Cardioprotective Effects of Astaxanthin against Isoproterenol-Induced Cardiotoxicity in Rats

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Abstract

Myocardial infarction (MI) is an important cause of mortality around the world. Isoproterenol (ISO) is a synthetic catecholamine found to cause toxicity leading to severe stress in the myocardium of experimental animals. The aim of the present article is to investigate cardioprotective activity of astaxanthin against ISO-induced cardiotoxicity in adult rats, in an attempt to understand its mechanism of action, which may pave the way for possible therapeutic applications. Oral administration of astaxanthin at a concentration of 50 and 100 mg/kg b.wt. daily for 58 days showed a significant protection against-induced alteration in plasma and cardiac SOD, Gpx, GSH and CAT as well as CK-MB, LDH, ALT and AST activities. In addition, astaxanthin reduced plasma CK-MB, LDH, ALT and AST as well as cardiac MDA and HP levels as compare to control group. In conclusion, astaxanthin renders resiliency against isoproterenol cardiotoxicity due to its antioxidant and free radical scavenging activity that might serve as novel adjuvant therapy with isoproterenol.

Keywords: Myocardial infarction; Isoproterenol; Astaxanthin; Antioxidant

Abbreviations: ISO: Isoproterenol; SOD: Superoxide Dismutase; Gpx: Glutathione Peroxidase; GSH: Reduced Glutathione and CAT: Catalase; CKMB: Creatine Phosphokinase-MB; LDH: Lactate Dehydrogenase; ALT: Alanine Transaminases; AST: Aspartate Transaminases; MDA Malondialdehyde; HP: Hydroperoxide

Introduction

Catecholamines are produced under stress conditions and are also administered in circumstances of cardiac stress to sustain blood pressure and cardiac function in patients. It is an important regulator of myocardial contractility and metabolism. However, excess amounts of catecholamines are responsible for cellular damage observed in clinical conditions like angina, transient myocardial hypoxia, acute coronary insufficiency and subendocardial infarct. Due to the generation of reactive oxygen species (ROS), catecholamines contribute to oxidative stress [1].

Isoproterenol [1-(3,4-dihydroxy phenyl)-2-isopropyl amino ethanol hydrochloride] (ISO), a synthetic catecholamine, acts as a β-adrenergic agonist and has been found to cause toxicity leading to severe stress in the myocardium, resulting in the depletion of energy reserve of cardiac muscle cells, and causes complex biochemical and structural changes leading to cell damage and necrosis [1]. ISO-induced myocardial damage is considered as one of the most widely used experimental models to study the beneficial effects of many drugs on cardiac function [2]. ISO undergoes auto-oxidation, which results in the generation of excess amount of electrons [3]. These electrons can reduce oxygen molecules, resulting in the generation of reactive oxygen species (ROS). ROS can initiate lipid peroxidation reactions and propagate cell membrane damage [4]. Astaxanthin is a red pigment carotenoid occurring naturally in a wide variety of living organisms and classified as a xanthophylls [5]. It has a chemical structure similar to that of the familiar carotenoid β-carotene. It has been suggested that astaxanthin protects muscle cells from damaging effects [6]. The presence of hydroxyl (OH) and ketone (C=O) moieties on each ionone ring along with an extension of conjugated double bond system explained the potency of astaxanthin with higher antioxidant activity [7]. Astaxanthin has been reported to possess anti-inflammatory [8], hepatoprotective [9] and antioxidant [10] activities via it’s inhibits nitric oxide production and inflammatory gene expression [11]. Though the antioxidant and hypolipidemic activity of astaxanthin was reported [7,10], the cardioprotective effect of astaxanthin has not been reported earlier. Based on the results of these references, the effect of astaxanthin on myocardial infarction has been evaluated in the present study.

Materials and Methods

Chemicals and reagents

Astaxanthin (92%) and isoproterenol hydrochloride (98%) were purchased from Sigma Chemical Co., St Louis, Missouri, USA. All other chemicals used were of analytical grade.

Animals

The experiments were carried out with male albino rats weighing 180 ± 20 gms were purchased from Faculty of Veterinary Medicine, Cairo University. They were acclimatized to animal house conditions. Animals were provided with standard diet and water ad libitum. Rats were kept under constant environmental condition and observed daily throughout the experimental work. They were housed in polypropylene cages (47 cm x 34 cm x 20 cm) lined with husk, replaced every 24 h, under a 12:12 h light: dark cycle at around 22°C and had free access to tap water and food. The rats were fed on a standard pellet diet (Nutri Lab-Rodent, Tetragon Chemicals Pvt Ltd, India). The pellet diet consisted of 22.30% crude protein, 3.44% crude fat, 3.9% crude...
Experimental set up

This experiment was carried out to examine the cardioprotective effects of astaxanthin against isoproterenol-induced cardiotoxicity in rats.

