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Oleic acid ameliorates adrenaline induced dysfunction of rat heart mitochondria by binding with adrenaline: An isothermal titration calorimetry study

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ABSTRACT

Aims: Our earlier studies revealed the cardio-protective effects of oleic acid, a monounsaturated fatty acid, against adrenaline induced myocardial injury. Moreover, it has been found to possess antioxidant properties. Thus, in the present study we have investigated the protective role of oleic acid on adrenaline induced mitochondrial dysfunction in vitro in rat heart mitochondria.

Main methods: Isolated rat cardiac mitochondria was incubated in vitro with adrenaline-bitartrate alone and with graded doses of oleic acid. Biomarkers of oxidative stress, mitochondrial Krebs cycle enzymes and respiratory chain enzymes along with mitochondrial morphology, membrane potential as well as intactness were analyzed. Isothermal titration calorimetric studies with pure adrenaline and oleic acid was also carried out.

Key findings: Incubation with adrenaline, in vitro, showed elevated levels of lipid peroxidation and protein carbonylation of mitochondrial membrane, a reduced level of glutathione content along with an altered profile of mitochondrial enzymes, morphology, membrane potential as well as intactness. All these changes were found to be ameliorated when cardiac mitochondria were co-incubated with adrenaline and oleic acid, in vitro.

Significance: Our earlier studies demonstrated the antioxidant properties of oleic acid. This study suggests that oleic acid binds adrenaline with high affinity gradual saturation of the binding sites of adrenaline. This prevents the generation of ROS and finally providing consequent protection of the cardiac mitochondria and ameliorating adrenaline induced mitochondrial dysfunction. Hence, oleic acid may be considered as a potent future cardio-protective antioxidant.

1. Introduction

Elevated levels of adrenaline, a medullary hormone involved in "fight or flight" mechanism [1], is generally also associated with cardiovascular risk. High levels of adrenaline in myocardial interstitial fluid have been reported to aggravate the progression of myocardial injury and increase the incidence of mitochondrial damage and development of heart failure [2,3]. Adrenaline acts by binding to a variety of adrenergic receptors present in our system. Epinephrine is a non-selective agonist of all adrenergic receptors, including the major subtypes a_1, a_2, $ß_1$, $ß_2$, and $ß_3$ and binding of epinephrine to these receptors triggers a number of metabolic changes [4]. Damage to mitochondria of cardiac myocytes has been observed as a result of adrenaline surge during myocardial ischemia [5]. A recent study from this laboratory has documented and recognized adrenaline as an endogenous oxidative stress inducer in cardiac myocytes mitochondria in sub-chronic treatment [6]. Excess adrenaline has traditionally been linked to increase in adrenoceptors and the subsequent oxidation of adrenaline can lead to generation of oxidative stress. The oxidatively modified adrenaline can generate highly reactive intermediates, like o-quinones, aminochrome,

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aminolutins, melanin and Reactive Oxygen Species (ROS) [7]. These ROS are free radicals and can induce cellular damage by acting on cell membrane proteins, lipids and nucleic acids. The large amounts of ATP required for the maintenance of aerobic life are generated predominantly by oxidative phosphorylation in mitochondria, a process that results in the conversion of oxygen to the superoxide radical, hydrogen peroxide and related ROS [8].

Mitochondria are known to be the major source of ROS production. An estimated 1–2% of the total consumed oxygen is converted into the superoxide anion ($O\cdot-$), reported to be generated at complexes I and III of the electron transport chain. Mitochondrial superoxide dismutase converts $O\cdot-$ to H_2O_2 [9–11]. Mitochondria are also critical regulators of cell metabolism and electron transport. ROS levels are kept in check by a variety of antioxidant systems, such as by small molecules like tocopherols, ascorbic acid and uric acid and also cellular antioxidant enzymes like superoxide dismutases, catalase and peroxidases. It is when this ROS production overwhelms the antioxidant defenses, leading to the generation of oxidative stress which is an important causative factor in pathological conditions, such as cancer, neurodegenerative diseases, atherosclerosis, autoimmune and inflammatory disorders [12–16]. ROS induced mitochondrial dysfunction has also been reported in various pathological conditions such as cardiac and liver related disorders [17]. The drugs available in the market for the treatment of such diseases may have serious side effects when used for longer periods, and thus it becomes necessary to look for alternatives with minimal or no side effects as well as with promising antioxidant potential. The aqueous extracts or isolated bioactive fractions from different parts of traditionally significant medicinal plants may potentially serve as a good source of alternative medicine against such oxidative stress related diseases.

Terminalia arjuna (TA), an important medicinal plant used in the preparation of various ayurvedic formulations for centuries, has recently found use as a cardioprotective substance in India [18–20]. We have previously shown that orally administered aqueous extract of bark of *Terminalia arjuna* (TA) exhibited protection against adrenaline induced myocardial injury in male albino rats [21]. Augmentation of endogenous antioxidant activity in vitro in rat heart by orally administrated crude bark of *Terminalia arjuna* against adrenaline induced oxidative stress mediated ischemic-reperfusion (IR) injury has also been reported [22–25]. The GCMS analysis of the ethyl acetate partitioned fraction of aqueous extract of TA identified 11 major phytocomponents with the retention time ranging from 9.042 to 21.951. They are benzoic acid, 1,2-benzene diol, D-glucuronolactone, nonanoic acid, glutethimide, azelaic acid, hexadecanoic acid, tridecanoic acid, tetradecanoic acid, oleic acid and cyclopropanenonanoic acid. The phyrocomponents with high peak areas (65.43%) found in ethyl acetate partitioned fraction of *Terminalia arjuna* bark was benzoic acid. Although benzoic acid is the major component of ethyl acetate partitioned fraction of *Terminalia arjuna* bark, it is very toxic and carcinogenic. In TA it may remain in a conjugate form that masks its toxicity and exerts its beneficial actions. Oleic acid, an 18 carbon monounsaturated omega 9 fatty acid, is also one of the major components of the ethyl acetate partitioned fraction of *Terminalia arjuna* and no adverse effects of oleic acid are reported till date. Various studies have acknowledged the role of saturated and unsaturated fatty acids and their potential effects at the cardiovascular level. Of these, Mono Unsaturated Fatty Acids or MUFAs, such as oleic acid, have been shown to improve lipid profile [26], sustain a balance of body weight [27] and also inhibit palmitate-induced mitochondrial dysfunction, insulin resistance and inflammatory signaling in neuronal cells [28] and skeletal muscle [29]. Several studies have also identified that oleate, a fatty acid ester, which is a condensation product of oleic acid, possesses numerous effects on the cardiovascular system [30,31]. Our latest research has also provided significant information regarding the cardioprotective effects of oleic acid, derived from the ethyl acetate partitioned fraction of aqueous extract of bark of *Terminalia arjuna* against adrenaline induced myocardial injury in male albino rats [6]. However, the mechanism by which oleic acid is able to provide such protection is not clearly understood. Hence, the present study is aimed to investigate and elucidate the mechanism by which oleic acid is able to provide protection against adrenaline induced oxidative stress mediated myocardial injury in rat heart mitochondria in vitro and to illuminate the antioxidant pathway involved in such protection.

2. Materials and methods

Oleic acid, adrenaline bitartrate and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Sigma-Aldrich. All other chemicals used including the solvents, were of analytical grade obtained from Sisco Research Laboratories (SRL), Mumbai, India, Qualigens (India/Germany), SD fine chemicals (India), Merck Limited, Delhi, India.

2.1. Isolation of mitochondria

Male albino rats of Wistar strain, weighing 150–200 g were handled as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Govt. of India. All the experimental protocols had the approval of the Institutional Animal Ethics Committee (IAEC) of the Department of Physiology, University of Calcutta [Approval No. IAEC/Proposal/Ph. D./DB -01, 2013 dated 23.03.2013]. Animals were sacrificed by cervical dislocation following mild ether anesthesia. The hearts were surgically extirpated after carefully opening the thoracic cavity, thoroughly washed in cold saline, soaked properly in blotting paper for further analysis.

Mitochondria were isolated from rat heart according to the method of Hare et al. with some modifications [32]. Rat hearts were collected just after animal sacrifice, homogenized with 50 mM Tris sucrose buffer (pH 7.8) at 4 °C with Teflon homogenizer. This 10% homogenate was first centrifuged at 600*g* at 4 °C to remove the nucleus as pellet. The supernatant was collected and again centrifuged at 16000*g* for 45 min at 4 °C using cold ultracentrifuge. The pellet was collected and re-suspended in the same buffer and preserved at-20 °C, and the supernatant was finally discarded.

2.2. Standardization of the dose of adrenaline bitartrate and oleic acid

Isolated rat heart mitochondria were first incubated with graded doses of adrenaline bitartrate (0.125 μM, 0.25 μM, 0.5 μM, 1.0 μM & 2.0 μM) only to standardize and determine the dose of adrenaline at which significant damage to the cardiac mitochondria occurred.

Similarly, isolated rat heart mitochondria were also incubated with graded doses of oleic acid (0.0625 μM, 0.125 μM, 0.25 μM, 0.5 μM, 1.0 μM & 2.0 μM) along with a defined dose of adrenaline (0.5 μM) to standardize the dose of oleic acid at which the significant protection against adrenaline treatment was observed.

2.3. Incubation of mitochondria in in vitro system

After the standardization of the doses of adrenaline bitartrate and oleic acid had been carried out through assessment of biomarkers of oxidative stress, freshly isolated cardiac mitochondria were re-suspended in phosphate buffer and divided into four groups. These were as following-.

GROUP I: Control group (**CON**): Mitochondrial sample (50%) was mixed with only phosphate buffer.
GROUP II: Positive control group (**OA**): Mitochondrial sample was mixed with only oleic acid (0.25 μM) and Phosphate Buffer.
GROUP III: Adrenaline bitartrate treated group (**ADR**): Mitochondrial sample was mixed with only adrenaline bitartrate (0.5 μM) and Phosphate Buffer.
GROUP IV: Adrenaline bitartrate and oleic acid co-incubation group (**OA + ADR**): Mitochondrial sample was mixed with 0.5 μM adrenaline bitartrate, 0.25 μM oleic acid and Phosphate Buffer.

