysed/min/mg protein.

Thioredoxin Reductase (TrxR, EC 1.8.1.9)

TrxR assay kit (Sigma-Aldrich) was used to determine the TrxR activity by reducing 5,5'-ditiobis(2-nitrobenzoic) acid with NADPH to 5-thio-2-nitrobenzoic acid producing yellow color. The activity of TrxR was assayed by adding the kit reagents to the supernatant containing 50 mg of protein and absorbance was measured in a spectrophometer at 412nm and activity is expressed in terms of units/mg protein.

Glutathione system

Tissue homogenates (10%) was prepared in 50mM tris-HCl (Sigma-Aldrich) buffer and centrifuged. The resulting supernatants were stored at -80 °C for protein and glutathione-related enzyme assays.

Reduced Glutathione (GSH)

GSH concentration was measured by Ellman's reagent as described by Tietze [24]. To 500 µl of supernatant, 2 ml of 0.3 M disodium hydrogen phosphate (Himedia, India) and 500 µl of 0.04% DTNB (Sigma-Aldrich) in 10% sodium citrate were added. The absorbance was measured at 412 nm immediately after development of yellow color. A calibration curve was prepared using GSH as a standard. GSH is expressed as µmol / mg protein.

Glutathione Peroxidase (GPx, EC 1.11.1.9)

GPx activity was assayed as described by Flohe and Gunzler [25]. To 5 volumes 0.1 M PBS, equal volume of enzyme solution, glutathione reductase (Sigma-Aldrich) and glutathione (Sigma-Aldrich) were added incubated for 15 mins at 37 °C. 1.5 mM β-Nicotinamideadenine diucleotide phosphate tetrasodium salt hydrate (Sigma-Aldrich) and 12 mM t-butyl hydroperoxide solution (Sigma-Aldrich) were added to the enzyme solution mixture and decrease in absorbance was recorded at 340 nm for 3 mins and further decrease was recorded for 5 mins at interval of 30 sec by adding equal volume of pre-warmed hydrogen peroxide. A molar extinction coefficient of 6.22×10^3 M/cm was used to determine enzyme activity. One unit of GPx activity is equal to µM NADPH oxidized/min/mg protein.

Glutathione Reductase (GR, EC 1.8.1.7)

GR activity was assayed as described by Carlberg and Mannervik [26]. In brief, equal volumes of enzyme solution, 50 mM glutathione oxidized (Sigma-aldrich), 250 µM flavin adenine dinucleotide (Sigma-Aldrich) were added to 2 ml of 0.1 M PBS and incubated for 15 mins at 37^{O}C. 4mM β-Nicotinamide adenine diucleotide 2'-phosphate tetrasodium salt hydrate (Sigma-Aldrich) was added and decrease in absorbance was read at 340 nm for 2 mins. A molar extinction coefficient of 6.22 x 10^3 M/cm was used to determine enzyme activity. One unit of GR activity is equal to µM NADPH oxidized / min / mg protein.

Heat Shock Protein (HSP*72*)

A 10% tissue homogenate was prepared in the buffer containing 15 mM tris-HCL (Sigma-Aldrich), 600 mM NaCl, 1 mM PMSF (Sigma-Aldrich), pH 7.5 and centrifuged. The resultant supernatant was stored at -80 °C for protein assay and detection of HSP.

Western Blot Analysis

Electrophoreses on 12% PAGE-SDS gel was conducted essentially as described by Laemmli [27]. Equal amounts of protein (100 mg) samples were mixed with the loading buffer and added into each well of the gel and separated electrophoretically. Pre-stained molecular mass markers (BIO-RAD Labs, Inc., USA) were used to estimate the positions of various proteins on the gel. Proteins were electro-blotted onto PVDF membrane using a semi-dry transblot unit set (Atto Corporation, Japan) at 25V or 100 mA for 60 mins. Membranes were blocked overnight at 4°C in 5% non-fat dry milk in phosphate buffer saline-Tween 20 (PBS-T). The blots were washed in PBS-T for 1x2mins and 2x10mins. The blots were then incubated for 1 hr with primary antibody against HSP72 monoclonal antibody (C92F3A-5(AP conjugate, ENZO Life Sciences, Switzerland), diluted 1:5,000 with TBS-T and washed for 1x2mins and 2x10mins. Further, blots were treated with anti-rabbit IgG-alkaline phosphatase (Sigma-Aldrich) diluted 1:10,000 with PBS-T, washed for 1x2mins and 3x10mins. Immunoreactivity was visualized using nitrobluetetrazolium and 5-bromo-4-chloro-3-indolylphosphate (NBT/BCIP) (Sigma-Aldrich) western blotting detection reagents. The blots were imaged and analyzed in gel-documentation system (Alpha-Imager, USA).

