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ORIGINAL ARTICLE

Fucoxanthin Averts Isoprenaline Hydrochloride-Induced Myocardial Infarction in Rats

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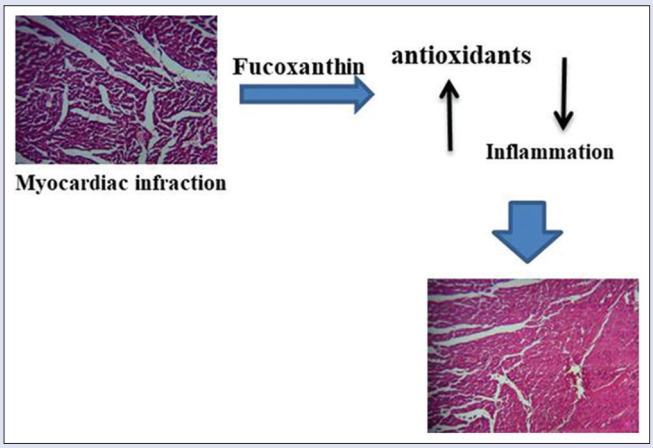
ABSTRACT

Background: In this current investigation, we aimed to assess the efficacy of natural antioxidant fucoxanthin against the myocardial infarction since it can be consumed as a regular diet. **Objective:** In this scientific investigation, we planned to investigate the curative effectual of fucoxanthin on isoprenaline hydrochloride provoked myocardial infarction on the experimental rats. **Materials and Methods:** Healthy Wistar albino rats were grouped into control, fucoxanthin alone, myocardial infarction induced, and myocardial infarction induced pretreated with fucoxanthin. The control rats were treated with a standard food diet, whereas fucoxanthin alone group rats were treated with 50 mg/kg/bwt of fucoxanthin along with standard diet. Myocardial infarction-induced groups were treated with 85 mg/kg/bwt of isoprenaline hydrochloride to induce myocardial infarction on the 29th and 30th days of the treatment period. Group IV rats were pretreated with 50 mg/kg/bwt of fucoxanthin from day 1 of experiment and on the 29th and 30th days of treatment period treated with 85 mg/kg/bwt of isoprenaline hydrochloride to induce myocardial infarction. **Results:** The fucoxanthin possessed effective cardioprotective activity in mycardiac infarction-induced rats. Fucoxanthin treatment reduced the statuses of cardiac troponin T and cardiac troponin I on myocardial infarction provoked rats; also, it exhibited the reduced Thiobarbituric acid reactive substances (TBARS) level in the rats. The antioxidant status such as superoxide dismutase, catalase, and glutathione peroxidase was extensively elevated in the myocardial infarction-induced fucoxanthin pretreated rats. Myocardial infarction-induced fucoxanthin pretreated rats shows decreased statuses of Na^+/K^+ ATPase and increase on the Na^+/K^+ ATPase level. **Conclusion:** Our results confirmed that fucoxanthin is a potent cardioprotective drug which increases the antioxidant levels and decreases the oxidative stress and inflammation during myocardial infarction.

Key words: Antioxidant, fucoxanthin, *in vivo,* inflammatory proteins, isoprenaline, myocardial infarction

SUMMARY

- Increased oxidative stress and decreased antioxidant status are the key markers for the induction of myocardial infarction
- Fucoxanthin is a potent cardioprotective drug which increases the antioxidant levels and decreases the oxidative stress and inflammation during myocardial infarction.



Abbreviations used: NF-κB: Nuclear factor kappa B; TNF-α: Tumor necrosis factor-alpha; IL-6: Interleukin-6; WHO: World Health Organization; ELISA: Enzyme-linked immunosorbent assay; TBARS: Thiobarbituric reactive substance; LOOHs: Lipid hydroperoxides; MDA: Malondialdehyde.

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INTRODUCTION

Myocardial infarction is one of the threatening crisis of both developed and developing countries.[1] The World Health Organization had reported that the cardiovascular and stroke will be the major cause for global morbidity and mortality rate by the year 2020.[2] As compared to developed countries, developing countries show increased mortality rates due to lifestyle-related non-communicable diseases. Indians are more prone to myocardial infarction due to their genetic structure which is provoked due to the sedentary lifestyle. In 2011, the incidence of cardiovascular-related mortality had increased 31%.[3]

The blockade in the coronary arteries due to plaque formed with low-density lipoprotein cholesterol leads to necrosis of myocardial cells, thereby induces myocardial infarction.[4] Hypertension and cardiomyopathy induce fibrosis in myocytes resulting in ventricular arrhythmia, heart attack, and eventually death.[5] Sedentary lifestyle, lack of exercise, obesity, stress, dyslipidemia, diabetes mellitus, and hypertension are the major risk factors of myocardial infarction. The most prescribed drugs of myocardial infarction include antiplatelet, antithrombotic, and steroidal anti-inflammatory drugs.[6] All these drugs possess severe side effects; hence, it is a need of today to formulate a potent phytomedicine with nil side effects.