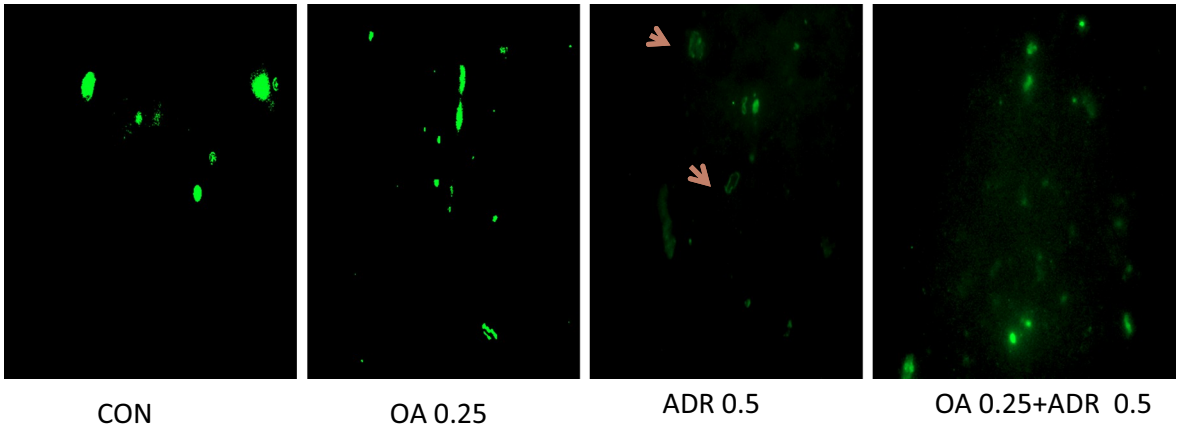

Fig. 1. Protective effect of oleic acid against adrenaline bitartrate induced changes in mitochondrial intactness. Janus Green B stained mitochondrial smears of control (CON), only oleic acid treated (OA 0.25 μM), adrenaline bitartrate treated (ADR 0.5 μM) and adrenaline bitartrate and oleic acid treated group (OA 0.25 + ADR 0.5) of isolated rat heart mitochondria taken at 200× magnification. 0.25 μM of oleic acid was found to be the most effective dose from in vitro experiments. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

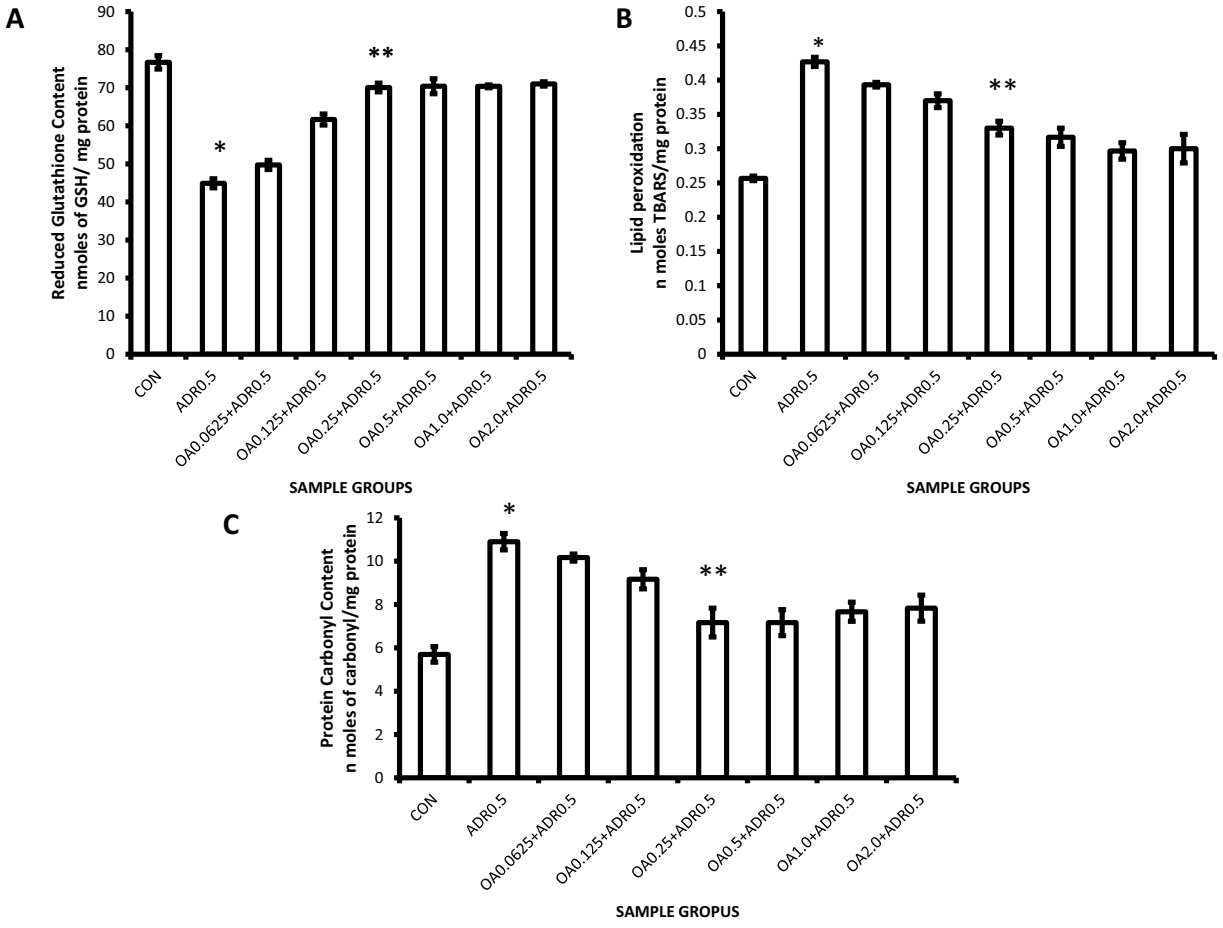

Fig. 2. Effect of adrenaline bitartrate–induced alterations in rat heart mitochondria with graded doses of adrenaline (ADR-0.125,0.25,0.5,1.0,2.0) (A) Reduced glutathione content, (B) Level of lipid peroxidation and (C) Protein Carbonyl content. 0.5 μM of adrenaline was found to be the most effective dose from in vitro experiments. Values are expressed as means ± S.E. *P < 0.001 control vs adrenaline- treated group.

All the above groups were incubated at 37 °C and pH 7.4 for 1 h. In each case the reaction was terminated with addition of 0.02 ml 35 mM EDTA after completion of 1 h.

To examine the protective effect of oleic acid against oxidative stress induced by other oxidants, in vitro, cardiac mitochondria was incubated with (a) 0.2 mM $CuCl_2$ and 1 mM ascorbic acid (b) 0.16 mM $FeSo_4$, 0.9 mM ascorbic acid and 30 μM H_2O_2 at pH 7.4 and at 37 °C for 1 h, respectively. Both systems were also co-incubated with 0.25 μM concentration of oleic acid in presence and absence of copper-ascorbate and iron-ascorbate-H_2O_2 system. After completion of the incubation period the reaction was terminated with 0.02 ml of 35 mM EDTA.

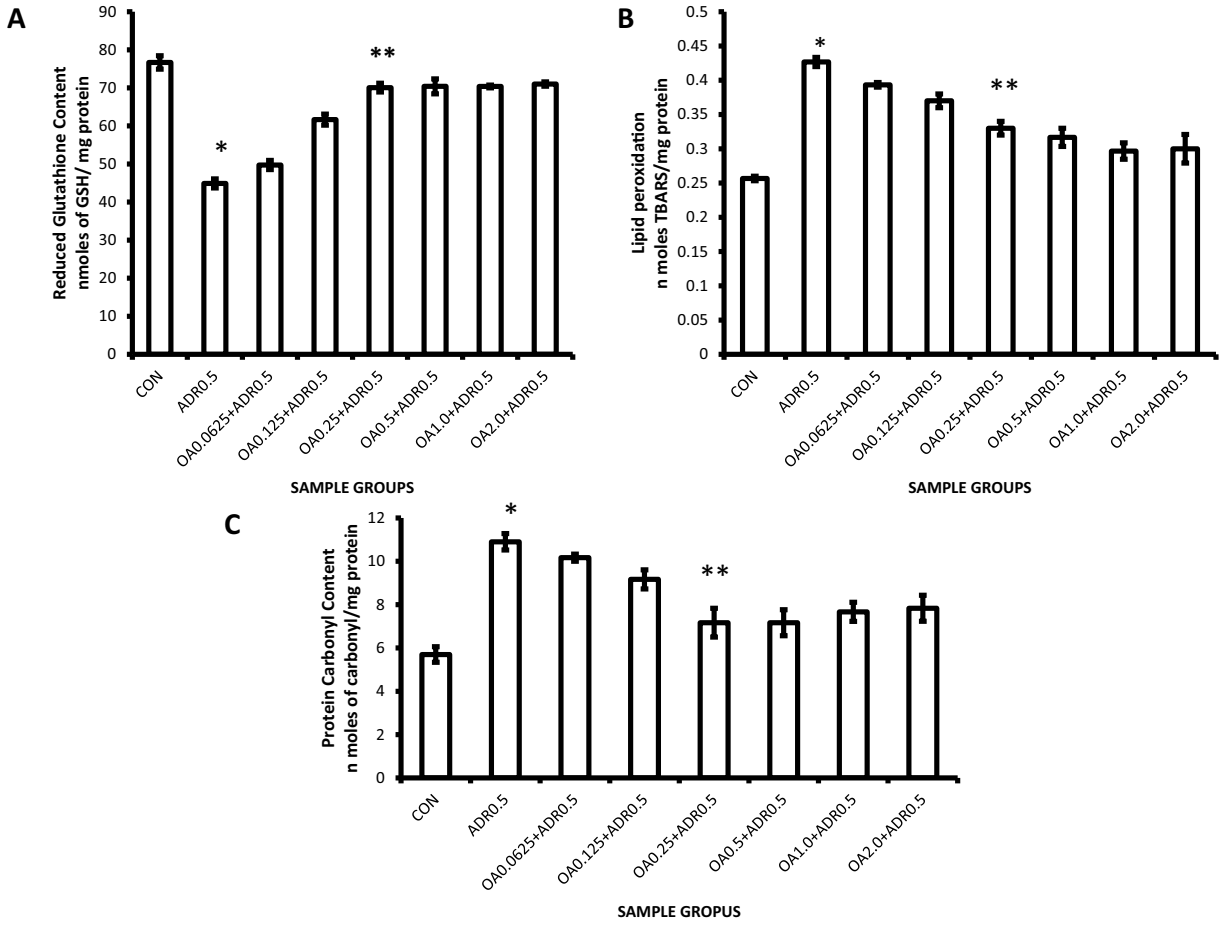

Fig. 3. Protective effect of graded doses of oleic acid (OA 0.0625,0.125,0.25,0.5,1.0,2.0 μM) against adrenaline (ADR 0.5) induced alterations in rat heart mitochondria on (A) Reduced glutathione content, (B) Level of lipid peroxidation and (C) Protein Carbonyl content. 0.25 μM of oleic acid was found to be the most effective dose from in vitro experiments. Values are expressed as means ± S.E. *P < 0.001 versus control, **P < 0.001 vs. adrenaline- treated group.

2.4. Determination of viability of mitochondria

Viability of mitochondria was determined according to the method of Mukherjee et al.[33]. 0.2 ml from each group of incubated mitochondrial suspension was spread uniformly on glass slide and dried. 0.1% Janus green B stain was applied to them. The slides were then kept in the dark for 40 min and then rinsed with distilled water carefully for removal of excess stain. Ultimately the slides were mounted with DPX and observed under Olympus BX 51 fluorescence microscope using green filter at 40× magnification (excited by application of blue filter).

2.5. Measurement of biomarkers of oxidative stress

2.5.1. Lipid peroxidation level (LPO)

Lipid peroxidation level (LPO) of mitochondria was measured by the method of Buege and Aust [34]. 2 ml TBA-TCA-HCl was added to 0.5 mg protein containing mitochondrial suspension and heated at 80 °C for 20 min and then centrifuged at 2000 rpm for 10 min to remove the protein debris. The absorbance of the colored supernatant was recorded at 532 nm. Lipid peroxidation level was finally calculated in terms of nmoles TBARS/mg protein using 1.56×10^5 as molar extinction coefficient of malondialdehyde (MDA).

2.5.2. Reduced glutathione level (GSH)

Reduced glutathione level (GSH) of mitochondria was measured from mitochondria using the Ellman's reagent (DTNB) according to the method of Sedlak and Lindsay [35]. In this method proteins of incubated mitochondria were precipitated with 10% ice cold TCA, and centrifuged at 5000 rpm for 20 min at 4 °C. To one volume supernatant, two volume 0.8(M) Tris-HCl-EDTA(pH 9.0) and one tenth volume 10 mM DTNB were added and kept at room temperature for 10 min. Then the absorbance of each sample was recorded at 412 nm. Finally reduced glutathione was estimated from the standard curve and evaluated in terms of nmoles GSH/mg protein.