Protein Assay

Protein content of RV was assayed by Folin-Phenol reaction as described by Lowry et al**.** [28]. The standard curve of bovine serum albumin (BSA) was included in each assay to determine linearity and measure the standard value.

Statistical Analyses

All data are presented as the mean ± SEM. To determine the type of analysis of variances (ANOVA), at the beginning the data was subjected Levene's test of homogeneity of variances (IBM SPSS® version 20.0 Inc., Chicago, Illinois, USA) and subsequently subjected to statistical analysis using the two-way ANOVA (GraphPad PRISM® version 6.01Software Inc., San Diego, California, USA). Differences among the group means were evaluated by Tukey's multiple comparison post-hoc test. Mean values were considered to be statistically significant at $P< 0.05$.

III. RESULTS

Changes in body weight and tissue somatic index (TSI)

Changes in body, heart and RV mass and TSI in different age groups and as a function of cold stress and GSPE supplementation are represented in Table I. Late-adult and old rats showed significant changes in the body, heart and ventricle mass in comparison to adult.

Superoxide

The two-way ANOVA revealed, age [$F_{(2,70)}$=786.7, *p<0.0001*], intervention [$F_{(5,70)}$=179.0, *p<0.0001*], age x intervention interaction [$F_{(10,70)}$=22.02, *p<0.0001*] in $O_2^{\bullet-}$ levels. In CCS group, the levels increased in adult (18%, p<0.001), in late-adult (28%, p<0.0001) and (17%, *p<0.0001*) in comparison with their age-matched controls. However, the extent of increase was higher (p<0.0001) in the ACS group of adults (60%), late-adult (40%), and old (21%, p<0.05).

GSPE supplementation reduced $O_2^{\bullet-}$ levels in CCS groups significantly in adult (35%, *p<0.0001*), late-adult (6%, p<0.001) and old (4%) in comparison to their respective stressed groups. However, supplementation was ineffective in the ACS groups (Table II).

Hydrogen peroxide

The two-way ANOVA revealed, age [$F_{(2,70)}$=1131, *p<0.0001*], intervention [$F_{(5,70)}$ = 220.1, *p<0.0001*], age x intervention interaction [$F_{(10,70)}$=5.704, *p<0.0001*] in H_2O_2 levels. In CCS group, the levels were significantly (p<0.0001) increased for adult (94%), late-adult (19%) and old (22%) when compared to controls. The highest increase was seen in ACS group at p<0.0001 for adults (36%), late-adult (46%), old (31%) when compared to their age-matched controls.

GSPE supplementation reduced the H_2O_2 levels in CCS groups of adult (15%, p<0.0001), late-adult (7.3%, *p<0.01*), and old (8.6%, p<0.01)) when compared with their unsupplemented counterparts. GSPE supplementation did not change the H_2O_2 level in the ACS group, (Table II).

Nitric oxide

The two-way ANOVA revealed, age [$F_{(2,70)}$=1839, *p<0.0001*], intervention [$F_{(5,70)}$=153.1, *p<0.0001*], age x intervention interaction [$F_{(10,70)}$=4.833, *p<0.0001*] in $NO^{\bullet}$ levels. In the CCS groups, the levels significantly (p<0.0001) increased in the adult (9%) and old (20%) rats and less significantly in the late-adult (25%, p<0.01) in comparison with their controls. Among the ACS groups, significant increases (p<0.0001) of higher magnitude than CCS groups were seen in the adult (28%), late-adult (52%) and old (45%) rats in comparison with their respective age-matched controls..

GSPE supplementation reduced $NO^{\bullet}$ levels in the CCS group (13%, p<0.01) and ACS groups (13%, P<0.0001) of the old in comparison to their unsupplemented stressed group (Table II).