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Marine Algae possess an immense number of phytochemicals that are renowned for their medicinal values. Fucoxanthin is one such phytochemical belongs to the family of carotenoid present in algae such as *Phaeodactylum tricornutum* and *Undaria pinnatifida*. More than 10% of carotenoids in the marine environment are fucoxanthin; they impart orange color pigment to the algae.[7] Fucoxanthin possesses various properties such as anti-aging, anti-inflammatory, antimutagenic, anticancer, antiobesity, hepatoprotective, and cardioprotective properties.[8-12] It also effectively suppresses the differentiation of preadipocytes to adipocytes, thereby decrease hyperlipidemia.[13] Therefore, in the present study, we evaluated the efficacy of marine carotenoid against the myocardial infarction-induced changes in rats.

There are several *in vivo* models to induce myocardial infarction; isoprenaline-induced myocardial infarction is the paramount model to study the biochemical changes induced during the myocardial infarction. Increased oxidative stress and decreased antioxidant status are the key markers for the induction of myocardial infarction. Isoprenaline was a synthetic catecholamine and an agonist of beta-adrenergic receptors which increases the oxidative stress on the myocardium, thereby resulting in the cell death of cardiac muscle as well as myocardial membrane integrity loss.[14] Hence, on this current exploration, we induced myocardial infarction in young healthy Wistar rats with a renowned myocardial infarction model using isoprenaline. The rats were then assessed to check the efficacy of marine carotenoid fucoxanthin against biochemical, molecular, and histopathological changes occurring during the myocardial infarction.

MATERIALS AND METHODS

Chemicals

Fucoxanthin (F6932), isoprenaline hydrochloride and whole additional fine requirements of diagnostic range were bought from Sigma Aldrich, USA. The enzyme-linked immunosorbent assay (ELISA) kits for creatine kinase MB isoenzyme (CK-MB-MBS705376), cardiac troponin I (cTnI-MBS 705158), and cardiac troponin T (cTnT-MBS 163975) were procured from MyBioSource, USA, and lipid hydroperoxide (LOOH) assay (NWK-LHP01) was obtained from Northwest Life Science Specialties. Antibodies were purchased from SantaCruz Biotech, USA.

Animals

Young and healthy male Wistar Albino rats (120–160 g weight) are selected for present work. Rats were bought from the institutional animal house facility, and it was adapted to 1 week in a laboratory condition with 12 h dark and light cycles, 60% humidity and at a temperature of 25°C ± 3°C. The rats were housed in sterile plastic cages and fed *ad libitum* with sterile standard rat food pellets, reverse osmosis water. All the procedures were followed as stated by the rules of Institutional Animal Ethical Committee and with human ethics (Animal ethical approval number: 201910-17).

Experimental protocol

Subsequent to the adaptation period, rats were grouped into four, as of every group having 6 rats. Group I rats were considered controls and were treated with standard food pellets for 30 days, whereas the Group II drug control rats were supplemented orally with 50 mg/kg/bwt of fucoxanthin along with standard diet. Group III rats are myocardial infarction-induced rats; these rats were treated with the standard diet until the 28th day and on last 2 days of treatment period (29th and 30th day), and the rats subcutaneously challenged with 85 mg/kg/bwt of isoprenaline hydrochloride to induce myocardial infarction. Group IV rats drug pretreated, these rats supplemented with 50 mg/kg/bwt of fucoxanthin along with standard diet till the 28th day and on the last 2 days of treatment period, myocardial infarction was induced as similar as Group III rats. Twenty-four hours after the experimental period and the second dosage of isoprenaline hydrochloride injection, rats were killed. Whole blood was gathered in ethylenediaminetetraacetic acid painted tubes from the jugular veins, and then serum and plasma were prepared. Heart tissues were excised from the rats and cleaned through chilled saline buffer and dehydrated using the tissue paper. The heart tissue was weighed and hoarded instantly at −80°C for additional studies.

Biochemical examinations

Myocardial enzymes evaluation

Creatine kinase

Serum CK activity was assessed using the colorimetric protocol of Wagner *et al.*[15] Creatine phosphate and adenosine phosphate formed due to the catalytic activity of creatine enzyme were measured colorimetrically and expressed as IU/L.