2.5.3. Protein carbonyl content (PCO)

The protein carbonyl content (PCO) of incubated mitochondria was determined according to the method of Levine et al. [36] as modified by Reznick et al. [37]. The sample was incubated with 10 mM DNPH for 45 min in the dark. At the end of incubation 10% TCA was added, and the mixture was centrifuged at 7000 rpm for 15 min, after which the supernatant was discarded and the pellets were washed carefully with ethanol: ethyl acetate mixture (1:1) thrice. Then equal volume of 6(M) guanidine hydrochloride and 0.5(M) potassium dihydrogen phosphate (pH 2.5) were added to the washed pellets, mixed thoroughly and centrifuged at 7000 rpm for 15 min. The supernatant was collected and the absorbance was determined at 375 nm and the calculated values were expressed in terms of nmoles protein carbonyl/mg protein.

2.6. Determination of activities of Krebs cycle enzymes

Pyruvate dehydrogenase activity (PDH) of incubated mitochondria was measured following the reduction of NAD to NADH at 340 nm for

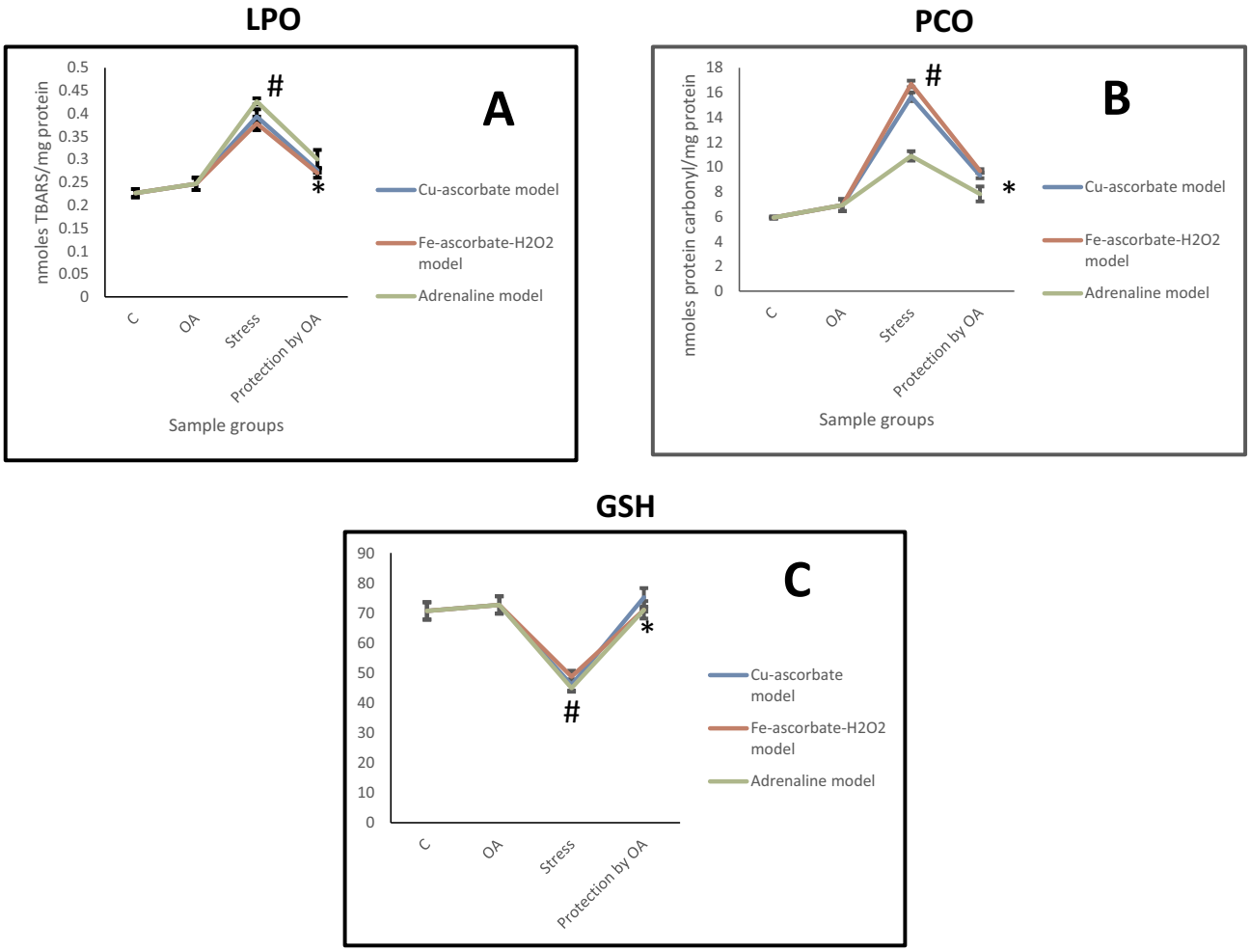

Fig. 4. Protective effect of oleic acid (0.25 μM) against adrenaline (ADR 0.5), Copper-Ascorbate and Fe-Ascorbate-H_2O_2 induced alterations in rat heart mitochondria on (A) Level of lipid peroxidation, (B) Protein Carbonyl content and (C) Reduced glutathione content. 0.25 μM of oleic acid was found to be the most effective dose from in vitro experiments. Values are expressed as means ± S.E. $^{\#}P < 0.001$ versus control, $^{*}P < 0.001$ vs. oxidants like adrenaline, Copper-Ascorbate and Fe-Ascorbate-H2O2 treated group.

90 s according to the method of Chreiten et al. [38]; using UV/Vis Bio Rad spectrophotometer. The 0.5 ml reaction mixture contained.

0.1 M phosphate buffer pH 7.5,0.5 mM sodium pyruvate, 0.5 mM NAD and suitable aliquot of mitochondrial suspension. Specific activity was calculated in terms of Units/mg protein.

Isocitrate dehydrogenase activity (ICDH) of incubated samples was measured according to the method of Duncan et al. [39]. The 0.5 ml reaction mixture contained 0.1 M phosphate buffer pH 7.5, 10 mM isocitrate, 2.5 mm $MnSO_4$ and incubated mitochondria. To start the reaction 5 mM NAD was added and the reaction was monitored following the increase in absorbance at 340 nm for 90 s at an interval of 10 s. Specific activity was evaluated in terms of Units/mg protein.

α-Ketoglutarate dehydrogenase (α-KGDH) activity was determined according to the method of Duncan et al. [39]. This assay was performed in 0.5 ml reaction mixture that contained 0.1 M phosphate buffer pH 7.5, 0.5 mM α-ketoglutarate and suitable aliquot of incubated mitochondria and, 0.35 mM NAD. The increase in absorbance at 340 nm was monitored for 90 s at interval of 10 s. Specific activity was expressed in terms of Units/mg protein.

Succinate dehydrogenase (SDH) activity of incubated mitochondria was measured through monitoring the reduction of potassium ferricyanide spectrophotometrically at 420 nm for 2 min according to the method of Veeger et al. [40]. The 0.5 ml reaction mixture contained 0.1 M phosphate buffer pH 7.5, 2% BSA, 2.5 mM potassium ferricyanide and 4 mM succinate and suitable volume of mitochondrial suspension. The specific activities were expressed in terms of Units/mg protein.

Aconitase activity was measured spectrophotometrically by monitoring the formation of cis-aconitate from added isocitrate (20 mM) at 240 nm and 25 °C following the method of Gardner et al. [41]. One unit was defined as the amount of enzyme necessary to produce 1 μmol cis aconitate per minute ($e_{240} = 3.6\ mM^{-1}\ cm^{-1}$).

Fumarase activity was determined spectrophotometrically by measuring the increase in absorbance at 240 nm at 25 °C in the reaction mixture to which 30 mM potassium phosphate (pH 7.4), and 0.1 mM Lmalate and suitable amount of mitochondrial suspension (the source of enzyme)were added following the method of Racker et al. [42]. One unit (U) was defined as the amount of enzyme necessary to produce 1 μmol fumarate per minute ($e_{240} = 3.6\ mM^{-1}\ cm^{-1}$).

2.7. Determination of activities of Electron transport chain (ETC) linked enzymes

NADH-cytochrome C oxidoreductase activity was measured spectrophotometrically by following the reduction of oxidized cytochrome C at 565 nm according to the method of Goyal et al. [43] with some modifications as done by Mitra et al.[44]. 0.5 ml of assay mixture contained in addition to the incubated mitochondrial suspension as the source of enzyme, 50 mM phosphate buffer pH 7.4, 0.5 μM NADH, 1 mg/ml BSA and 20 mM oxidized cytochrome C. The decrease in absorbance of cytochrome C was monitored for 90 s at 10 s interval.