Protein sulphydryl

The two-way ANOVA revealed, age [$F_{(2,70)}$=103.9, *p<0.0001*], intervention [$F_{(5,70)}$=72.98, *p<0.0001*], age x intervention interaction [$F_{(10,70)}$=1.029, *p=0.4285*] in P-SH levels. In the CCS group, the levels significantly (p<0.001) decreased in the adult (9%), late-adult (6.6%) and old (10%) in comparison to their respective controls. Significant decreases (p<0.0001) of higher magnitude were seen in the ACS groups of adults (18%), late-adult (21%) and old (18%) in comparison to their respective controls.

GSPE supplementation increased P-SH levels in the CCS groups of adult (6%, p<0.05), and late-adult *(*3.4%, *p<0.0001*) in comparison to their unsupplemented stressed group (Table II). Supplementation revealed no significant change in ACS group (Table II).

Superoxide dismutase

The two-way ANOVA revealed, age [$F_{(2,70)}$=160.8, *p<0.0001*], intervention [$F_{(5,70)}$=128.2, *p<0.0001*], age x intervention interaction [$F_{(10,70)}$=2.757, *p=0.0063*] in SOD activity. In the CCS group, activity decreased significantly (p<0.0001) in adult (19%), late-adult (15.4%) and old (17%) rats in comparison to their respective controls. Significant (p<0.0001) decreases and of higher magnitude were seen in the ACS groups in adults (23%), late-adult (23%) and old (24%).

Activity increased with GSPE supplementation in CCS groups significantly (P<0.0001) for adult (18%,), p<0.05 for late-adult (9%) and old (10%) when compared with their respective stressed group. GSPE supplementation did not significantly change SOD activity in the ACS groups (Fig. 1A).

Catalase

The two-way ANOVA revealed, age [$F_{(2,70)}$=290.5, *p<0.0001*], intervention [$F_{(5,70)}$=257.4, *p<0.0001*], age x intervention interaction [$F_{(10,70)}$=3.878, *p=0.0003*] in CAT activity. In the CCS group, activity increased significantly (p<0.0001) in

adult (21%), late-adult (17%), old (15%) rats in comparison to their respective controls. Significant (*p<0.0001*) increases were observed in the ACS groups of adults (28%), late-adult (21%), old (17%) when compared to their controls.

GSPE supplementation significantly (p<0.05) decreased the CAT activity in the CCS groups of adult (5%), late-adult (7%, p<0.001) and old (4%, p<0.0001) in comparison to their unsupplemented stressed group (Fig. 1B).

Thioredoxin reductase

The two-way ANOVA revealed, age [$F_{(2,36)}$=255.1, *p<0.0001*], intervention [$F_{(5,36)}$=867.5, *p<0.0001*], age x intervention interaction [$F_{(10,36)}$=37.84, *p<0.0001*] in TrxR activity. In the CCS group, activity increased significantly (p<0.0001) in adult (165%), late-adult (477%) and old (590%) in comparison to their respective controls. The extent of increase was, however, higher (p<0.0001) in the ACS than CCS groups of adult (474%), late-adult (885%) and old (997%). GSPE supplementation reduced TrxR activity significantly (p<0.0001) in the CCS groups of adult (28%), late-adult (48%) and old (68%) in comparison to their unsupplemented counterparts.

The decreases observed in GSPE supplemented ACS rats when compared to their unsupplemented counterparts were significant (p<0.0001) in the adult (18%), late-adult (69%) and old (45%) rats (Fig. 1C).

Reduced glutathione

The two-way ANOVA revealed, age [$F_{(2,70)}$=756.9, *p<0.0001*], intervention [$F_{(5,70)}$=136.5, *p<0.0001*], age x intervention interaction [$F_{(10,70)}$=9.863, *p<0.0001*] in GSH levels. In CCS group, levels decreased significantly in adult (9%), p<0.0001 for late-adult (29%) and old (20%) when compared to their respective controls. The extent of decrease in the ACS group was higher (p<0.0001) than the CCS groups of adult (25%), late-adult (42%) and old (24%).

The increases observed in GSPE supplemented CCS rats when compared to their unsupplemented counterparts were 9% (p<0.01) in the adult rats, 18% (p<0.05) in the late-adult rats, and 13% (p<0.05) in the old (13%) rats (Fig. 1D).

Glutathione peroxidase

The two-way ANOVA revealed, age [$F_{(2,70)}$=601.9, *p<0.0001*], intervention [$F_{(5,70)}$=136.5, *p<0.0001*], age x intervention interaction [$F_{(10,70)}$=3.860, *p=0.0003*] in GPx activity. In the CCS group, GPx activity decreased significantly (p<0.0001) in the adult (20%), late-adult (34%) and old (69%) rats when compared to their respective controls. The extent of decrease in the ACS group was higher than CCS groups (p<0.0001) of adult (33%) and late-adult (39%) rats while old rats showed an almost equal extent (68%) of decrease as of CCS rats.