Creatine kinase MB isoenzyme

CK MB isoenzyme action was examined using a quantitative sandwich ELSIA kit brought from MyBiosource, USA. The protocol was adopted as stated by the manufacturer's instruction manual; serum samples of control and treated rats were pipette into the CK-MB antibody coated plates. The CK-MB isoenzyme present in the serum bounds to the CK-MB antibody and the unbound substances were removed. Biotin conjugated CK-MB antibody was added to the microtiter wells to quantify the CK-MB antigen-antibody complex Avidin-conjugated HRP enzyme was added to develop the color which is directly proportional to the present CK-MB antigen-antibody complex amount. Color intensity was studied at 450 nm through ELISA microplate reader.

Cardiac trophins

Cardiac isoform of Troponin T and I (cTnT and cTnI) enzymes were assessed using a double-sandwich ELISA assay kit procured from MyBiosource, USA. The sample serum was amalgamated to the microtiter wells prepainted with the rat cTnT and CTnI monoclonal antibodies, respectively. After the incubation period, the plates were rinsed to remove the unbound substance and then treated with biotin-coated rat cTnT and CTnI polyclonal antibodies, respectively. Further, the wells were treated with avidin-conjugated horseradish peroxidase enzyme, and the 3,3',5,5' tetramethyl-benzidine were added to exhibit color change by the antigen-antibody complex. The reaction was terminated using the stop solution, and the color intensity was measured at 450 nm using enzyme-linked immunosorbent assay microtire plate reader.

Oxidative stress estimation

Thiobarbituric acid reactive substance assay

The levels of lipid peroxidation were estimated using the TBARS assay using the protocol of Yagi (1978).[16] Oxidative stress statuses induced by control and treated rats were estimated by assessing the malondialdehyde levels of the present in the plasma rats. The secondary product of lipid peroxidation malondialdehye responds to the thiobarbituric acid reactive substance (TBARS) to produce pink chromogen which estimated at 532 nm using the microplate reader.

Lipid hydroperoxide assay

The unstable hydroperoxides generated as the result of lipid peroxidation were assessed using the LOOH assay kit procured from Northwest Life Science Specialties. The test was performed as said by the kit's guideline, the LOOH present in the sample reacts with the ferrous ion to form ferric

ion, and it subsequently combines with xylenol to form a chromogen. The color strength formed was studied at 560 nm through microtiter plate reader.

Antioxidant estimation

Antioxidant enzyme activities superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reduced glutathione (GSH) were measured in both plasma and heart tissue homogenates of control and experimental rats. Heart homogenate was prepared using phosphate-buffered saline, pH 7.4.

SOD (EC.1.15.1.1) function to block auto-oxidation of pyrogallol was studied through Marklund and Marklund[17] technique, and the value was portrayed as a dosage of the enzyme needed to restrain the chromogen accreted by 50% in 1 min beneath regular circumstance. CAT function (EC. 1.11.16) was examined through Sinha[18] procedure, and the values were expressed as μmole of hydrogen peroxide decomposed/min. GPx (EC.1.11.1.9) action was measured using the protocol of Rotruck *et al.*,[19] and the values are portrayed as μmole of GSH utilized/min. Abridged GSH status was investigated through Ellman and Fiches method,[20] in accordance with color strength formed through sulfhydryl groups existed in diminished GSH unites with 5, 5' dithio 2-nitro benzoic acid. Values were depicted in microgram of abridged GSH formed/min.

Assessment of sodium–potassium ATPase

The levels of Na^+/K^+ ATPase were measured spectroscopically using the protocol of Daemen *et al.*,[21] and the level was portrayed as micromoles of Pi liberated/mg/protein. In the presence of Na^+/K^+ ions, the ATPase splits the ATP molecules to adenosine diphosphate (ADP) which releases phosphate group which further responds to ammonium molybdate to phosphomolybdate. Phosphomolybdate reduces the ANSA reagent to form blue color which is measured at 620 nm.

Immunoblotting

Cardiac tissue homogenates of normal and tested rats were arranged by radioimmuno test buffer. The levels of whole protein were examined using the method of Lowry *et al.*,[22] and 40 μg of protein was subjected to electrophoresis on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The electrophoresed gel was transferred to Polyvinylidene difluoride (PVDF) membrane, and the unbound pores on the membrane were masked with 5% skimmed milk for 2 h. The membrane was then incubated overnight with primary rat monoclonal antibodies tumor necrosis factor-alpha (TNF-α) (sc-52746), interleukin-6 (IL6) (sc-57315), and nuclear factor kappa B (NFκB) (sc-166588). After incubation, the membranes were rinsed with tris buffer and then incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h. The membranes were then washed tris buffer and then subjected to chemdoc analysis using enzyme chemiluminiscence kit (Millipore, USA). β actin (sc-517582) antibody was used as an internal control.