Animals were randomly assigned to five groups, eight rats in each.

**Group I:** Normal (was given 1 ml normal saline orally)

**Group II:** Control (was given similar volume of saline orally)

**Group III:** Was treated with astaxanthin (50 mg/kg b.w.) suspended in normal saline orally in a single daily dose for 58 days [12].

**Group IV:** Was treated with astaxanthin (100 mg/kg b.w.) suspended in saline orally in a single daily dose for 58 days.

**Group V:** Was treated with vitamin C (1 g/kg b.w.) suspended in normal saline orally in a single daily dose for 58 days [13].

On the 58th day 1 hour after the administration of test sample, isoproterenol (85 mg/kg) dissolved in normal saline was injected subcutaneously to all rats, other than the group I, at an interval of 24 hours for 2 days to induce experimental cardiotoxicity [14]. On 60th day, blood was collected from the retro-orbital vein of each animal and each sample was collected into 2 heparinized tubes. The first part of heparinized blood samples were divided into 2 aliquots. The first aliquot was used for determination of GPx and CAT activity.

The second aliquot was haemolyzed using bidistilled water and the haemolysate of each sample was divided into two portions was treated with chloroform/ethanol (3:5 V/V) mixture to precipitate and the resultant supernatant was used for the determination of SOD activity. The second portion was deproteinized with meta-phosphoric acid and the clear supernatant was used for the estimation of GSH level. Haemoglobin levels were determined in the heparinized blood samples and used in the calculation of the enzyme activity. The second part of heparinized blood samples were allowed to centrifuged at 1000g for 20 min. The separated plasma was used for the estimation of serum activity of ALT, AST, LDH and CKMB as well as levels of TNF-α, NO, GR and NADPH. The oxidation of NADPH to NADP+ is accompanied by a decrease in absorbance at 340 nm. Under conditions in which the GPx activity is rate limiting, the rate of decrease in the A₃₄₀nm is directly proportional to the GPx activity. One unit (U) of GPx activity is defined as the amount of enzyme that will cause the formation of 1.0nmol of formaldehyde per minute at 25°C.

**Statistical analysis**

The Statistical Package for the Social Sciences (SPSS for WINDOWS, version 11.0; SPSS Inc, Chicago). Results were expressed as mean ± standard error (SE) and values of P>0.05 were considered non-significantly different, while those of P<0.05 and P<0.01 were considered significantly and highly significantly different, respectively [27].

**Results**

Tables 1 and 2 show the level of blood and heart SOD, GPx, CAT and GSH of control and experimental groups of rats. Isoproterenol (85 mg/kg), injection subcutaneously to all group of rats markedly decreased plasma and heart SOD, GPx, CAT and GSH level (p<0.01) indicating acute cardiotoxicity compared with saline-treated "normal" rats. Oral treatment with astaxanthin (50 and 100 mg/kg) for a period of 58
Isoproterenol was given subcutaneously as a single dose of 85 mg/kg b.w. to 18 h fasted animals. It was given to all groups except the normal one. astaxanthin (50 and 100 mg/kg) were orally given daily for 58 days and the last dose of each was given 1 h before Isoproterenol administration. Blood samples were collected 24 h after isoproterenol administration. Activity is expressed as: 50% of inhibition of pyrogallol autooxidation per min for SOD and the obtained values were divided by the haemoglobin (Hb) concentration. Values are given as mean ± SD for groups of eight animals each.

* Significantly different from normal group at p<0.01. @ Significantly different from control group at p<0.05.

Table 1: Level of GSH, SOD, GPx and CAT in blood of normal and experimental groups of rats.

| Groups                                      | GSH (mM/g protein) | SOD (U/g Hb) | GPx | CAT (U/g Hb) |
|---------------------------------------------|--------------------|--------------|-----|--------------|
| Normal (1 ml saline)                        | 42.61 ± 4.11       | 1.82 ± 0.24  | 2.15 ± 0.21 | 55.20 ± 3.25 |
| Control (ISO, 85 mg/kg)                     | 23.74 ± 6.99       | 0.93 ± 0.17  | 0.76 ± 0.33 | 17.75 ± 2.54 |
| Astaxanthin (50 mg/kg) + ISO (85 mg/kg)     | 31.85 ± 5.22       | 1.24 ± 0.35  | 1.35 ± 0.53 | 39.45 ± 3.94 |
| Astaxanthin (100 mg/kg) + ISO (85 mg/kg)    | 39.14 ± 4.84       | 1.64 ± 0.45  | 1.80 ± 0.42 | 48.18 ± 6.08 |
| Vitamin C (1 g/kg.b.w) + ISO (85 mg/kg)     | 35.25 ± 3.77       | 1.43 ± 0.26  | 1.63 ± 0.37 | 40.22 ± 5.16 |

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* Significantly different from normal group at p<0.01. @ Significantly different from control group at p<0.05.

