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EFFECT OF CADMIUM ON ANTIOXIDANT METABOLIC MODULATIONS IN HEART AND MUSCLE OF FEMALE RABBITS

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ABSTRACT

Disturbance in pro-oxidant/anti-oxidant systems and oxidative challenges of cadmium induced altering the antioxidant system in both heart and muscle tissue of female rabbits were studied. The cadmium induced oxidative stress leads to the tissue destruction by the elevated free radicals by suppression of antioxidant scavenger system. The imbalance between the free radicals and scavenger antioxidants leads to the tissue damage caused by the free radicals and peroxidants in rabbits. The present findings suggest that cadmium induction decreased Superoxide dismutase (SOD) activity is observed in both tissues like myocardial heart and muscle of rabbits over control rabbits. The decreased SOD activity on induction of cadmium envisages reduced neutralization of superoxide anions which might leads to cause an increase in superoxide radicals. The Glutathione-S-transferase (GST) activity was significantly inhibited in both heart and muscle on induction of cadmium. The rate of inhibition was maximum in heart myocardial tissue than in muscle when compared to control. Suppressed Reactive oxygen species (ROS) formation in both heart and muscle tissues by inhibiting Xanthine oxidase (XOD) activity on induction of cadmium. Decreased XOD can be envisaged that the XOD probably inter converting into xanthine dehydrogenase enzyme either by reversible sulphydryl oxidation or by irreversible photolytic modification. We concluded that the cadmium toxicity could have induced oxidative damage in both heart and muscle by enhancing peroxidation of membrane lipids by inducing inhibition of the antioxidant enzymes.

KEY WORDS

Cadmium, antioxidants, Reactive oxygen species (ROS)

INTRODUCTION

The antioxidant system plays an important role in the prevention of oxidative damage caused by free radicals. Antioxidants are also widely used to preventing diseases such as cancer, coronary heart disease and neurological diseases [1]. Cadmium causes hypertension and arteriosclerosis without renal dysfunction in rats [2, 3, 4]. Recent investigation have established that free radicals may be important contributors to cardiac dysfunction and myocardial damage [5, 6] and free radical mediated cellular damage and reactive oxygen species (ROS) had been the raised as contributing to metal mechanism of toxicity [7], cadmium plays an important role on antioxidant system in different animals [8, 9], it is essential to understand the impact of cadmium on antioxidant cascade. The superoxide dismutase is a primary antioxidant enzyme and is essential to the organism to fight against oxidative effects of free radicals. It is well established that catalase and glutathione peroxidase also plays a vital role in reducing the risk of oxidative stress [10, 11]. The highly

reactive free hydroxyl radicals (OH^-) by the Fenton reaction, which is widely believed to be mainly responsible for oxidative damage [12,13]. Glutathione peroxidase converts H_2O_2 and/or other lipid peroxides to H_2O and hydroxyl lipids and in the process glutathione is converted to oxidized glutathione (GSSG). The GST catalyses the conjugation of glutathione (GSH) with wide variety of several other organic compounds, including hydroperoxides thereby shares peroxidase activity [14]. The inhibition in the GST activity indicates that the breakdown of toxic compounds such as glutathione and hydroperoxide could have been reduced.

In this present study we conducted an experiment to understand the role of cadmium toxicity on antioxidant system which includes the enzymes superoxide dismutase, catalase, glutathione-S-transferase, xanthine oxidase and lipid peroxidation status was tested in heart myocardial tissue and muscle tissues of rabbits.

MATERIALS AND METHODS

Twelve healthy female New Zealand white breed rabbits (*Oryctolagus cuniculus*) with an age group of 2 Months, (weight: 1.0 ± 0.2 kg) were obtained from Veterinary College, Tirupati, India. And divided into two groups, each group consists of six individuals, group one is kept as control and another as experimental group. After procurement they were thoroughly examined and acclimatized to lab conditions prior to start the experiment. The rabbits were maintained at laboratory conditions (26 ± 2°C; 12 h light & 12 h dark cycle) throughout the study. The animals had free access to standard laboratory chow food and clean water *adlibitum.* All care and management procedures for rearing of rabbits were in accordance with the National Institutes of Health Guidelines on the Care and Use of Laboratory Animals, and the approval of the Ethical Committee of the S.V. University was obtained prior to the study. Rabbits in group 1(control) were administered single dose of cadmium chloride (25mg/kg body weight) every day orally for a period of two weeks. Rabbits in group 2 receive only normal water was given orally. The cadmium in form of $CdCl_2$ dissolved in normal saline was administered to the rats at a dose of 25mg/kg body weight two weeks to the termination of the experiment. At the end of 2 weeks experimental study period animals were sacrificed by cervical dislocation. The biochemical estimations and myocardial tissue damage was excised after two weeks period of exposure.