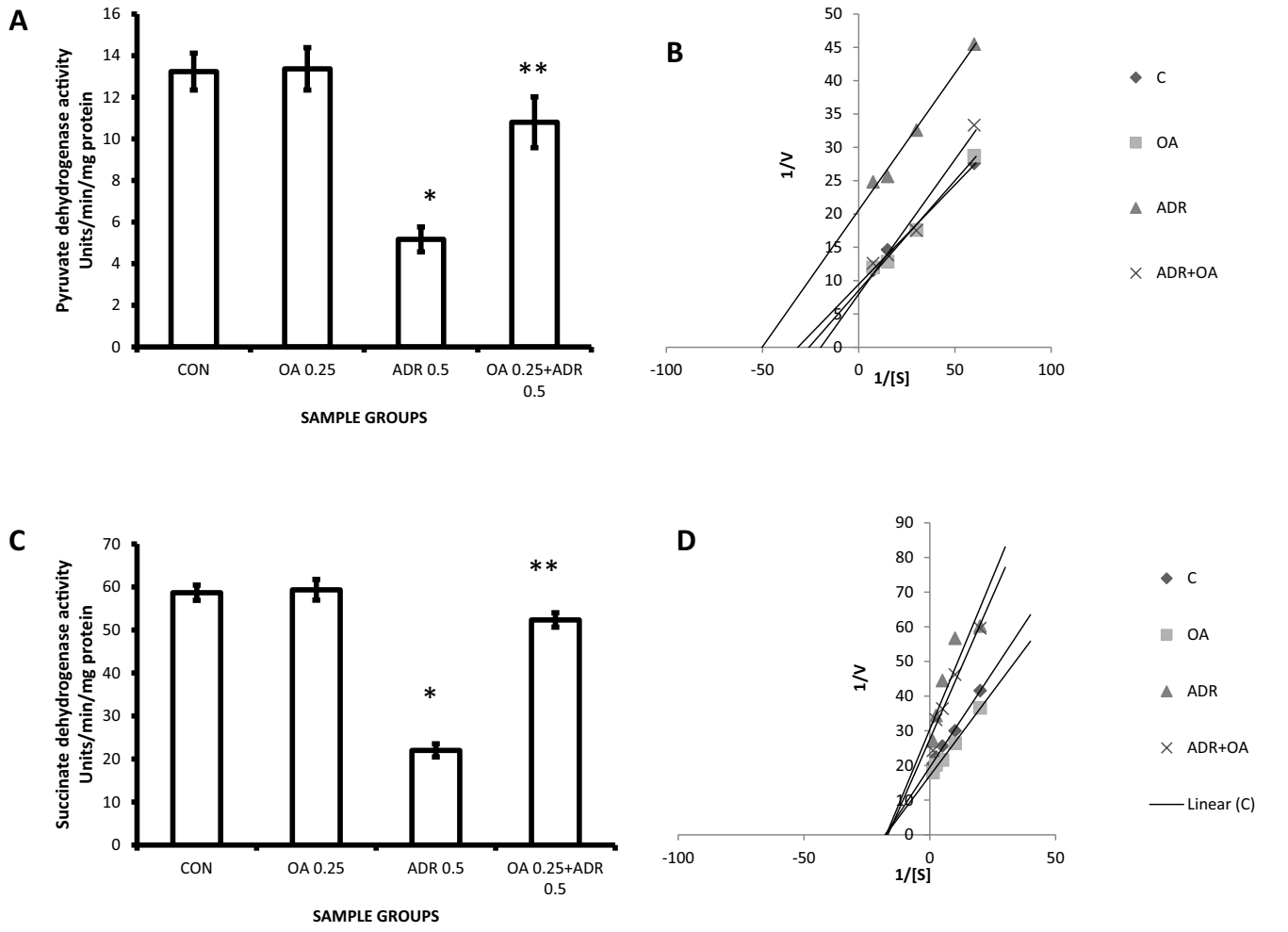

Fig. 5. Protective effect of oleic acid (0.25 μM) against adrenaline bitartrate induced alterations in rat heart mitochondria on (A) PDH activity (B) Lineweaver-Burk plot of PDH activity. K_m values were calculated from the above curve using the straight line equation $y = mx + c$. V_{max}: *Con* = 0.106 mM/min, ADR = 0.049 mM/min, OA = 0.117 mM/min, ADR + OA = 0.125 mM/min; K_m: *Con* = 0.031 mM, ADR = 0.02 mM, OA = 0.038 mM, ADR + OA = 0.05 Mm (C) SDH activity with (D) Lineweaver-Burk plot of SDH activity. K_m values were calculated from the above curve using the straight line equation $y = mx + c$. V_{max}: *Con* = 0.059 mM/min, ADR = 0.033 mM/min, OA = 0.051 mM/min, ADR + OA = 0.036 mM/min; K_m: *Con* = 0.057 mM, ADR = 0.057 mM, OA = 0.057 mM, ADR + OA = 0.057 mM. (E) α-KGDH activity (F) Lineweaver-Burk plot of α-KGDH activity. K_m values were calculated from the above curve using the straight line equation $y = mx + c$. V_{max}: *Con* = 0.117 mM/min, ADR = 0.056 mM/min, OA = 0.117 mM/min, ADR + OA = 0.126 mM/min; K_m: *Con* = 0.026 mM, ADR = 0.015 mM, OA = 0.026 mM, ADR + OA = 0.034 mM. (G) ICDH activity. Values are expressed as means ± S.E. $^{*}P < 0.001$ versus control, $^{**}P < 0.001$ vs. adrenaline- treated group.

Specific activity was expressed in terms of Units/mg protein.

Cytochrome C oxidase activity was determined spectrophotometrically by following the oxidation of reduced cytochrome C at 550 nm according to the method of Goyal et al. [43] with some modifications as done by Mitra et al. [44]. Oxidized cytochrome C was reduced by 1 M dithiothreitol for overnight. Then OD550/OD560 ratio was observed to determine the degree of reduction. Then in 0.5 ml of assay mixture suitable volumes of 50 mM phosphate buffer, pH 7.4, 40 mM reduced cytochrome C and the incubated mitochondrial suspension as the source of enzyme were taken and decrease in absorbance at 550 nm was monitored for 90 s at interval 10 s. Specific activity was calculated in terms of Units/mg protein.

2.8. Determination of mitochondrial DNA damage

2.8.1. DAPI staining of mitochondria

DAPI staining of mitochondrial sample was done according to the method of Mukherjee et al. [45]. Incubated mitochondria was uniformly spread on clean glass slides, 300 nm DAPI stain was applied and kept in the dark for 15 min. The excess stain was washed away by applying PBS and the slides were mounted by DPX and observed under Olympus Fluoview IX 81confocal laser scanning microscope at 40× magnification (Excitation wavelength 358 nm, emission wavelength 461 nm).

2.9. Determination of surface topology of mitochondria

Surface topology of mitochondria was studied according to the method of Ghosh et al. [46] using Scanning Electron Microscopy (SEM). 0.5 ml of incubated mitochondrial suspension was mixed with equal volume 2% glutaraldehyde and kept at 40 °C for two nights for fixation. These samples were then dehydrated with gradual washing with increasing concentration of ethanol and n-amyl alcohol, dried and observed under scanning electron microscope at 40× magnification.

2.10. Binding study between oleic acid and adrenaline bitartrate through isothermal titration calorimetry (ITC)

Binding of pure oleic acid with adrenaline bitartrate was analyzed by isothermal titration calorimetry using Microcal ITC-200, Malvern. In this assay, in the sample cell 0.35 ml oleic acid (at 40 nmoles/ml concentration) was titrated with 0.06 ml 1 mM adrenaline bitartrate.

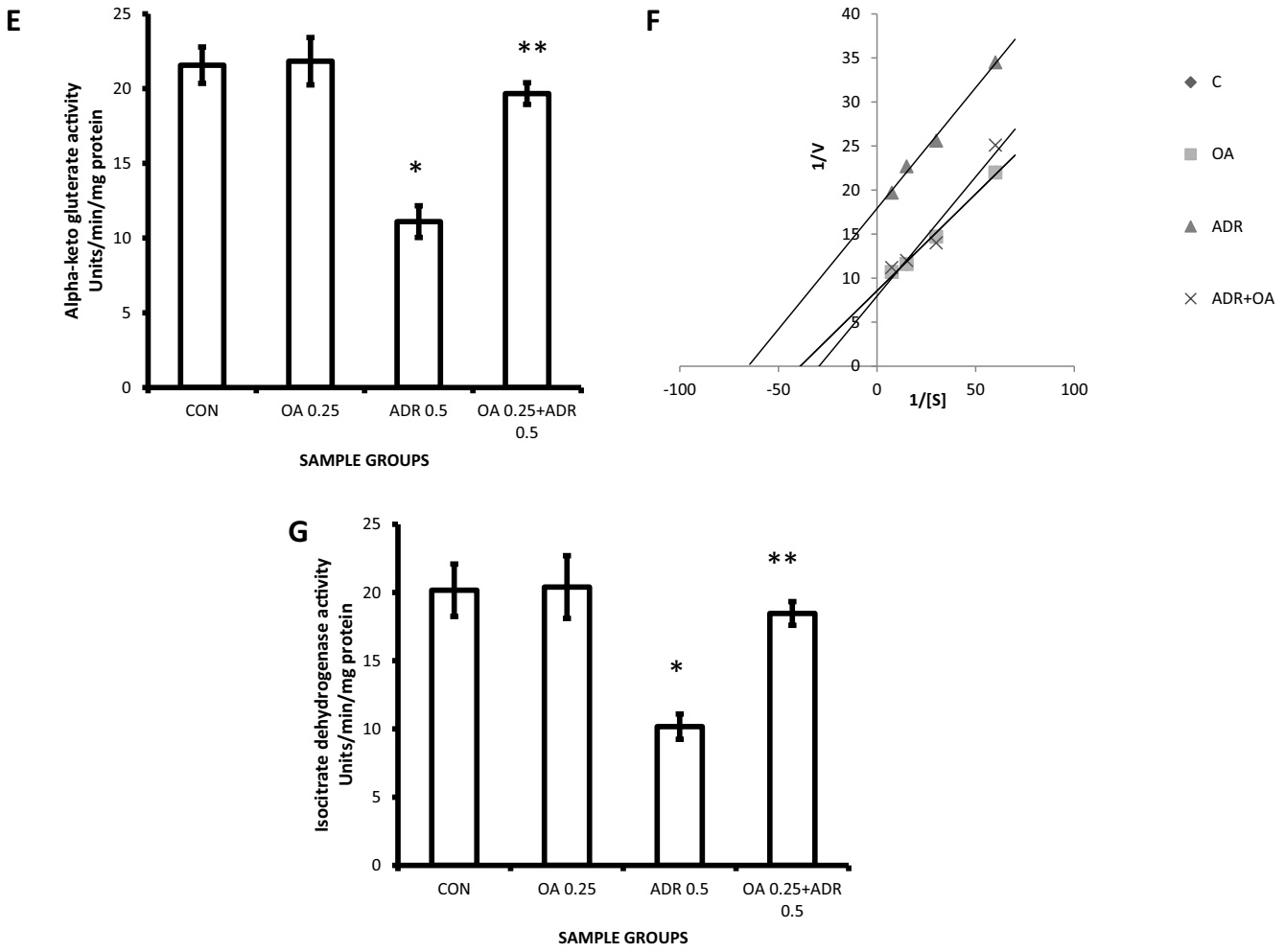

Fig. 5. (*continued*)

Titration was conducted with twenty injections each with 2 µl volume with 150 s spacing between two injections for approximately 1 h [47].

2.11. Determination of mitochondrial membrane potential (DΨm)

Changes of mitochondrial membrane potential was determined by using JC-1 (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanineiodide) dye (Sigma-Aldrich Co. LLC, St. Louis, MO,USA). Incubated mitochondrial sample was stained with help of JC-1 dye. Final concentration of JC-1 staining solution was 0.2 µg/ml. The stained mitochondrial sample was analyzed and an excitation wavelength of 488 nm and an emission wavelength of band pass filter586/42 nm were used to measure the mitochondrial membrane potentiality with help of flow Cytometry (BDFACS Versa, USA) [48].

2.12. Estimation of protein

Protein concentration of mitochondria was determined by the method of Lowry et al. [49].

2.13. Statistical evaluation

Each experiment was repeated at least three times with different groups. Data are presented as means ± S.E. Significance of mean values of different parameters between the treatment groups were analyzed using one way post hoc tests (Tukey's HSD test) of analysis of variances (ANOVA) after ascertaining the homogeneity of variances between the treatments. Pair wise comparisons were done by calculating the least significance. Statistical tests were performed using Microcal Origin version 7.0 for Windows.

3. Results

3.1. Mitochondrial viability and generation of oxidative stress by adrenaline

Fig. 1 shows the mitochondrial viability and intactness of isolated rat heart mitochondria, in vitro, after staining with a vital dye, Janus green B, to ascertain that whole and intact mitochondria have been isolated for the study. When seen under a fluorescent microscope, intact mitochondria appear as green fluorescence whereas damaged mitochondria do not exhibit any fluorescence. Incubation of mitochondria with adrenaline bitartrate at a concentration of 0.5 µM (ADR0.5) resulted in a significant decline in the intactness of mitochondria as compared to control (see red arrows) whereas co-incubation with 0.25 µM oleic acid significantly protected the mitochondria from losing its structural integrity as compared to adrenaline treated mitochondria which is evident from the increased fluorescent intensity as compared to only adrenaline treated group. Positive control groups containing only oleic acid in mitochondrial samples however did not show any difference as compared to control ($P < 0.001$ vs control).