The increases observed in GSPE supplemented CCS rats when compared to their unsupplemented counterparts were 7% (p<0.05) in the adult rats, 29% (p<0.01) in the late-adult rats and 32% (p<0.01). Insignificant changes were seen in ACS groups with supplementation (Fig. 1E).

Glutathione reductase

The two-way ANOVA revealed, age [$F_{(2,70)}$=627.6, *p<0.0001*], intervention [$F_{(5,70)}$=104.6, *p<0.0001*], age x intervention interaction [$F_{(10,70)}$=9.433, *p<0.0001*] in GR activity. In the CCS group, GR activity decreased significantly for adult (10%, p<0.05), late-adult (21%, p<0.0001) and old (36%, p<0.0001) rats when compared to their controls. However, the extent of decrease in the ACS group was higher (p<0.0001) than the CCS group of adult (32%), late-adult (45%) and old (35%).

The increases observed in GSPE supplemented CCS rats when compared to their unsupplemented counterparts were 20% (p<0.0001) in the adult rats, 20% (p<0.05) in the late-adult rats and 15% (p<0.01) in 28% in the old (p<0.01) Supplementation revealed insignificant changes in ACS group (Fig. 1F).

Heat shock protein *72*

The two-way ANOVA revealed, age [$F_{(2,36)}$=16.11, *p<0.0001*], intervention [$F_{(5,36)}$=3.103, *p=0.0198*], age x intervention interaction [$F_{(10,36)}$=0.3567, *p=0.9573*] in HSP 72 expression. In CCS group, increased expression was noticed in adult (6%, p<0.05), *p<0.01* for late-adult (22%) and old (27%) when compared to controls. The ACS group exhibited highest increase (p<0.001) for late-adult (26%) and old (48%) in comparison to controls. GSPE supplementation decreased expression levels in CCS groups significantly (p<0.05) for late-adult (9%) and old (11%) when compared respective stressed group. The ACS group exhibited insignificant change with supplementation (Fig. 2A).

IV. DISCUSSION

In this study, intermittent cold stress was induced in aging rats to study the redox state in the right ventricle and the intervention of a natural polyphenol extract to overcome stress effects. Our results showed increased body weight in adult rats exposed to chronic stress compared to their controls. Right ventricular weight of the two stressed groups, chronic and acute, were slightly higher than the control group. However, the changes were insignificant irrespective of age.

The results showed that chronic cold stress increases superoxide, hydrogen peroxide and nitric oxide in the RV of adult, late-adult and old rats. This trend is not surprising since heart is sensitive to cold and can increase its metabolic rate and oxygen consumption [29]. However, the extent of increase in the levels of FRs in the RV was more under acute stress than chronic stress. These findings may be explained based on an earlier study wherein we have reported that acute cold stress rather than chronic stress elevates greater serum corticosterone level in parallel with hyperlipidemia and more proniunced in the old [**16**]. It is also known that hyperlipidemic states actually alter physical properties of cell membranes resulting in of free radicals from the damaged mitochondria.

The present study demonstrating reduced GR activity may be due to the less availability of NADPH implying the prevalence of imposed oxidative stress by the increased cellular free radicals under cold stress. Interestingly, our study has shown attenuation of mitochondrial FRs in the RV myocardium by GSPE against cold-induced oxidative stress. Further, the lowered SOD and GPx activities along with GSH levels is in agreement with the findings in hypercholesterolemic rabbits wherein SOD and GPx activities are related to higher lipid levels [30]. Further, the low SOD and GPx activities accompanied by high protein oxidation during cold stress is in accordance with the observations of Condell et al [31], wherein GPx activity is inactivated by high levels of ROS in vitro.

In the present study, expression of HSP*72* was significantly reduced with age. However, overexpression of HSP*72* in cold -induced stress was evident along with the weakened SOD and GPx activities. These results bear a somewhat similar trend reported in chicken heart for maintaining normal metabolism during cold stress [32].