BIOCHEMICAL ESTIMATIONS

The scavenger enzymes which includes the Superoxide dismutase, Catalase, Glutathione-S-transferase, Xanthine oxidase all these enzymes activity studied in the present investigation, and the assays were standardized in both experimental and control tissues by conducting preliminary tests to determine the optimal P^H, temperature, enzyme and substrate concentrations and these optimal conditions were subsequently followed for each enzyme assay. Superoxide dismutase activity was measured as the inhibition of photo reduction of nitro blue tetrazolium (NBT) by the enzyme as per the method of [15]. The reaction samples were placed under fluorescence light for 30 minutes and the resulting color was read at 560 nm against the reagent blank and kept in dark place. The activity of the enzyme was expressed as units/ mg protein/ min. Catalase activity was determined by a slightly modified version of [16]. In this method decreased optical density of Catalase was measured at 240 nm for 60 seconds. The molar extinction coefficient of 43.6 M cm^{-1} was used to determine Catalase activity. Glutathione-s-transferase activity was measured with its conventional substrate 1-chloro 2, 4-

dinitrobenzene (CDNB) at 340 nm as per the method of [17]. In this method the reaction mixture glutathione and enzyme source was initiated by the addition of glutathione and the absorbance was read at 340 nm against reagent blank. Xanthine oxidase activity was estimated by the dye reduction method of [18]. The reaction formazan formed was estimated extracted over night in to 5 ml of toluene and intensity of the color was read at 495 nm in spectrophotometer against toluene blank.

ESIMATION OF LIPID PEROXIDATION

Lipid peroxidation can be determined by estimating the levels of thiobarbituric reactive substances (TBARS) levels following the method of Miller and Aust (1989). Tissue homogenate (1 mg protein) in 0.15 M KCl, 0.25 M Tris-HCl buffer, 2 m mole ADP and 10 µ moles $FeSO_4$ were incubated at 37 °C for 5 min. The reaction (final volume of 1.0 ml) was initiated by adding 0.1 mM ascorbic acid. The reaction was terminated after 30 min by adding 2 ml of thio-barbituric acid reagent (0.375% TBA, o.2 N HCL, 15% TCA) and aldehydes formed were determined.

STATISTICAL ANALYSIS

Statistical analysis (ANOVA) analysis of variance used to test the differences between treatment effects and the control to analyze various enzymes was studied at the sub cellular level in heart and muscle of both control and experimental female rabbits was tested and performed by using statistical package, SSPS 16.0.1 to determine means and standard deviations were calculated. The percent change over control was calculated and presented for each parameter. Comparison between means was made with the help of Independent –Sample t-Test and the $P<0.01$ was considered as significant.

RESULTS

Decreased antioxidant system which includes superoxide dismutase (SOD) after two weeks dosing with Cd 25mg/kg body weight significantly ($P>0.01$) The level of experimental rabbits was lowered in heart (-34.207) and muscle (-26.984) over control animals. (**Table-1**).followed by of catalase level of experimental rabbits was reduced in heart (-36.269) and muscle (-28.482) than control were observed and the percent change of glutathione-s-transferase level of experimental rabbits was decreased in heart (-39.429) and muscle (-43.951) over control animals. And finally of xanthine oxidase level of experimental rabbits was considerably reduced in heart (-59.561) and muscle (-20.00) over control. Which includes evidenced that lipid peroxidation increased in the level of experimental rabbits was considerably in heart (+76.923) and muscle (+93.061) over control. The oxidative stress may be due to imbalance of antioxidants and peroxidants which might be induced due to several factors and heart and muscle of myocardial tissue destruction observed by the continues exposure of free radicals induced by cadmium. The antioxidant system consists of glutathione, superoxide dismutase (SOD), catalase and xanthine oxidase (XOD).The data is presented in **(Table 1)**. The analysis of various enzymes was studied at the sub cellular level in heart and muscle of both control and experimental female rabbits.

Table-1: Antioxidant Enzyme levels heart and muscle tissues.

Parameters	Tissue	Control	Experimental	%Change
Superoxide dismutase (SOD)(Superoxide anion reduced /mg protein/min)	Heart	7.63±0.241	5.02 ± 0.259	-34.207 P<0.001
	Muscle	8.82±0.280	6.44±0.420	-26.984 P<0.001
Catalase (μ moles of H_2O_2 reduced /mg protein/min)	Heart	1.93±0.082	1.23±0.092	-36.269 P<0.001
	Muscle	1.12±0.029	0.801±0.022	-28.482 P<0.001
Glutathione -S-Transferase (GST) (μ moles of NADPH oxidized / mg protein/ min)	Heart	3.83±0.35	2.32±0.117	-39.429 <0.001
	Muscle	2.48±0.124	1.39±0.083	-43.951 P<0.001
Xanthine oxidase μ moles of formazan formed (XOD) (Units/mg protein/min)	Heart	0.319±0.026	0.129±0.008	-59.561 P<0.001
	Muscle	0.165±0.012	0.132±0.010	-20.00 P<0.001
Lipid peroxidation (μ moles of TBARS cleaved/g wet/of tissue/h	Heart	1.95±0.036	3.42±0.053	+76.923 P<0.001
	Muscle	2.45±0.023	4.73±0.083	+93.061 P<0.001