Histopathological investigation

Excised heart tissue from normal and tested animals was examined by the histopathological study. Heart tissue was processed through 10% formalin, and then tissues were dehydrated with the aid of xylene and ethanol. Then, tissues were entrenched with paraffin and sliced to thin slivers at 5μ thickness. Then, sections were stained with hematoxylin and eosin. Finally, slides were examined beneath the optical microscope, and the images were investigated for histological alterations.

Statistical study

Data portrayed as mean ± standard deviation of six rats of all groups. Whole tests were conducted in triplicates, and the results were evaluated statistically through one-way ANOVA subsequent to Diploma in Medical Radiotherapy study through GraphPad Prism software (GraphPad Software Inc., San Diego, CA, USA). $P < 0.05$ was regarded as statistically relevant.

RESULTS

Effect of antioxidant fucoxanthin on heart weight

Momentous elevation of the weight of the heart was noted in myocardial infarction-induced rats while comparing it to normal and fucoxanthin alone treated [Figure 1]. Even though comparing it to control, significant elevation in heart weight was observed in fucoxanthin pretreated myocardial infarction-induced rats, it is significantly decreased compared to myocardial infarction alone induced rats.

Effect of antioxidant fucoxanthin on creatine kinase and cardiotropins

CK, an enzyme expressed by various organs and specifically CK-MB, is expressed by the heart muscle. During heart injury, the rapid increase in this enzyme was observed in the present study compared to myocardial infarction-induced rat expressed 208.65 ± 15.23 and 135.36 ± 06.57 IU/L of CK and CK-MB, respectively. Whereas, significantly decreased levels of CK (158.36.17 ± 08.67) and CK-MB (97.17 ± 3.56) were measured in myocardial infarction-induced rats pretreated with fucoxanthin. The levels of cardiotroponin in both the isoforms cTnT and cTnI were drastically increased in myocardial infarction-induced rats cTnT (1.18 ± 0.09) and cTnI (0.60 ± 0.04), whereas significantly reduced levels of cTnT (0.68 ± 0.04) and cTnI (0.36 ± 0.01) were observed in myocardial infarction-induced rats pretreated with fucoxanthin [Table 1].

Effect of antioxidant fucoxanthin on oxidative stress markers

Effectual of antioxidant fucoxanthin on oxidative stress markers of both normal and tested groups is depicted in Table 2. Increased statuses

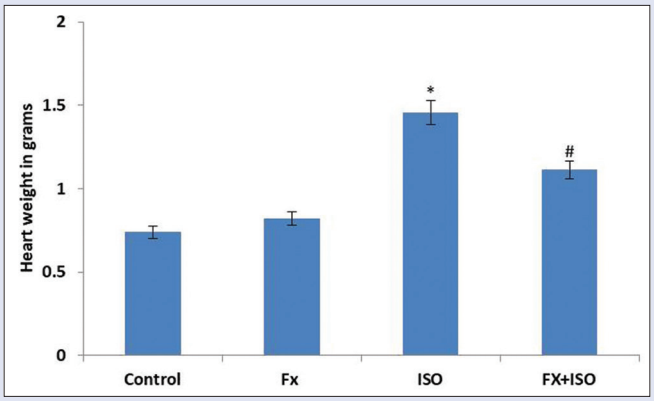

Figure 1: Effect of antioxidant fucoxanthin on the control and experimental rats cardiac weight. The values illustrated in the histogram are the mean ± standard deviation of six rats in each group. Values not sharing a common superscript differ significantly at $P \leq 0.05$ (Diploma in Medical Radiotherapy) is considered as statistically significant. * and # - compared with control and ISO respectively

of oxidative stress markers TBARS (0.35 ± 0.03, 1.68 ± 0.14) and LOOH (19.57 ± 1.05, 110.62 ± 8.24) were observed in plasma and the cardiac tissue of myocardial infarction-induced rats, respectively, whereas, it is significantly reduced in the rats myocardial infarction-induced rats pretreated with fucoxanthin TBARS (0.22 ± 0.02 and 7.89 ± 0.03) and LOOH (0.68 ± 0.04 and 62.86 ± 3.28).

Effect of antioxidant fucoxanthin on cardiac antioxidants

Compared to the control, the both circulatory levels and the cardiac tissue levels of antioxidants were drastically decreased SOD (5.08 ± 0.02; 4.26 ± 0.18), CAT (131.89 ± 11.19; 28.46 ± 1.09), GPx (09.28 ± 0.40; 4.53 ± 0.13), and GSH (20.39 ± 1.61; 3.08 ± 0.15) in the myocardial infarction-induced rats, respectively. Whereas the antioxidant levels were significantly increased SOD (7.08 ± 0.32; 6.38 ± 0.26), CAT (170.32 ± 07.69; 40.59 ± 1.93), GPx (12.05 ± 0.95; 6.19 ± 0.26), and GSH (33.68 ± 0.95; 4.98 ± 0.29) in the myocardial infarction-induced fucoxanthin pretreated rats. None noteworthy differences were noted among the control and fucoxanthin alone supplemented rats [Tables 3 and 4].