V. CONCLUSION

The present study demonstrates that GSPE can attenuate mitochondrial generation of free radicals in the RV of rats exposed to cold stress and that supplementation is beneficial with age, more so, in the old. The data provides an insight into the possible benefits that may be derived from GSPE in analogous situations such as hypothermic stress -related rapid cooling in ischaemia-reperfusion and heart surgeries, apart from rescuing the RV from occupational hazards.

ACKNOWLEDGEMENT

This work was supported by research grants under the Promotion of University Research and Scientific Excellence (PURSE)-Department of Science and Technology (DST) (SR/59/Z-23/2010/38 dt.27.06.2011), New Delhi.

REFERENCES

[1] Maejima M, Adachi S, Ito H et al (2008) Induction of premature senescence in cardiomyocytes by doxorubicin as a novel mechanism of myocardial damage. Aging Cell 7: 125-136.

[2] Sabbah HN, Sharov VG, Goldstein S (2000) Cell death, tissue hypoxia and the progression of heart failure. Therefore, a reduction in the total number of viable cells may lead to an accelerated decline in heart function. Heart Fail Rev 5: 131-138.

[3] Asha Devi S, Manjula KR, Subramanyam MVV (2012) Protective effects of vitamin E and C against oxidative stress caused by intermittent cold exposure in ageing rat's frontoparietal cortex. Neurosci Lett. 529: 155-160.

[4] Asha Devi S, Manjula KR (2014) Intermittent cold-induced hippocampal oxidative stress is associated with changes in the plasma lipid composition and is modifiable by vitamins C and E in old rats. Neurochem Int 74:46-52.

[5] Wei H, Zhang R, Su Y et al (2016). Effects of acute cold stress after long-term cold stimulation on antioxidant status, heat shock proteins, inflammation and immune cytokines in broiler heart. Front. Physiol. 9:1589.

[6] Samuels SE, Thompson JR, Christopherson RJ (1996) Skeletal and cardiac muscle protein turnover during short-term cold exposure and rewarming in young rats. J Physiol. 2:R1231-1239.

[7] Schluter KD (ed.) (2016) "Ways to study the biology of cardiomyocytes" In Cardiomyocytes-Active Players in Cardiac Disease (Cham: Springer Int Publishing).3-23.

[8] Chang YM, Chang HH, Lin HJ et al (2017) Inhibition of cardiac hypertrophy effects in D-galactose-induced senescent hearts by alpinate oxyphyllae fructus treatment. Evid Based Compliment Alternat Med 2624384.

[9] Yu JM, Bao ED (2008) Effect of acute heat stress on heat shock protein 70 and its corresponding mRNA expression in the heart, liver, and kidney of broilers. Asian Australas J Anim Sci 21 1116–1126. 10.5713/ajas.2008.70703.

[10] Zhao FQ, Zhang ZW et al (2014) Cold stress induces antioxidants and Hsps in chicken immune organs. Cell Stress Chaperones 19635–648. 10.1007/s12192-013-0489-9 (2014).

[11] Manjula KR, Subramanyam MVV, Asha Devi S (2013) Protection against oxidative stress caused by intermittent cold exposure by combined supplementation with vitamin E and C in the aging rat hypothalamus. Neurochem Res 38: 876-885.

[12] Fu J, Liu CP, Zhang ZW et al (2013) Influence of inflammatory pathway markers on oxidative stress induced by cold stress in intestine of quails. Res Vet Sci 95 495–501. 10.1016/j.rvsc.2013.05.006.

[13] Fine AM (2000) Oligomeric proanthocyanidin complexes: history, structure, and phytopharmaceutical applications. Altern Med Rev 5: 144-151.

[14] Varzakas T, Zakynthinos G, Verpoort F (2016) Plant food residues as a source of nutraceuticals and functional foods. Foods 5:88

[15] Khurana S, Venkataraman K, Hollingsworth A et al (2013) Polyphenols: Benefits to the cardiovascular system in health and in aging. Nutrients 5: 3779-3827.

[16] Jyothi B, Subramanyam MVV, Asha Devi S (2019) Grape seed proanthocyanidin extract alleviates cold stress-induced hyperlipidemia and lipid peroxidation in the aging rat heart. Int J Sci Res 8: 902-911.

[17] Geller BL,Winge D (1984**)** Subcellular distribution of superoxide dismutase in rat liver. Methods in Enzymol 105:105-114.