3.2. Changes associated with the biomarkers of oxidative stress

Fig. 2 represents the changes associated with biomarkers of oxidative stress after adrenaline bitartrate treatment. Graded doses of

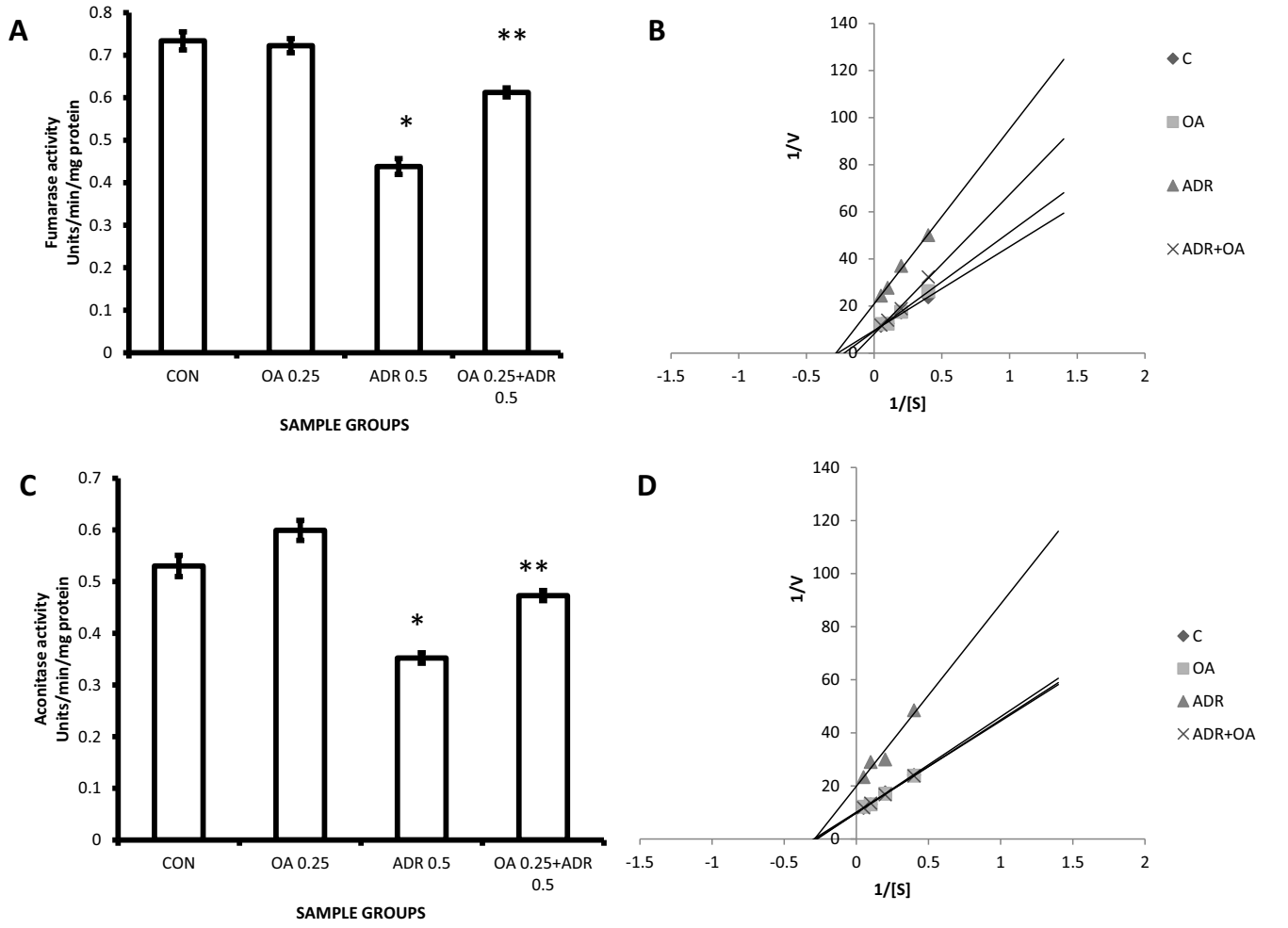

Fig. 6. Protective effect of oleic acid (0.25 μM) against adrenaline bitartrate induced alterations in rat heart mitochondria on (A) Fumarase activity (B) Lineweaver-Burk plot of Fumarase activity. K_m values were calculated from the above curve using the straight line equation $y = mx + c$. V_{max}: *Con* = 0.11 mM/min, ADR = 0.052 mM/min, OA = 0.11 mM/min, ADR + OA = 0.134 mM/min; K_m: *Con* = 3.1 mM, ADR = 2.57 mM, OA = 3.77 mM, ADR + OA = 6.8 mM (C) Aconitase activity (D) Lineweaver-Burk plot of Aconitase activity. K_m values were calculated from the above curve using the straight line equation $y = mx + c$. V_{max}: *Con* = 0.104 mM/min, ADR = 0.05 mM/min, OA = 0.11 mM/min, ADR + OA = 0.12 mM/min; K_m: *Con* = 3.7 mM, ADR = 3.7 mM, OA = 3.7 mM, ADR + OA = 3.7 mM. Values are expressed as means ± S.E. *P < 0.001 versus control, **P < 0.001 vs. adrenaline- treated group.

adrenaline bitartrate (0.125, 0.25, 0.5, 1, 2 μM) was incubated with rat heart mitochondria and the assays that express the biomarkers of oxidative stress namely, GSH (Fig. 1A), LPO (Fig. 1B) and PCO (Fig. 1C) were performed to verify the occurrence of oxidative stress in heart mitochondria in vitro. A significant decrease (92.74% decrease,*P < 0.001 vs Control group) in GSH content (Fig. 1A) and subsequent increase in both LPO (Fig. 1B) and PCO (Fig. 1C) levels was observed following adrenaline treatment in a dose dependent manner (72.41%, 91.04%, increase respectively, *P < 0.001 vs Control). From the following assays it was concluded that adrenaline at a dose of 0.5 μM exhibited maximum damage to the mitochondria and was thus chosen as the most effective dose for the study. No further damage was observed at the higher doses of adrenaline. Thus, 0.5 μM dose of adrenaline bitartrate was used throughout the study to induce oxidative stress, in vitro, and to investigate the protective effect of oleic acid on it.

In Fig. 3, rat heart mitochondria was incubated with 0.5 μM adrenaline alone and also along with six graded doses of oleic acid (0.0625, 0.125, 0.25, 0.5, 1 and 2 μM) to induce oxidative stress, in vitro, and to study the protection afforded by oleic acid against such oxidative insult. As compared to control, the GSH content (Fig. 3A) was found to be decreased (40.90% decrease,*P < 0.001 vs Control group) and the LPO level (Fig. 3B) and PCO content (Fig. 3C) were found to be increased (94.28%, 107.14%, increase respectively, *P < 0.001 vs Control) respectively in adrenaline bitartrate treated rat heart mitochondria (ADR 0.5). These parameters were found to be protected in a dose-dependent manner when the mitochondria was co-incubated with oleic acid with significant protection being observed at a dose of 0.25 μM of oleic acid (OA 0.25 + ADR 0.5) (22.64%, 34.31%, decrease respectively,**P < 0.001 vs. adrenaline- treated group).

Thus, it was observed that oleic acid afforded protection to adrenaline treated rat heart mitochondria at a minimum effective dose of 0.25 μM. Similarly, in Fig. 4, the assays that signify the biomarkers of oxidative stress, namely LPO (Fig. 4A), PCO (Fig. 4B) and GSH (Fig. 4C) were repeated using only the minimum effective dose of oleic acid (0.25 μM) in rat heart mitochondria along with a positive control group (OA 0.25 μM) to ascertain the protection observed in Fig. 3. Adrenaline induced significant increase in LPO and PCO levels (99.25%, 96.70% increase respectively,*P < 0.001 vs Control) and a significant decrease in GSH content (43.61% decrease,*P < 0.001 vs Control group) as compared to control were found to be significantly protected by oleic acid at a dose of 0.25 μM (OA0.25). However, positive control groups with only oleic acid did not show any change as compared to control groups indicating that oleic acid alone has no effect on any of the biomarkers analyzed.

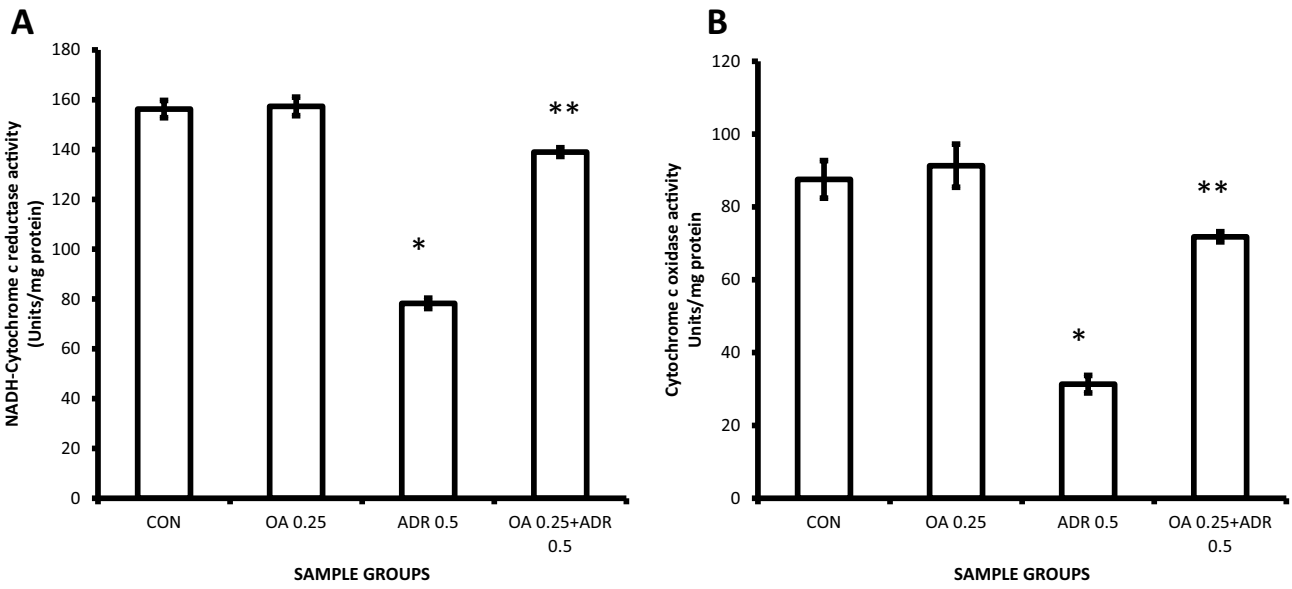

Fig. 7. Protective effect of oleic acid (0.25 μM) against adrenaline bitartrate induced alterations in rat heart mitochondria on (A) NADH-Cytochrome c oxidoreductase activity and (B) Cytochrome c oxidase activity. Values are expressed as means ± S.E. *P < 0.001 versus control, **P < 0.001 vs. adrenaline-treated group.

There were significant elevations in lipid peroxidation and protein carbonylation levels in both Cu-ascorbate (LPO 73.7% and PCO 3.28 fold vs. control) and Fe-ascorbate-H_2O_2 (LPO 69.7% and PCO 3.63 fold vs. control) treated cardiac mitochondria compared to control along with a significant decline in reduced glutathione (GSH) level (35% decreased in case of Cu-ascorbate and 31% in case of Fe-ascorbate-H_2O_2 system vs. control). In both the cases, in presence of 0.25 μM concentration of oleic acid, all of the parameters were protected from being altered. Thus, these observations clearly confirm the antioxidant role of oleic acid as it not only provides protection against adrenaline induced oxidative stress but also oxidative stress induced by other oxidants like Cu-ascorbate and Fe-ascorbate-H_2O_2.

3.3. Changes in the activities of some of the Krebs's cycle and mitochondrial respiratory chain enzymes of rat heart mitochondria, in vitro

Incubation of rat heart mitochondria with only adrenaline bitartrate (ADR 0.5) at a dose of 0.5 μM decreased the activities of mitochondrial enzymes such as PDH (Fig. 5A), SDH (Fig. 5C), α-KGDH (Fig. 5E), ICDH (Fig. 5G), Fumarase (Fig. 6A) and Aconitase (Fig. 6C) by 60.92%, 62.50%, 48.51%, 49.60%, 41.09% and 33.96% respectively (*P < 0.001 vs. Control group). However, a dose-dependent protection in the activities of these enzymes was observed following oleic acid treatment (109.30%, 137.86%, 77.11%, 81.69%, 39.26% and 34.28% increases **P < 0.001 vs. adrenaline- treated group respectively) when the rat heart mitochondria was co-incubated with oleic acid at a dose of 0.25 μM (OA 0.25 + ADR 0.5) which exhibited significant protection in the enzymatic activity of all the above enzymes. However, oleic acid alone did not show any effect on the activities of Krebs cycle enzymes as compared to control.

Fig. 5B depicts that treatment of cardiac mitochondria with adrenaline has decreased the V_{max} of pyruvate dehydrogenase (PDH) enzyme with significant increases in Km as compared to control mitochondria. However, when the mitochondria were co-incubated with oleic acid (OA) with adrenaline, V_{max} of this enzyme was protected from being altered. Treatment of cardiac mitochondria only with OA did not show any significant alteration in V_{max} of PDH.