Effect of antioxidant fucoxanthin on Na^+/K^+ ATPase

Figure 2 illustrates the statuses of Na^+/K^+ ATPase measured in the cardiac tissue of control and myocardial infarction-induced rats. Even though compared to control rats, Group III myocardial infarction-induced rats and Group IV myocardial infarction-induced fucoxanthin pretreated rats show

Table 1: Cardioprotective effect of antioxidant fucoxanthin on control, experimental rat's serum cardiac enzymes

Groups	CK (IU/L)	CK-MB (IU/L)	cTnT (ng/mL)	cTnI (ng/mL)
Group I	146.85±09.27[a]	90.58±4.20[a]	0.54±0.02[a]	0.36±0.02[a]
FX control (50 mg/kg b.wt.)	142.20±07.54[a]	91.81±3.39[a]	0.56±0.04[a]	0.34±0.01[a]
ISO (85 mg/kg b.wt.)	208.65±15.23[b]	135.36±06.57[b]	1.18±0.09[b]	0.60±0.04[b]
FX (50 mg/kg b.wt.) + ISO (85 mg/kg b.wt.)	158.36.17±08.67[a]	97.17±3.56[a]	0.68±0.04[a]	0.36±0.01[a]

The values of creatine kinase, CK-MB, cardiotroponins isoforms cTnT and cTnI were illustrated in Table 1. The values depicted in table are the mean±SD of six rats in each group. Values not sharing a common superscript differ significantly at $P \leq 0.05$ (DMRT) is considered as statistically significant. CK-MB: Creatine kinase MB; SD: Standard deviation; cTnT: Cardiac troponin T; cTnI: Cardiac troponin I; DMRT: Diploma in Medical Radiotherapy

Table 2: Effect of antioxidant fucoxanthin on oxidative stress markers of control and experimental rats

Groups	Control	FX control (50 mg/kg b.wt.)	ISO (85 mg/kg b.wt.)	FX (50 mg/kg b.wt.) + ISO (85 mg/kg b.wt.)
Plasma (mmol/dL)				
TBARS	0.18±0.02[a]	0.17±0.01[a]	0.35±0.03[b]	0.22±0.02[a]
LOOH	7.25±0.50[a]	7.35±0.46[a]	19.57±1.05[b]	7.89±0.03[a]
Heart (mmol/100 g wet tissue)				
TBARS	0.60±0.04[a]	0.62±0.05[a]	1.68±0.14[b]	0.68±0.04[a]
LOOH	58.18±2.71[a]	56.98±3.58[a]	110.62±8.24[b]	62.86±3.28[a]

The levels of oxidative stress markers TBARS and LOOH measured in both plasma and cardiac tissue of control, experimental rats were shown in Table 2. The values depicted in table are the mean±SD of six rats in each group. Values not sharing a common superscript differ significantly at $P \leq 0.05$ (DMRT) and is considered as statistically significant. TBARS: Thiobarbituric acid reactive substance; LOOH: Lipid hydroperoxides; SD: Standard deviation; DMRT: Diploma in Medical Radiotherapy

Table 3: Effect of antioxidant fucoxanthin on control, experimental rats circulatory antioxidants levels of control and experimental rats

Groups	SOD (U*/mg of Hb)	Catalase (U**/mg of Hb)	GPx (U@/mg of Hb)	GSH (mg/dL)
Control	7.35±0.20[a]	178.26±6.59[a]	12.95±0.89[a]	39.24±1.22[a]
FX control (50 mg/kg b.wt.)	7.30±0.28[a]	180.67±11.69[a]	12.79±0.79[a]	37.95±1.05[a]
ISO (85 mg/kg b.wt.)	5.08±0.02[b]	131.89±11.19[b]	09.28±0.40[b]	20.39±1.61[b]
FX (50 mg/kg b.wt.) + ISO (85 mg/kg b.wt.)	7.08±0.32[a]	170.32±07.69[a]	12.05±0.95[a]	33.68±0.95[a]

The levels of circulatory antioxidants SOD, CAT, GPx, and GSH measured in the plasma of control and experimental rats are shown in Table 3. The values depicted in the table are the mean±SD of six rats in each group. Values not sharing a common superscript differ significantly at $P \leq 0.05$ (DMRT) is considered as statistically significant. The values of SOD, CAT, GPx, and GSH were expressed as U* Enzyme concentration required to inhibit the chromogen produced by 50% in 1 min under standard condition. The values of U** µmole of hydrogen peroxide decomposed/min, U@ µmole of GSH utilized/min, respectively. SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; GSH: Reduced glutathione; SD: Standard deviation; DMRT: Diploma in Medical Radiotherapy