[18] Das UN, Padma M, Sagar PS et al (1990) Stimulation of free radical generation in human leukocytes by various agents including tumor necrosis factor is a calmodulin dependent process. Biochem Biophys Res Commun 167: 1030–1036.

[19] Josephy PD, Elingg T, Mason RP (1982) The horseradish peroxidase-catalyzed oxidation of 3,5,3',5'-tetramethylbenzidine. J Biol Chem 257: 3669–3675.

[20] Yucel AA, Gulen S, Dincer S et al (2012) Comparison of two different applications of the Griess method for nitric oxide measurement. J Exp Integr Med 2:167-171.

[21] Habeeb AFSA (1972) Reaction of protein sulfhydryl groups with Ellman's reagent. Methods Enzymol. 34: 457-464.

[22] Misra HP, Fridovich I (1972) The role of superoxide anion in the epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247: 3170–3175.

[23] Aebi H (1984) Catalase in vitro. Methods Enzymol. 105: 121–126.

[24] Teitze F (1969) Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal Biochem 27: 502-522.

[25] Flohé L, Gonzler WA (1984) Assays of glutathione peroxidase. Methods Enzymol 105:114 –120.

[26] Carlberg I, Mannervik B (1985) Glutathione reductase assay. Methods Enzymol. 113: 484-495

[27] Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.

[28] Lowry OH, Rosebrough NJ, Farr L, Randall RJ, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:: 265–275.

[29] Wang X, Che H, Zhang W et al (2015) Effects of mild chronic intermittent cold exposure on rat organs. Int J Biol Sci 11: 1171-1180.

[30] Aikawa M, Sugiyama S, Hill CC et al (2002) Lipid lowering reduces oxidative stress and endothelial cell activation in rabbit atheroma. Circulation 106: 1390-1396.

[31] Condell RA, Tappel AL (1983) Evidence for suitability of glutathione peroxidase as a protective enzyme: studies of oxidative damage, restoration and proteolysis. Arch Biochem Biophys 223: 407-476.

[32] Zhao FQ, Zhang ZW, Wang C et al. (2013) The role of heat shock proteins in inflammatory injury induced by cold stress in chicken hearts. Cell Stress Chaperones 18: 773–783.

APPENDICES-A

Table I: Changes in body weight and TSI of right ventricle as a function of cold stress and GSPE supplementation in aging rats

Body Indices	Age (mo)	Groups					
		CON	CON+PA	CCS	CCS+PA	ACS	ACS+PA
BW (g)	4	236 ± 8.0	268 ± 10.6	281 ± 12.6$^{!}$	253 ± 17.4	283 ± 12.1$^{\#}$	276 ± 12.3$^{\#}$
	12	408 ± 14.6	437 ± 22.5	460 ± 25.6$^{\#}$	422 ± 27.5	400 ± 23.6	422 ± 23.1
	24	412 ± 15.0	423 ± 29.0	467 ± 12.6$^{\#}$	432 ± 24.5	412 ± 14.0	462 ± 28.3$^{\#}$
HW (g)	4	0.76 ± 0.02	0.85 ± 0.03	0.94 ± 0.01$^{\#}$	0.84 ± 0.04	0.86 ± 0.04	0.90 ± 0.01
	12	1.20 ± 0.03	1.18 ± 0.03	1.26 ± 0.04	1.27 ± 0.04	1.23 ± 0.04	1.32 ± 0.04
	24	1.39 ± 0.01	1.29 ± 0.04	1.42 ± 0.04	1.34 ± 0.05	1.32 ± 0.05	1.49 ± 0.05
RV (g)	4	0.18 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.24 ± 0.02
	12	0.28 ± 0.01	0.25 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.24 ± 0.02	0.26 ± 0.02
	24	0.32 ± 0.02	0.27 ± 0.02	0.34 ± 0.02	0.28 ± 0.02	0.28 ± 0.01	0.33 ± 0.01
RV-TSI	4	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.09 ± 0.01
	12	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
	24	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01

Values are mean ± SEM of 5 animals/group. BW, body weight; HW, heart weight; RV, right ventricle; TSI, tissue somatic index; CON, control; CON+PA, control supplemented with GSPE; CCS, chronically cold-stressed; CCS+PA, chronically cold- stressed and GSPE supplementation ; ACS, acutely cold-stressed; ACS+PA, acutely cold-stressed and GSPE supplementation. Values are statistically significant at # $p<0.01$; ! $p<0.05$ in comparison to respective controls