Fig. 5D depicts that treatment of cardiac mitochondria with adrenaline has decreased the V_{max} of succinate dehydrogenase (SDH) enzyme with unaltered Km as compared to control mitochondria. However, when the mitochondria were co-incubated with oleic acid (OA) with adrenaline, V_{max} of this enzyme was protected from being altered. Treatment of cardiac mitochondria only with OA did not show any significant alteration in V_{max} of SDH.

Fig. 5F shows that a decline in V_{max} and Km were also observed in alpha ketoglutarate dehydrogenase (α-KGDH) in presence of adrenaline (compared to control), but these parameters of this enzyme were protected from being altered upon co-incubation with OA. Treatment of cardiac mitochondria only with OA showed an enhancement in V_{max} and decline in Km of this enzyme as compared to control.

Fig. 6B shows that a decline in V_{max} and Km were also observed in fumarase in presence of adrenaline (compared to control), but these parameters of this enzyme were protected from being altered upon co-incubation with OA. Treatment of cardiac mitochondria only with OA showed an enhancement in V_{max} and decline in Km in of this enzyme as compared to control.

Fig. 6D depicts that treatment of cardiac mitochondria with adrenaline has decreased the V_{max} of aconitase enzyme with unaltered Km as compared to control mitochondria. However, when the mitochondria were co-incubated with oleic acid (OA) with adrenaline, V_{max} of this enzyme was protected from being altered. Treatment of cardiac mitochondria only with OA did not show any significant alteration in V_{max} of PDH.

Fig. 7 shows that incubation of rat heart mitochondria with adrenaline bitartrate (ADR 0.5) significantly decreased the activities of both NADH cytochrome-c-oxido-reductase (Fig. 7A) and cytochrome-c-oxidase (Fig. 7B) by 68% and 60.20% decreased respectively, *P < 0.001vs. control group. However, a significant protection in the activity of both these enzymes was observed upon co-incubation with oleic acid at a dose of 0.25 μM [(OA 0.25 + ADR 0.5) by 63.80% and 55.37% increase **P < 0.001 vs. adrenaline- treated group respectively].

3.4. DAPI staining of rat heart mitochondria

The blue fluorescence spots formed by intercalation of DAPI with DNA were highly abundant in the slides of ADR (0.5) treated mitochondria in comparison to control, which was confirmed by evaluation of fluorescence intensity. But in the slides of mitochondria that was co-incubated with OA (0.25) in presence of ADR(0.5), the blue

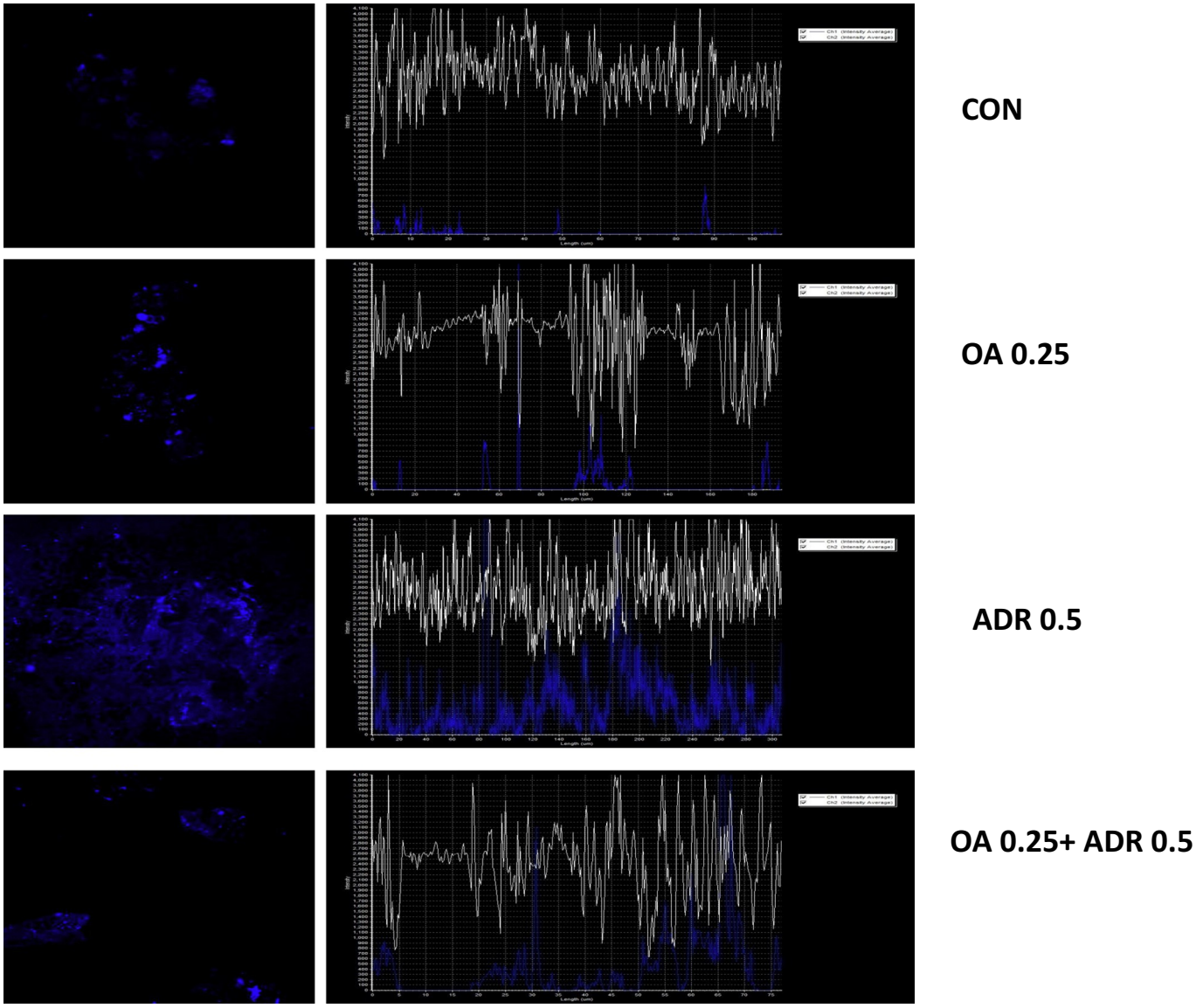

Fig. 8. Protective effect of oleic acid(0.25 μM) against adrenaline- induced alterations in damages of cardiac mitochondrial DNA, in vitro. CON = Control. OA 0.25 = rat heart mitochondria treated with0.25 μM only; ADR0.5: rat heart mitochondria treated with adrenaline at the dose of 0.5 μM; OA0.25 + ADR0.5: rat heart mitochondria co-treated with oleic acid at the dose of 0.25 μM and with adrenaline at the dose of 0.5 μM.

fluorescence spots were comparatively less abundant. In the slides of positive control, that is mitochondria incubated only with OA, the abundance of these fluorescence spots was almost identical with the slides of control mitochondria as evident from Figs. 8 and 1.

3.5. Scanning electron microscopy (SEM) of cardiac mitochondria

Fig. 9 depicts the SEM images of rat heart mitochondria of different incubated groups at 20000× magnification. Mitochondria incubated with adrenaline demonstrated a damaged outer membrane with a highly corrugated and irregular outer surface (as is evident from graph) suggesting loss of intactness of their architecture and intense damage to membrane (ADR 0.5) as compared to control mitochondria (CON). Here also, we find that the protection offered by oleic acid, as evidently seen in (OA 0.25+ ADR 0.5) group, indicates that oleic acid could help protect against adrenaline induced damage to vital membranes of rat heart mitochondria. Interestingly, the mitochondria incubated with only OA at a dose of 0.25 μM (OA 0.25) did not show any sign of morphological decline.

3.6. Isothermal titration calorimetric (ITC) profile of adrenaline and oleic acid

Fig. 10 shows the raw data from the titration between adrenaline bitartrate and oleic acid. Downward peaks indicate an endothermic reaction. The smaller peak from the first injection is due to diffusion of titrant into the titrate during equilibration. Injection peaks 2 to 19 decrease gradually in height from about −3.00 μcal/s to −1.0 μcal/s., indicating binding approaching saturation to adrenaline bi-tartrate with oleic acid. Each successive peak after the tenth is small and of similar magnitude suggesting no further binding. The integrated area under each peak is to determine the binding affinity (K), enthalpy (dH) and stoichiometry (n). The sum of the two models is shown as the dotted line in the lower plot.

The association constant (Ka) of 4.32E3 ± 1.27E3M^{-1} corresponds to a very tight binding interaction. The enthalpy change (ΔH) of binding is −2.274E5 cal/mol and stoichiometry of binding (n) is 0.6 mol of OA per mole of ADR. The effectiveness of ITC in analyzing the assembly of OA is demonstrated by determination of the enthalpy, binding constant and stoichiometry of the interaction. To understand

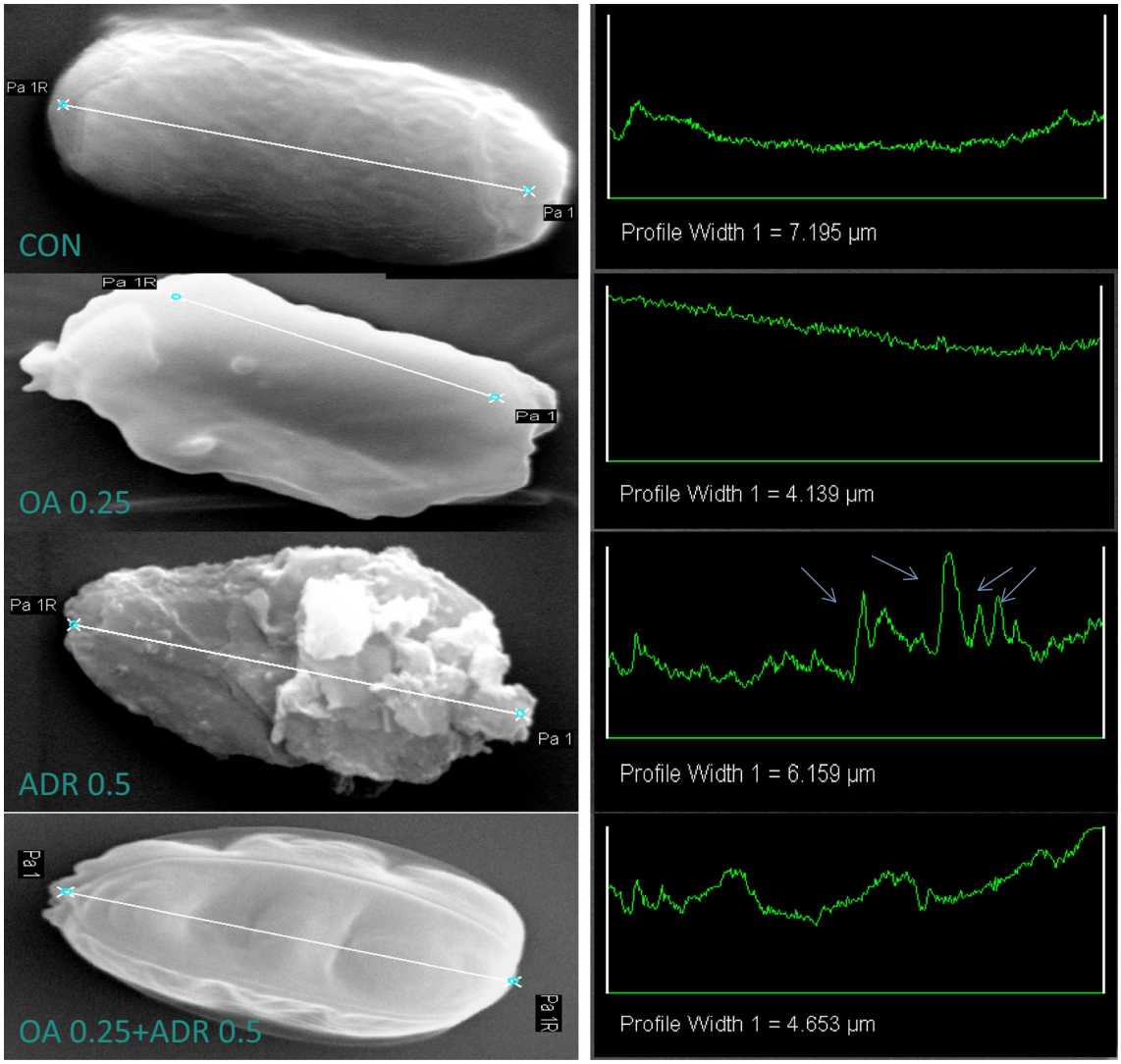

Fig. 9. Protective effect of oleic acid against adrenaline- induced alterations in intactness of cardiac mitochondria in vitro. CON = Control. rOA 0.25 = rat heart mitochondria treated with0.25 µM only; ADR0.5: rat heart mitochondria treated with adrenaline at the dose of 0.5 µM; OA0.25 + ADR0.5: rat heart mitochondria co-treated with oleic acid at the dose of 0.25 µM and with adrenaline at the dose of 0.5 µM. Magnification of images = 10 KX.

the thermodynamics of OA assembly, replacement of an OA with an ADR on the in colloidal suspension was measured with a TA Instruments ITC.