Table 4: Effect of antioxidant fucoxanthin on control, experimental rats' cardiac antioxidants of the control and experimental rats

Groups	SOD (U*/mg protein)	Catalase (U**/mg protein)	GPx (U@/mg protein)	GSH (µg/mg protein)
Control	6.98±0.48[a]	43.17±2.17[a]	6.61±0.31[a]	5.04±0.04[a]
FX control (50 mg/kg b.wt.)	6.95±0.52[a]	44.50±2.07[a]	6.58±0.34[a]	5.10±0.12[a]
ISO (85 mg/kg b.wt.)	4.26±0.18[b]	28.46±1.09[b]	4.53±0.13[b]	3.08±0.15[b]
FX (50 mg/kg b.wt.) + ISO (85 mg/kg b.wt.)	6.38±0.26[a]	40.59±1.93[a]	6.19±0.26[a]	4.98±0.29[a]

The levels of antioxidants SOD, CAT, GPx, and GSH measured in the cardiac tissue of control and experimental rats are shown in Table 4. The values depicted in the table are the mean±SD of six rats in each group. Values not sharing a common superscript differ significantly at $P \leq 0.05$ (DMRT) is considered as statistically significant. The values of SOD, CAT, GPx, and GSH were expressed as U* Enzyme concentration required to inhibit the chromogen produced by 50% in 1 min under standard condition. The values of U** µmole of hydrogen peroxide decomposed/min, U@ µmole of GSH utilized/min, respectively. SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; GSH: Reduced glutathione; SD: Standard deviation; DMRT: Diploma in Medical Radiotherapy

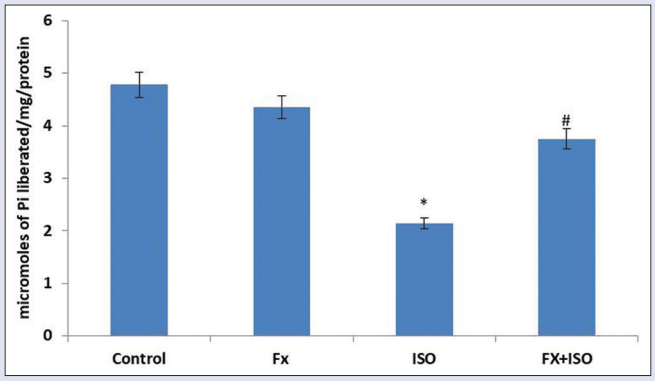

Figure 2: Effect of antioxidant fucoxanthin on control, experimental rats Na^+/K^+ ATPase. The values illustrated in the histogram are the mean ± standard deviation of six rats in each group. Values not sharing a common superscript differ significantly at $P \leq 0.05$ (Diploma in Medical Radiotherapy) is considered as statistically significant. * and # compared with control and ISO respectively

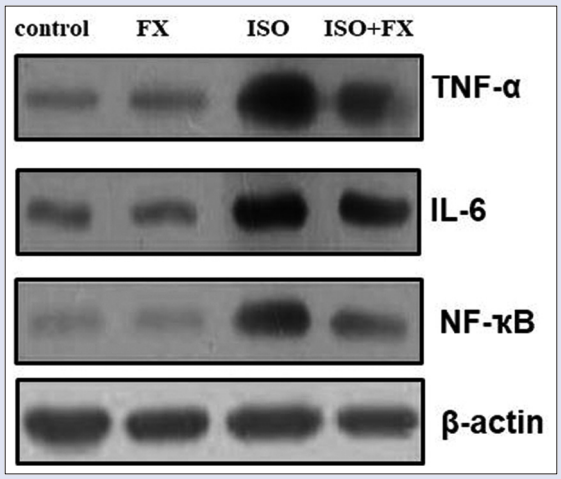

Figure 3: Cardioprotective effect of antioxidant fucoxanthin on inflammatory cytokines protein expression in cardiac tissue of control and experimental rats. Forty microgram of total proteins from control and experimental rats' cardiac tissue homogenate were subjected to electrophoresis and immunoblotting analysis with specific inflammatory cytokine proteins tumor necrosis factor-alpha, interleukin-6, and nuclear factor-kappa B. Values not sharing a common superscript differ significantly at $P \leq 0.05$ (Diploma in Medical Radiotherapy) is considered as statistically significant

decreased levels of Na^+/K^+ ATPase; and considerable increase in the Na^+/K^+ ATPase level was noted in Group IV rats. None noteworthy variations were noted among the control and fucoxanthin only supplemented rats.