Table 2: Level of cardiac GSH, SOD, GPx and CAT in normal and experimental groups of rats.

| Groups                                      | CK-MB (U/L) | LDH (IU/L) | ALT (IU/L) | AST (IU/L) |
|---------------------------------------------|-------------|------------|------------|------------|
| Normal (1 ml saline)                        | 63.46 ± 4.65 | 126.45 ± 7.43 | 25.16 ± 2.30 | 32.64 ± 3.40 |
| Control (ISO, 85 mg/kg)                     | 526.75 ± 11.37 | 243.85 ± 9.44 | 42.68 ± 1.35 | 57.33 ± 4.11 |
| Astaxanthin (50 mg/kg) + ISO (85 mg/kg)     | 292.25 ± 10.55 | 205.61 ± 11.36 | 35.18 ± 2.40 | 46.22 ± 3.53 |
| Astaxanthin (100 mg/kg) + ISO (85 mg/kg)    | 210.30 ± 9.86 | 143.56 ± 7.18 | 28.24 ± 3.06 | 37.48 ± 3.50 |
| Vitamin C (1 g/kg.b.w) + ISO (85 mg/kg)     | 235.12 ± 7.65 | 152.33 ± 9.40 | 31.25 ± 1.53 | 40.64 ± 2.57 |

Isoproterenol was given subcutaneously as a single dose of 85 mg/kg b.w. to 18 h fasted animals. It was given to all groups except the normal one. astaxanthin (50 and 100 mg/kg) were orally given daily for 58 days and the last dose of each was given 1 h before Isoproterenol administration. Activity of LDH (μM of pyruvate liberated/ min/mg of protein); ALT as (mM of pyruvate formed/min/mg of protein) Values are given as mean ± SD for groups of eight animals each.

* Significantly different from normal group at p<0.01. @ Significantly different from control group at p<0.05.

Table 3: Level of plasma CK-MB, LDH, ALT and AST in normal and experimental groups of rats.

| Groups                                      | CK-MB (U/mg protein) | LDH (mM/mg protein) | ALT (mM/mg protein) | AST (mM/mg protein) |
|---------------------------------------------|----------------------|---------------------|---------------------|---------------------|
| Normal (1 ml saline)                        | 183.84 ± 15.60       | 85.64 ± 5.54        | 425.88 ± 14.25      | 410.35 ± 20.50      |
| Control (ISO, 85 mg/kg)                     | 66.58 ± 27.44        | 53.25 ± 7.88        | 246.55 ± 25.74      | 205.22 ± 11.74      |
| Astaxanthin (50 mg/kg) + ISO (85 mg/kg)     | 105.40 ± 23.80       | 63.65 ± 4.26        | 318.77 ± 15.60      | 297.19 ± 14.85      |
| Astaxanthin (100 mg/kg) + ISO (85 mg/kg)    | 165.66 ± 17.45       | 76.18 ± 5.49        | 410.70 ± 18.47      | 386.29 ± 13.78      |
| Vitamin C (1 g/kg.b.w) + ISO (85 mg/kg)     | 115.40 ± 32.44       | 70.37 ± 4.17        | 396.06 ± 22.84      | 367.05 ± 14.27      |

Isoproterenol was given subcutaneously as a single dose of 85 mg/kg b.w. to 18 h fasted animals. It was given to all groups except the normal one. astaxanthin (50 and 100 mg/kg) were orally given daily for 58 days and the last dose of each was given 1 h before Isoproterenol administration. Activity of LDH (μM of pyruvate liberated/ min/mg of protein); ALT and AST as (mM of pyruvate formed/min/mg of protein) Values are given as mean ± SD for groups of eight animals each.

* Significantly different from normal group at p<0.01. @ Significantly different from control group at p<0.05.

Table 4: Level of cardiac (CK-MB), LDH, ALT and AST in normal and experimental groups of rats.

days significantly increase in the activities of these cardiac marker enzymes (p<0.05) compared with ISO-treated rats. Furthermore, vitamin C a proven heart protecting agent, also significantly enhanced the cellular antioxidant enzymes (p<0.05). The effect was more pronounced in astaxanthin (100 mg/kg) (p<0.01) compared to vitamin C (p<0.05) as well as astaxanthin (50 mg/kg) (p<0.05).