Table II: Free radicals and protein oxidation in the right ventricle as a function of cold stress and GSPE supplementation in aging rats

Parameters	Age (mo)	Groups					
		CON	CON+PA	CCS	CCS+PA	ACS	ACS+PA
Free Radicals							
Superoxide	4	57.6 ± 1.5	51.2 ± 1.3	67.9 ± 1.6$^{\$}$	44.4 ± 1.2*	92.6 ± 1.2*	86.6 ± 3.3*
	12	64.7 ± 1.6	59.3 ± 0.9	82.6 ± 2.4*	77.7 ± 1.8*	90.7 ± 0.8*	87.9 ± 1.3*
	24	93.9 ± 1.8	90.4 ± 1.3	110 ± 2.1*	105 ± 1.7*	113 ± 1.5*	111 ± 1.2*
Hydrogen peroxide	4	17.1 ± 1.0	14.3 ± 1.2	33.3 ± 1.1*	28.2 ± 0.8*	40.4 ± 1.1*	32.5 ± 0.6*
	12	36.7 ± 1.1	31.7 ± 0.7$^{!}$	43.7 ± 1.0$^{\$}$	40.5 ± 0.5	53.6 ± 0.7*	51.3 ± 1.0*
	24	49.9 ± 1.2	42.8 ± 0.6*	60.8 ± 1.4*	55.6 ± 0.6$^{\#}$	65.2 ± 1.1*	58.2 ± 1.2*
Nitric oxide	4	1.2 ± 0.05	1.1 ± 0.04	2.4 ± 0.11*	1.9 ± 0.04$^{!}$	3.8 ± 0.05*	3.1 ± 0.15*
	12	3.2 ± 0.08	2.4 ± 0.14$^{!}$	4.0 ± 0.11$^{\#}$	3.4 ± 0.07	4.9 ± 0.17*	4.3 ± 0.18$^{\$}$
	24	6.7 ± 0.11	5.5 ± 0.22*	7.9 ± 0.10*	6.9 ± 0.41	9.6 ± 0.18*	8.4 ± 0.11*
Protein Oxidation							
Total thiols (P-SH)	4	49.6 ± 1.1	53.9 ± 0.8$^{!}$	44.7 ± 0.9$^{!}$	47.2 ± 1.1	40.5 ± 1.1*	42.0 ± 1.3*
	12	48.2 ± 0.9	51.9 ± 1.0	40.2 ± 1.3*	41.5 ± 0.7$^{\$}$	37.7 ± 0.9*	39.7 ± 1.3*
	24	40.5 ± 0.9	45.8 ± 0.5$^{\#}$	36.5 ± 1.2$^{!}$	37.1 ± 1.1	33.4 ± 0.9*	34.8 ± 0.9$^{\#}$

Results are mean ± SEM of 5 animals/group. P-SH, protein sulfhydryl. All other abbreviations are similar to Table I. Values are statistically significant at * $p<0.0001$; $ $p<0.001$; # $p<0.01$; !$p<0.05$ in comparison to respective controls

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 3, pp: (159-168), Month: July - September 2019, Available at: www.researchpublish.com