Effect of antioxidant fucoxanthin on inflammatory cytokines

The immunoblotting results of inflammatory cytokines assessed in control and myocardial infarction-induced rats are represented in Figure 3. Compared to control cardiac tissue, all the three inflammatory cytokines TNF-α, IL-6, and NF-κB were increased in myocardial infarction provoked rats. Whereas the levels were significantly decreased Group IV myocardial infarction-induced fucoxanthin pretreated rats. Both the control and antioxidant fucoxanthin treated rats show significant differences.

Effect of antioxidant fucoxanthin on histoarchitecture of the cardiac muscle

The histopathological changes induced by isoprenaline treated and the control rats are illustrated in Figure 4. Normal endocardium, pericardium, and myocardial cells without any infiltration of inflammatory cells were observed in control and fucoxanthin alone treated rats. Estranged cardiac fibers, infracted edemal zone, and necrotic myocardial cells with an increased number of inflammatory cells were seen in the myocardial infarction-induced rats. Compared to myocardial infarction-induced rats, the Group IV myocardial infarction induced rats pretreated with fucoxanthin depicts decreased the infiltration of inflammatory, necrotic myocardial cells, and the reduced myocardial edema.

DISCUSSION

Myocardial infarction or heart attack is an impulsive block occurring on the arteries of the heart, leading to the necrosis of cardiomyocytes. Various risk factors are associated with myocardial infarction; the main causes are hyperlipidemia which along with the macrophages forms the atherosclerotic plaque.[23,24] Isoprenaline-induced myocardial infarction model is the standard model used to assess the efficacy of cardioprotective drugs since it aptly mimics the condition of myocardial infarction animals.[25]

In the present study, the myocardial infarction-induced rats showed increased heart weight compared to the control, whereas the fucoxanthin pretreated rats showed decreased heart weight compared to myocardial infarction-induced rats. The elevation in the heart mass of myocardial infarction rats is as a result of the hypertrophy, inflammation of cardiomyocytes induced by the isoprenaline, whereas the fucoxanthin pretreatment had scavenged the free radicals generated by the isoprenaline, thereby preventing the hypertrophy, edema of cardiomyocytes. Zaafan *et al.*[26] reported that phloroglucinol inhibits the cardiac hypertrophy induced by the isoprenaline in the rat model which correlates with our present findings.

CK enzymes, especially CK-MB, play a vital role in the conversion of ATP to ADP during the transportation of high-energy phosphate from the mitochondria to cardiac myofibrils.[27] The estimation of CK-MB measured within 24–36 h of cardiac arrest shows 95% sensitivity, and it is a more specific biomarker of myocardial infarction.[28] Cardiac troponins isoform (both cTnT and cTnI) are the sensitive biomarkers of myocardial infarction.[29] In the current drastic increase in the levels, cardiac enzymes were observed in myocardial infarction-induced rats, whereas it is significantly decreased in fucoxanthin pretreated rats. This proves that fucoxanthin is a potent cardio protectant that inhibited the cardiomyocytes necrosis induced by isoprenaline.

Oxidative stress takes a crucial function on the atherogenesis mechanism since it induces endothelial dysfunction there leading to the formation of atherosclerotic plaque.[30] Isoprenaline induces myocardial infarction through increasing the cardiomyocytes oxygen demand and by the overload of calcium in myocytes. The hydroxyl group present in catecholamines isoprenaline oxidized to form quinones and adrenochromes, respectively, thereby inducing cardiomyocytes necrosis. Therefore, assessing the levels of oxidative stress biomarkers in the fucoxanthin pretreated myocardial infarction-induced rats depicts the efficacy of fucoxanthin to scavenge the oxidative stress induced by isoprenaline. Thiobarbituric Reactive Substances (TBARS) and LOOH are the sensitive biomarkers to be estimated during the oxidative stress condition. Free radicals generated during the oxidative stress condition target the lipid molecules, leading to lipid peroxidation in turn synthesis of malondialdehyde. The levels of malondialdehye usually measured as the levels of TBARS.[31] Another most common product of lipid