Tables 3 and 4 show the activity of plasma and heart CK, LDH and ALT and AST of control and experimental groups of rats. Activity of CK, LDH, ALT and AST was observed to be decreased significantly in heart homogenate and increased in plasma of rats (group II) when injected with Isoproterenol (85 mg/kg) (p<0.01) compared with saline-treated rats. Pretreating animals with astaxanthin (50 and 100 mg/kg) decreased the activity of these enzymes in plasma and increased the same in heart tissue significantly against diseased animals (p<0.01) compared with ISO-treated rats. Moreover, the activities of enzymes in plasma and heart of astaxanthin (100 mg/kg) pretreated groups was comparable with that of normal and vitamin C pretreated group. The effect was more pronounced in astaxanthin (100 mg/kg) (p<0.01) compared to vitamin C (p<0.01) as well as astaxanthin (50 mg/kg) (p<0.01).

Table 5 depicts the concentration of plasma and cardiac MDA and HP of control and experimental groups of rats. The levels of plasma and heart TBARS and hydroperoxides in isoproterenol injected rats were significantly higher than control rats, whereas group of rats-

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treated with the astaxanthin (50 and 100 mg/kg b.w.) restored the altered values to the near normalcy (p<0.05). Furthermore, vitamin C a proven myocardial protecting agent, also significantly inhibited the lipid peroxidation of heart. The effect was more pronounced in the groups of rats administered with astaxanthin (100 mg/kg b.w.) than vitamin C. The effect was more pronounced in astaxanthin (100 mg/kg) (p<0.01) compared to vitamin C (p<0.01) as well as astaxanthin (50 mg/kg) (p<0.01).

**Discussion**

Oxidative stress has been associated with diverse pathophysiological events, including cancer, renal disease and neuro-degeneration [28]. It has been established that excessive oxidative stress caused by either increased ROS production or inadequate antioxidant defenses can lead to cardiac lesions [29]. Present study demonstrates the cardioprotective effect of astaxanthin in isoproterenol-induced cardiotoxicity as evidenced by improved antioxidant defense as well as inhibition of lipid peroxidation and prevention of leakage of myocytes injury marker enzymes from heart.

Isoproterenol is a widely used chemical in toxicological studies to induce cardiac muscle injury, through an exaggerated pharmacological effect [30]. The administration of isoproterenol is a well-established animal model of acute myocardial infarction [31]. Yates et al. [32] have explained that isoproterenol undergoing oxidation results in the formation of superoxide anion and the chain reactions propagate results in the formation of reactive oxygen species. In the present study, the increased level of oxidative stress markers observed in isoproterenol-injected rats might be due to the generated free radicals from auto-oxidation of isoproterenol. The primary antioxidant enzymes in mammals include SOD which converts superoxide to hydrogen peroxide, GPx and CAT which are responsible for converting hydrogen peroxide to water [33]. GSH is a very important non-enzymatic antioxidant which can react directly with free radicals or act as an electron donor in the reduction of peroxides catalyzed by GPx [34]. The present results show marked decreased plasma and heart SOD, GPx, CAT and GSH level (p<0.01) indicating acute cardiotoxicity in isoproterenol-treated group (Tables 1 and 2). The obtained data in the present study demonstrated that astaxanthin considered as a potent antioxidant agent when given simultaneously with isoproterenol since it could produce marked increase in plasma and heart SOD, GPx, CAT and GSH levels towards the normal values. Many authors have reported the effective antioxidant action of astaxanthin. Astaxanthin has unique chemical properties based on its molecular structure (6 and 7). As illustrated in Figure 1, the presence of hydroxyl (OH) and ketone (C=O) moieties on each ionone ring along with an extension of conjugated double bond system explained the potency of astaxanthin with higher antioxidant activity [6]. The two groups most prominent antioxidant activities of astaxanthin are quenching of singlet oxygen and inhibition of lipid peroxidation [7]. In our model, Astaxanthin provides cell membranes with potent protection against free radicals or other oxidative attack. Experimental studies confirm that this nutrient has a large capacity to neutralize free radicals or other oxidant activity in the nonpolar (hydrophobic) zones of phospholipid aggregates, as well as along their polar (hydrophilic) boundary zones [35].

Besides, Astaxanthin is able to restore the activities of antioxidant enzymes SOD, CAT and GPx as well as other non-enzymatic antioxidants such as GSH, vitamins C and E in plasma and other various tissues in pathological conditions [36-38].