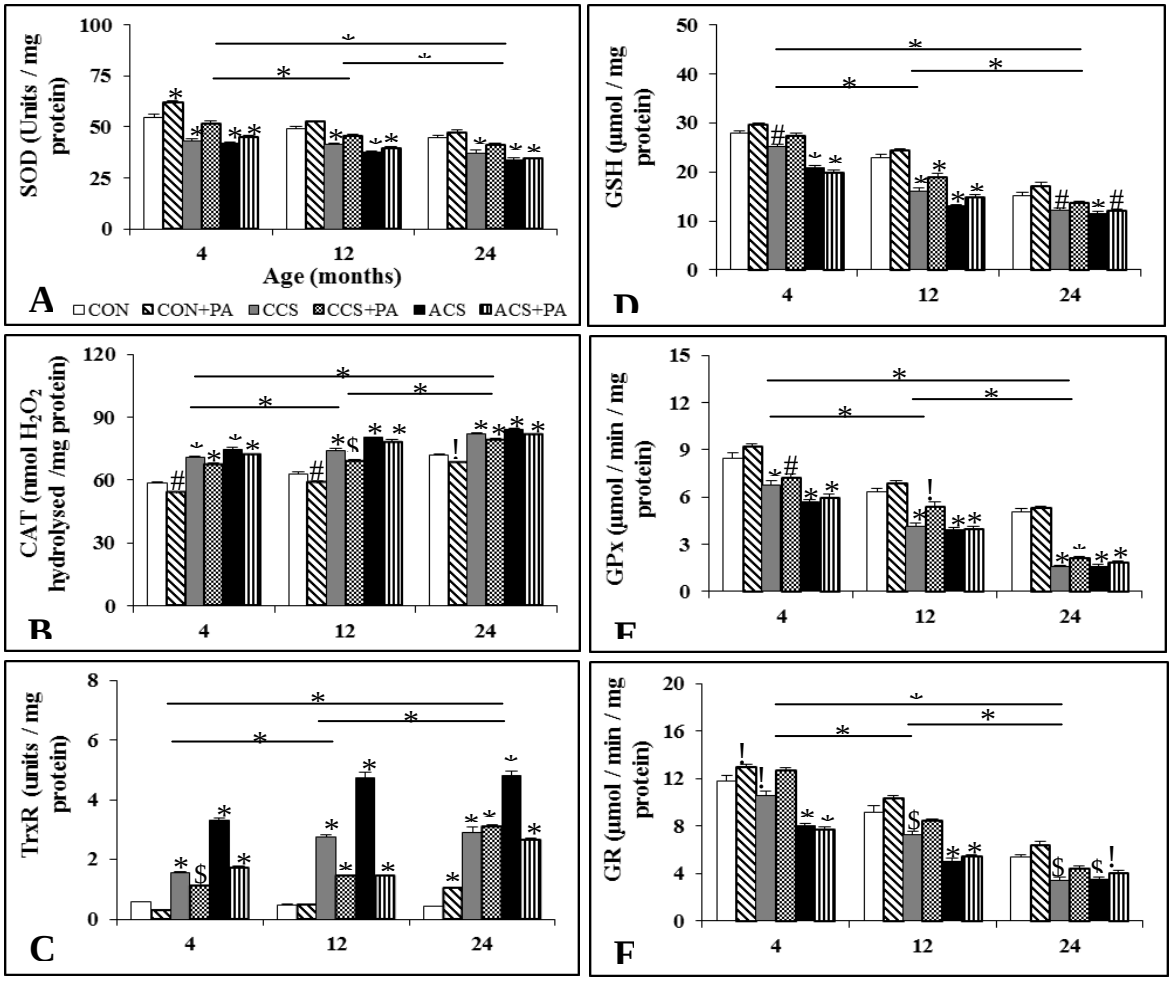

FIG. 1: Endogenous antioxidants in right ventricle of rat heart as a function of cold stress and GSPE supplementation. Results are mean ± SEM of 5 animals/group. GSPE, grape seed proanthocyanidin extract; SOD, superoxide dismutase; CAT, catalase; TrxR, thioredoxinreductase; GSH, reduced glutathione; GPx, glutathione peroxidase; GR, glutathione reductase; CON, control; CON+PA, control supplemented with GSPE; CCS, chronically cold-stressed; CCS+PA, chronically cold-stressed with GSPE supplementation; ACS, acutely-cold stressed; ACS+PA, acutely cold-stressed with GSPE supplementation. Values are statistically significant at * p<0.0001; $ p<0.001; # p<0.01; !p<0.05 as compared to control.

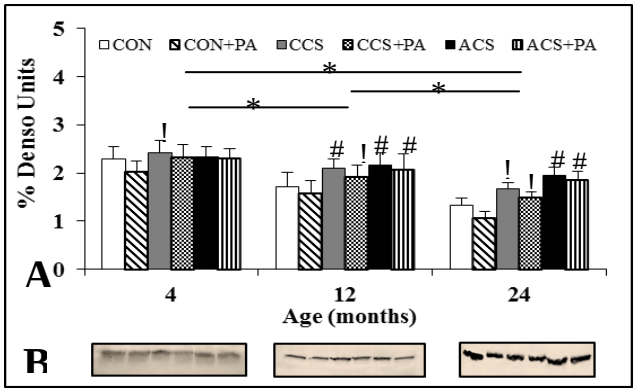

FIG. 2 Expression of HSP*72* (A) and western blot bands (B) in right ventricle of aging rat heart following cold stress. All other abbreviations are similar to Fig.1.Values are statistically significant at * p<0.0001; # p<0.01;! p<0.05 as compared to control.