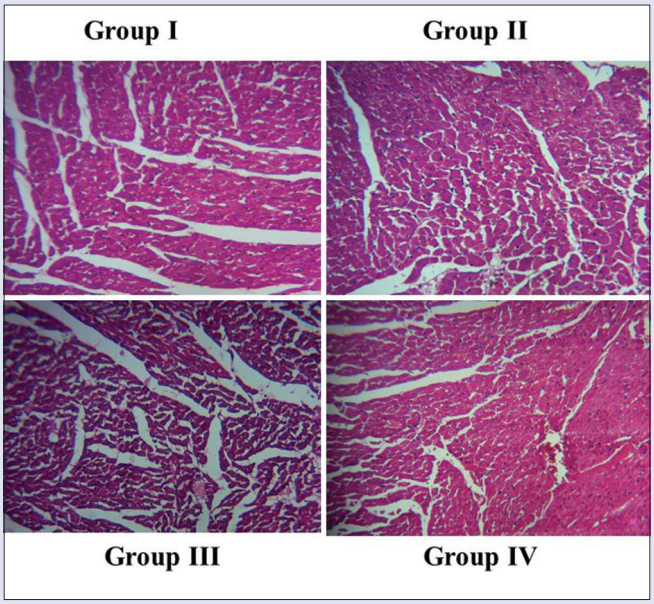

Figure 4: Cardioprotective effect of antioxidant fucoxanthin on cardiac muscle histoarchitecture of control and experimental rats. The control experimental rats' cardiac muscle were processed for histological analysis and sectioned into slices of 5μ thickness. The sectioned slides were stained with hematoxylin and eosin stains. The stained slides were viewed under the light microscope and photographed. The experiments were performed in triplicates

peroxidation is LOOHs which are generated as a result of peroxidation of polyunsaturated fatty acids. LOOH are unstable decomposes to form reactive epoxy-allylicperoxyl radicals[32] which are reported to be increased in various studies associated oxidative damage.[33] In our study, also momentous elevation on the statuses of both circulatory and cardiac tissue TBARS, LOOH was increased in myocardial infarction-induced rats, whereas considerable decline was noted on the fucoxanthin pretreated rats. It may be a result of antioxidant action of fucoxanthin present in the allenic bond, epoxide and hydorxy group[8] which efficiently scavenges the free radicals, thereby preventing the lipid peroxidation of the cardiac tissue.

Antioxidants defense mechanism takes an essential function in preventing caridomyocytes from the oxidative stress induced during myocardial infarction. In the present study, the first-line cellular defense mechanism imparted by endogenous antioxidants SOD, CAT, GPX, and reduced GSH was appreciably declined on myocardial provoked rats. Astounding reduction on the levels of Na^+/K^+ ATPase was noted on the biopsies of heart failure patients. The statuses of Na^+/K^+ ATPase were as well as influenced by drugs prescribed during a heart attack; aldosterone antagonist given to reduce hyperaldosteronism decreases the Na^+/K^+ ATPase.[34] On this current investigation, the fucoxanthin pretreated rats induced with myocardial infarction revealed the increased levels of Na^+/K^+ ATPase compared to myocardial infarction-induced rats; this confirms the potency of fucoxanthin as a cardioprotectant. Our findings as well correlate with the results of Ravi Kumar *et al.*[35] and stated that fucoxathin increases the levels of Na^+/K^+ ATPase and inhibits the lipid peroxidation in retinol-deficient rats.

Inflammation is the immediate response exhibited by the defense mechanism of cells against the external stimuli.[36] Inflammation is triggered by the inflammatory cytokines which in turn generates free radicals, thereby increasing the oxidative stress in cells. Estimating the levels of inflammatory cytokines will be valuable to assess the risk stratification of myocardial infarction patients.[37] Oxidative stress induced by isoprenaline triggers the complement activation, cytokines leading to the formation of inflammation in the cardiac tissue.[38] In the present, the statuses of inflammatory regulators, that is, TNF-α, IL-6, and NF-κB were significantly increased in the myocardial infarction induced, whereas drastically reduced in fucoxanthin pretreated rat cardiac tissue.

Our immunoblotting results were also confirmed with the histopathological analysis of control and experimental rat's cardiac tissue. Infiltration of inflammatory cells and myonecrotic cells were noted on the isoprenaline treated rat heart tissue, whereas the fucoxanthin pretreated rats reduced edema, necrotic cells compared to isoprenaline treated rats. Our results correlate with previous reports stating the inhibitory effect of fucoxanthin against nitric oxide, TNF-α, and interleukins.[39,40] The decline in the amount of necrotic cells on fucoxanthin pretreated rats was due to the anti-inflammatory property of fucoxanthin which efficiently scavenged the free radicals and prevented the cells from the induction of inflammatory cytokines.

CONCLUSION

The administration of antioxidant fucoxanthin efficiently prevented the rats from myocardial infarction induced by isoprenaline; an effective *in vivo* model mimics the condition of human myocardial infarction. Fucoxanthin alone treated rats does not show any biochemical, molecular, or histoarchitectural changes; therefore, it is confirmed that fucoxanthin is a potent cardio protectant with nil side effects and can be prescribed for the human trials of myocardial infarction.

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Conflicts of interest

There are no conflicts of interest.

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