3.7. Mitochondrial membrane potential (DΨm)

In the Fig. 11, a low JC-1 ratio was found which means that there will be a low amount of the aggregated form of JC-1 in the mitochondria and this correlates with a high amount of ROS. The aggregated form of JC-1 molecules (red fluorescence) accumulated in functional mitochondria. On the other hand, the green fluorescence signal from JC-1 monomers is evenly distributed in the cytoplasm. The mitochondrial inner membrane potential was studied from the uptake of the cationic carbocyanine dye, JC-1 into the mitochondrial matrix. In the control mitochondria, the dye concentrated in the matrix and bright red fluorescence was observed. In ADR(0.5) incubated mitochondria, a shift from red to green fluorescence was observed which indicates damage to the inner mitochondrial membrane potential. This prevents the accumulation of the JC-1 dye in the mitochondrial matrix. The data reveals that, when the mitochondria were co-incubated with ADR(0.5) and the OA(0.25), red and green fluorescence was observed which indicates that the mitochondrial inner membrane integrity was maintained.

4. Discussion

Cardiovascular diseases or CVDs poses a major threat to the global population. It is well known that mitochondria are highly sensitive to oxidative stress and their function is severely impaired in cases of myocardial ischemia induced cardiac failure [33]. Although a number of antioxidants are available, which provide protection to mitochondria, naturally occurring specific ROS-scavenging molecules with limited or no side effects to mitochondria becomes highly essential. Several translational studies have identified the differential role between saturated and unsaturated fatty acids at cardiovascular level. However, the molecular mechanisms that support the protective role of oleic acid in cardiovascular cells are poorly known.

Elevation of circulating levels of adrenaline in blood plays a major role in generation of oxidative stress induced myocardial ischaemia. Our earlier studies have demonstrated oleic acid as an anti-oxidant molecule with cardioprotective properties as previously several studies have reported its benefits relating to cardiovascular diseases. Mono unsaturated fatty acids (MUFA) such as oleic acid has been linked to amelioration of different types of oxidative stress induced disorders [50,51]. Since oleic acid is the chief fat in vegetable oils such as olive, canola and sunflower and is also found in various kinds of nut oils, meat

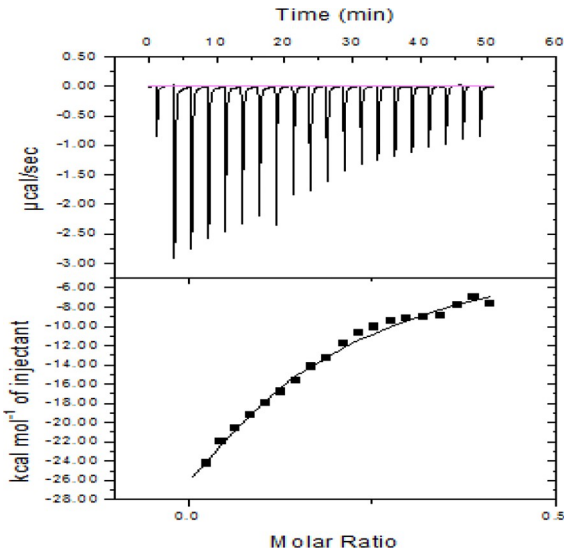

Fig. 10. A representative Isothermal Titration Calorimetric (ITC) data set of adrenaline and oleic acid. Raw titration data measured in µcal/s. Each peak corresponds to a single injection of oleic acid aqueous solution into the adrenaline bi-tartrate suspension. The amount of heat change per second (ΔH) is represented by the area under the curve (top curve) and the heat change in terms of kcal mol^{-1} of injectant against molar ratio is shown in bottom curve of each figure respectively. Values of ΔH, ΔS and no. of sites were expressed in terms of mean ± S.E.

and cheese, its consumption is said to aid in the recovery from oxidative stress induced damages to cardiomyocytes [52]. Incubation of rat heart mitochondria with adrenaline bitartrate in vitro can generate huge amount of oxidative stress as is evident from the significant increase in LPO, PCO and decrease in GSH level, the primary biomarkers of oxidative stress; indicating a possible ROS mediated damage of the rat cardiac myocytes. Usually, receptor-mediated responses exhibit saturability at one point. Our results demonstrated a concentration dependent effect of adrenaline on mitochondria. This indicates that the effect may be receptor-independent although possible involvement of receptor-mediated action may not be ruled out and needs further exploration. Co-incubation of oleic acid at six different doses (0.0625, 0.125, 0.25, 0.5, 1 and 2 µM) prevented all the above parameters from being altered in a dose dependent manner by obstructing the free radical generating steps of catecholamine metabolism or by scavenging the free radicals that are generated due to redox-active transition metals like copper or iron [6]. GSH, a major cellular antioxidant, known to scavenge free radicals and reactive oxygen species plays a very integral role in the repair of radical caused membrane damage. Thus, restoration of normal levels of GSH suggests immense antioxidant properties of oleic acid that not only protects the mitochondria from oxidative damage, it also boosts the intercellular defense system of the cell.

The cardiac cells contain a large number of mitochondria required for continuous aerobic respiration via oxidative phosphorylation [53]. Almost 90% of the energy requirement of the cell for proper functioning is supplied by the mitochondria. Interestingly, mitochondria are also considered the primary hub of reactive oxygen species (ROS) generation in mammalian cells [54]. Adrenaline is quite stable in acid solutions but gets oxidized with increasing pH. Excess of adrenaline can be auto-oxidized to form paramagnetic superoxide anion radical within the cell at pH 7.4 in the following manner:

(a) $RH_3^- + Me^n \rightarrow RH_3. + Me^{n-1}$
(b) $RH_3. + O_2 \rightarrow RH_2 + O_2^- + H^+$
(c) $RH_2 + O_2^- + H^+ \rightarrow RH. + H_2O_2$
(d) $RH. + O_2 \rightarrow R + O_2^- + H^+$

Here RH_3^- is adrenaline, R is adrenochrome and Me represents metal ion.

This superoxide anion is then converted to H_2O_2 by Mn-SOD in mitochondria, which in turn generate diamagnetic hydroxyl radical through interaction with Cu^+ or Fe^{2+} (the metal ions abundant in mitochondria) by Fenton reaction or with superoxide anion radical by Haber-Weiss reaction [55]. In our present study we have solubilized adrenaline bitartrate in 0.01 mM HCl and then applied to in vitro incubation medium at pH 7.4. As oxygen is abundant in cardiac mitochondria, the generation of superoxide anion and ·OH in the above mentioned manner is highly probable. Moreover, superoxide anion seems to catalyze the conversion of adrenaline to adrenochrome by acting as a propagating species in a cascade reaction during the univalent oxidation of the catecholamine [56]. Adrenaline has been reported to generate the most common forms of ROS which include hydrogen peroxide (H_2O_2), oxygen ions (O^{2-}), hydroxyl ions (·OH) and superoxide radicals ($\cdot O^{2-}$) [7]. Although moderate quantities of ROS induce cell apoptosis, but high amounts initiate cell necrosis.

Since mitochondria are the principal target of ROS mediated oxidative damage, inhibition of the activity of mitochondrial enzymes upon incubation with adrenaline is an important observation of the present study. Our current study reports that the activities of PDH and some of the Krebs cycle enzymes like α-KGDH and fumarase showed uncompetitive inhibition pattern whereas SDH and aconitase showed non-competitive inhibition. These Krebs cycle enzymes which are involved in energy metabolism and are also related to ATP production in mitochondria through oxidative phosphorylation, were found to be inhibited by adrenaline and this inhibition was found to be removed upon co-incubation with oleic acid. Direct effect of adrenaline on mitochondrial function has also been reported earlier [56].

Sufficient evidence exists which indicates that specific fatty acids, including MUFA, tend to reduce the risk of CVDs as they are less susceptible to oxidation as compared to PUFAs [57]. Dietary fat plays a very important role in the regulation of various components of the mitochondrial membrane. Diets rich in oleic acid tend to facilitate accumulation of oleic acid in mitochondrial membranes. Moreover, it has also been reported that oleic acid rich virgin olive oil is able to control mitochondrial oxidative stress than other n-6 polyunsaturated fatty acid (PUFAs) [58]. Removal of the adrenaline-induced inhibition of the activities of mitochondrial Krebs' cycle and respiratory chain enzymes suggests that adrenaline, through its free radical mediated action, interferes with the catalytic activities of the mitochondrial enzymes and binding of OA at a minimum effective dose of 0.25 mM is able to attenuate the damage incurred due to adrenaline.

Furthermore, from kinetic studies of the Krebs cycle enzymes under in vitro conditions it was revealed that in case of SDH and Aconitase both, treatment with adrenaline has changed its turnover number, which was confirmed by altered Vmax value of both of this enzymes, but did not disturb its active site directly, which was confirmed by unaltered Km value of both of this enzymes in presence and also absence of oleic acid. This resembled the noncompetitive mode of enzyme inhibition.

In case of another three Krebs cycle enzymes like α-KGDH, PDH and fumarase all of them were inhibited at their substrate bound condition, which was confirmed in presence of adrenaline by their alteration in both Km and Vmax. This resembled the uncompetitive mode of enzyme inhibition.