Vitamin C readily reacts with GSH radicals arising from the reaction of GSH and free radicals, thereby vitamin C spares GSH first by competing with GSH for free radicals and second by converting thiol radicals back to GSH [39].

Enzymes, the macromolecules that leak from the damaged tissue, because of their tissue specificity and catalytic activity, are the best markers of tissue damage. Hence, in isoproterenol-induced myocardial infarcted rats, there was a decrease in activities of the marker enzymes CK, LDH, AST and ALT in the heart homogenate, and inhibition of lipid peroxidation [7]. In our model, Astaxanthin provides cell membranes with potent protection against free radicals or other oxidative attack. Experimental studies confirm that this nutrient has a large capacity to neutralize free radicals or other oxidant activity in the nonpolar (hydrophobic) zones of phospholipid aggregates, as well as along their polar (hydrophilic) boundary zones [35].

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**Table 5:** Level of plasma and cardiac TBARS and HP in normal and experimental groups of rats.

| Groups                           | TBARS                     | Heart (nM/100 g of tissue) | Plasma (nM/ml) | Heart (nM/100 g of tissue) | Plasma (mM/dL) |
|----------------------------------|---------------------------|---------------------------|----------------|---------------------------|----------------|
| Normal (1 ml saline)             |                           |                           |                |                           |                |
| Control (ISO, 85 mg/kg)          | 2.10 ± 0.47 p<0.05        | 3.20 ± 0.18 p<0.01        | 2.56 ± 0.35    | 3.65 ± 0.54              |                |
| Astaxanthin (50 mg/kg) + ISO (85 mg/kg) | 1.83 ± 0.23 a 2.46 ± 0.29 a | 3.30 ± 0.25 a 3.94 ± 0.18 a | 4.84 ± 0.42 a 5.25 ± 0.36 a |                |
| Astaxanthin (100 mg/kg) + ISO (85 mg/kg) | 1.40 ± 0.35 a 1.97 ± 0.24 a | 2.44 ± 0.46 a 3.50 ± 0.22 a | 2.75 ± 0.11 a 3.79 ± 14.27 a |                |
| Vitamin C (1 g/kg,b.w) + ISO (85 mg/kg) | 1.66 ± 0.23 a 2.35 ± 0.31 a |                |                |                           |                |

*Isoproterenol was given subcutaneously as a single dose of 85 mg/kg b.w. to 18 h fasted animals. It was given to all groups except the normal one. astaxanthin (50 and 100 mg/kg) were orally given daily for 58 days and the last dose of each was given 1 h before isoproterenol administration. Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at p<0.01. ** Significantly different from control group at p<0.05.

**Figure 1:** Astaxanthin and its antioxidant chemical site.
followed by an increase in their levels in plasma [40] (Tables 3 and 4). These findings confirm the onset of myocardial necrosis and leaking out of the marker enzymes from heart to blood [41]. The amount of marker enzymes is directly proportional to isoproterenol-induced necrotic lesions present in the myocardium. In the present study, pretreatment of astaxanthin to myocardial infarction-induced rats reduce the cardiac damage and restrict the leakage of enzymes as evident from a significant reduction in the activities of cardiac marker enzymes in plasma. In the present study, isoproterenol significantly increased MDA and HP level (Table 5) with concomitant reduction of myocardial CKMB and LDH enzyme activity. Elevated MDA and HP level reflects an increase in membrane permeability, which could be responsible for leakage of myocardial enzymes (CKMB and LDH) from cardiomyocytes [42]. CK-MB and LDH, localized in myocytes, are released during isoproterenol-induced irreversible myocardial injury and are considered as characteristic of cardiac muscle injury [43]. The reduction in the leakage of CK-MB and LDH enzymes from heart as evidenced by increased levels of CK-MB and LDH in heart tissue, is suggestive of the cardioprotective effect of astaxanthin pretreatment. Due to disruption of endogenous antioxidant network, as observed in present study, myocardium may be more susceptible to free radicals induced ischemic injury and subsequent cascade of inflammation and injury. Inflammation is the very initial response of the immune system to infection. The anti-inflammatory effect of astaxanthin has been documented [8,9]. The present study findings demonstrate therapeutic benefits of astaxanthin an integrated approach as evidenced by increased levels of CK-MB and LDH in heart tissue, is suggestive of the cardioprotective effect of astaxanthin pretreatment. 

In conclusion, our study clearly demonstrates that astaxanthin administration to isoproterenol-induced rats for 58 days possess significant cardioprotection by minimizing the alterations in the activities of the antioxidant enzymes and decreasing the levels of MDA and HP. These effects could be due to membrane protective action of astaxanthin by scavenging the free radicals and its antioxidant action.

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