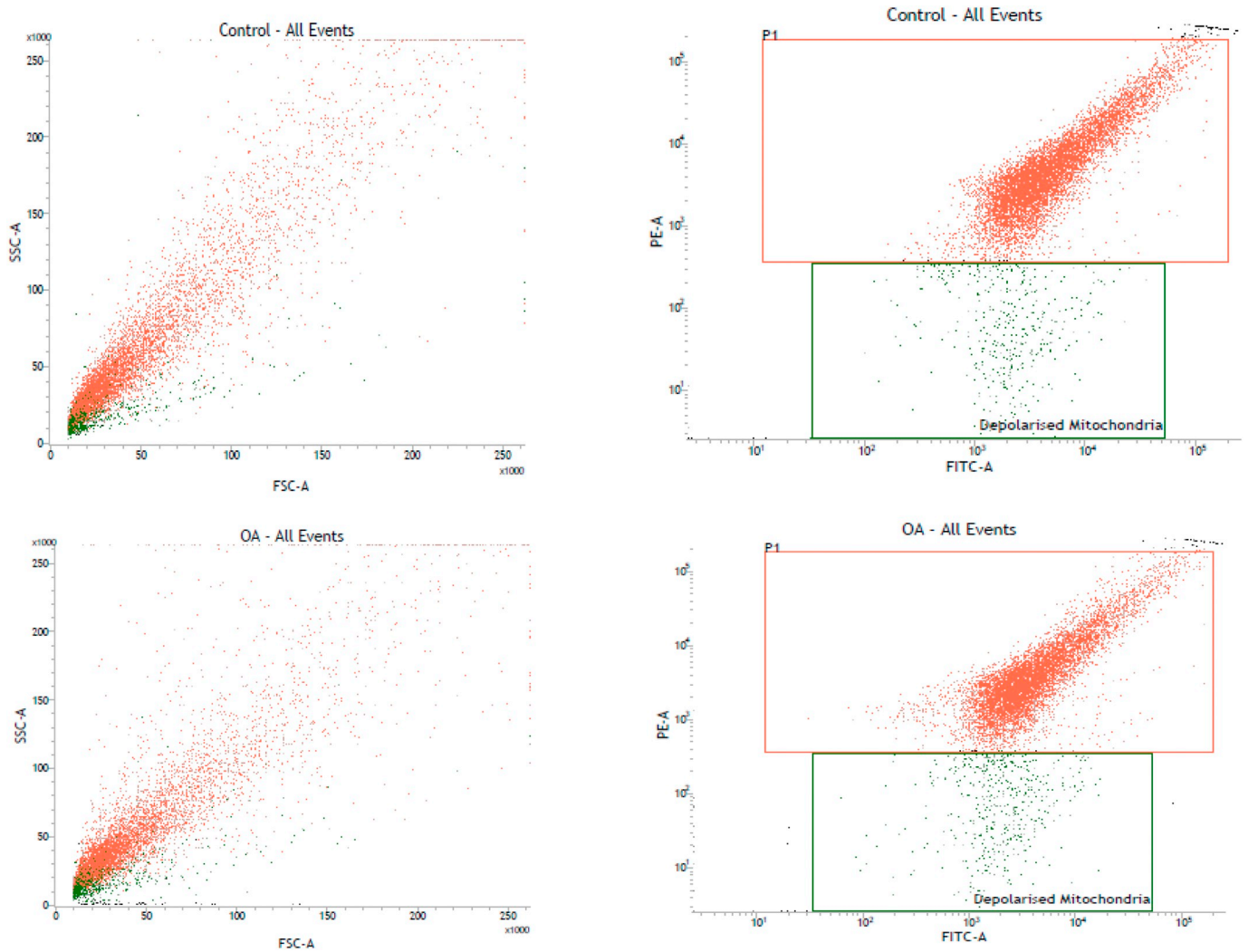

Fig. 11. Protective effect of oleic acid against adrenaline- induced alterations in depolarization events of cardiac mitochondria in vitro analyzed through FACS. CON = Control. OA 0.25 = rat heart mitochondria treated with 0.25 μM only; ADR0.5: rat heart mitochondria treated with adrenaline at the dose of 0.5 μM; OA0.25 + ADR0.5: rat heart mitochondria co-treated with oleic acid at the dose of 0.25 μM and with adrenaline at the dose of 0.5 μM.

As all of those pattern of inhibitions are reversible, so from these result it can be concluded that adrenaline interferes with the catalytic activities of the enzymes associated with Krebs cycle by not only generation of ROS, but also by binding with them and OA can also bind with those enzymes and attenuate the binding of adrenaline with them.

Scanning electron microscopic images of adrenaline incubated rat heart mitochondria indicates a highly corrugated and disintegrated outer membrane with loss of membrane integrity and intactness (as is evident from membrane roughness graph). Loss of structural integrity tends to deter the normal functions of the mitochondria along with intensive damage to key regulatory components and integral enzymes. Mitochondria are the primary ATP provider for fueling the metabolic processes that take place within the cell. It was found that upon co-incubation with oleic acid at a dose of 0.25 Mm, all the changes were found to be protected and the surface morphology returned to its normal configuration.

Presence of DNA in mitochondria has made it a semiautonomous organelle. Mitochondrial DNA of mammalian system also contains some genes that encode some proteins also responsible for regulation of physiological activity of mitochondria [59]. So oxidative damage induced by excess ADR (0.5) in the DNA can also alter mitochondrial physiology, that can be detected by intercalating of DAPI with AT rich sequence of DNA. Reactive oxygen species induced damage of DNA appears as smearing pattern in agarose gel and this breakdown is confirmed by DAPI staining, in which the appearance of the fluorescence spots is directly proportional to the breakdown of DNA as evident from Fig. 11. Co-incubation of mitochondria with OA (0.25) in presence of ADR (0.5) has inhibited this damage from occurring.

Membrane depolarization of cardiac mitochondria in adrenaline treated group occurs due to damages of integrity of both external and internal membrane. It may be due to leakage of mitochondrial membrane by excess adrenaline treatment [48]. Isothermal titration calorimetry allows simultaneous determination of binding constants (K_a), reaction stoichiometry, enthalpy (ΔH) and entropy (ΔS) during single experiments, providing a complete thermodynamic profile of investigated interaction [60]. ITC is becoming a method of choice for characterization of intermolecular interactions and recognizing reactions with exquisite sensitivity, since both low and high affinity interactions can be quickly and accurately characterized [61]. Hence, ITC is an attractive approach for the study of biomolecular interactions [62]. The isothermal titration calorimetric analysis of the interaction between antioxidant oleic acid and adrenaline bi-tartrate in a cell-free system has revealed new information regarding the antioxidant and free radical scavenging abilities of oleic acid. Highly exothermic single site interaction between oleic acid and adrenaline bi-tartrate suggests its high affinity towards the binding sites. This suggests that adrenaline binds with oleic acid effectively and much more strongly and with

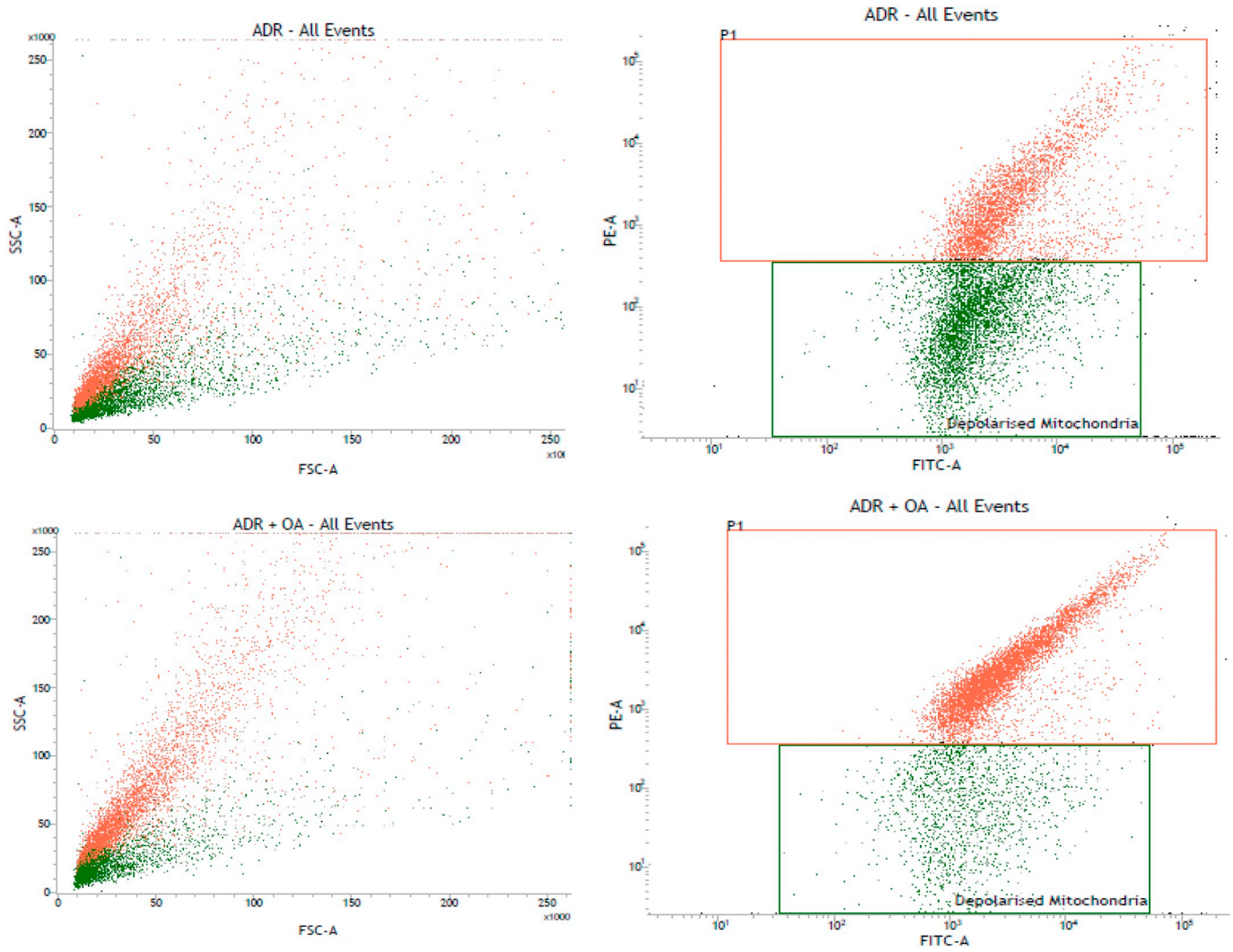

Fig. 11. (*continued*)

higher affinity as compared to β-adrenaline receptor. Almost all sites of adrenaline get saturated with oleic acid and hence gradual saturation is reached. Hence, it may be presumed that this strong binding of oleic acid with adrenaline prevents the oxidation of adrenaline to adrenochromes as well as generation of ROS thereby protecting the structural and consequently functional integrity of the cardiac mitochondria.

Over the past decades, the prevalence of cardiovascular disease (CVD) has increased worldwide and is also becoming a public health problem in India as well [63]. Thus, in order to optimize the dietary fat intake in order to achieve maximum health benefits, it is important to turn our attention to evidences that report that dietary monounsaturated fatty acids (MUFA) may have a beneficial health effect in relation to cardiovascular risk. Various Monounsaturated fatty acids are now known to not only play a role in the prevention of cardiovascular diseases via their antioxidant effects but they also influence the various factors that govern the development and progression of Cardiovascular Diseases (CVD). Previously, the underlying molecular mechanisms of the protective role of oleic acid in cardiovascular cells were poorly known. Hence, through the present study it can be concluded that oleic acid at a dose of 0.25 mM is able to provide adequate protection to rat heart mitochondria in vitro.

5. Conclusion

The beneficial effect of dietary fats such as oleic acid, which is natural constituent of nuts, fruits and olives, and is an essential component of recommended daily fat intake is well documented. Through the present study we have shown that oleic acid, besides acting as a potent antioxidant against adrenaline induced oxidative stress in rat heart mitochondria ameliorates adrenaline-bitartrate induced mitochondrial dysfunction by binding adrenaline strongly and effectively and with high affinity as evident from isothermal titration calorimetry study. This strong binding of oleic acid with adrenaline prevents the generation of ROS and consequently protects cardiac mitochondria from oxidative damage. This possibly helps in maintaining the structural and functional integrity of cardiac mitochondria. Thus, in the face of oxidative stress induced myocardial ischaemia and related cardiovascular disorders, diet derived oleic acid can be a welcome alternative and their health benefits are indisputable as per the research data available worldwide.

Conflict of interest

The authors declare that there are no conflicts of interest.

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