DISCUSSION

Cadmium could not generate free radicals by its own even though the byproducts of superoxide radical, hydroxyl radical and nitric oxide radicals could be generated indirectly through competitive binding kinetics with antioxidant defense enzymes. It does not participate in Fenton-type reactions [19] but can indirectly favour the production of different ROS, such as hydrogen peroxide (H_2O_2), superoxide ($O_2^{\cdot -2}$), and hydroxyl radical (·OH), by unknown mechanisms, giving rise to an oxidative burst [20,21,22].The enzymes superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) are involved in the detoxification of $O_2^{\cdot -2}$ (SOD) and H_2O_2 (CAT, GST), thereby preventing the formation of ·OH radicals.The present findings suggest that cadmium induction decreased antioxidant system which includes the primary antioxidant enzyme SOD decreased activity is observed in both the tissues like heart myocardial and muscle of rabbits over control rabbits. The primary reason could be attributed that this enzyme is naturally present in every living organism and its presence always regulates the formation of peroxidative free radicals and each in turn prevents the cells from damage caused by imbalance between oxidant and antioxidant systems. Hence the decreased SOD activity could have been accelerated the oxidative stress which might be induced by the cadmium treatment. Usually the superoxide anions are formed by addition of one electron with a negative electric

charge on molecular oxygen. This mechanism could have been obstructed in both heart and muscle tissues due to the inhibition of SOD activity [23] suggested acute cadmium exposure caused increased oxidative stress by producing superoxide anions. All the above reports provided ample evidence and support the result exhibits the decreased SOD activity due to increased ROS production in both heart myocardial tissue and muscle tissues [24]. The decreased SOD in heart and muscle might result in reduced conversion of free radicals. The decreased SOD activity might depend on the imbalanced expression of the antioxidative enzymes SOD, and elevated ROS leads to the cellular destruction by the continuous exposure of free radicals. These results initiated to study another important peroxidation enzyme, catalase to understand the impact of cadmium on free radical metabolism in heart and muscle. Another scavenger enzyme Catalase is present in the peroxisomes of nearly all aerobic cells and preserves to protect the cell from the toxic effects of H_2O_2 by catalyzing its decomposition into molecular oxygen and water without the production of free radicals. Catalase is one of the most important antioxidant enzymes which cleave the toxic H_2O_2 substrate into water. One molecule of catalase can convert millions molecules of H_2O_2 into water and oxygen per second [25]. Catalase also involves in the removal of toxic H_2O_2 from the cell. The inhibition of catalase activity could have altered the protection of cells from the toxic injury caused by free radicals in both heart and muscle. Catalase activity was inhibited maximum in heart tissue than muscle on induction of cadmium in the present study which indicates that the conversion of H_2O_2 is reduced more in both the tissues (**Table-1**). The cytosolic enzyme catalase activity was inhibited in both heart and muscle tissues on treatment with cadmium indicates that the cadmium induction might cause damage to the peroxisomes, which in turn might lead to the loss of detoxificatory mechanism. Numerous reports depicted that cadmium intoxication significantly increased the malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) Free radicals generated by cadmium were scavenged by GSH directly or via the GSH peroxidase/GSH system. Acute intoxication of animals with cadmium has shown increased activity of antioxidant defense enzymes like copper-zinc containing superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase. Glutathione-s-transferase (GST) is another important detoxification enzyme which regulates the formation of glutathione compounds in the cells. Especially the GST participates in the detoxification of reactive electrophilic compounds by catalyzing their conjugation to glutathione. The GST activity was significantly inhibited in both heart and muscle on induction of cadmium. The rate of inhibition was maximum in heart myocardial tissue than in muscle when compared to control (**Table 1**). It indicates that the cadmium induction caused maximum loss of detoxificatory mechanism in heart myocardial tissue than in muscle. The GST catalyses the conjugation of glutathione (GSH) with wide variety of several other organic compounds, including hydroperoxides thereby shares peroxidase activity [14]. It indicates that GST not only participates independently in the detoxificatory mechanism by cleaving the glutathione compound but also associates peroxidases to convert certain species of hydroperoxides. The inhibition in the GST activity in the present study indicates that the breakdown of toxic compounds such as glutathione and hydroperoxide could have been reduced. Xanthine oxidase enzyme activity (XOD) which is another important enzyme which

involving in the cascade of ROS generation, it is well known that the XOD enzyme catalyzed the oxidation of hypoxanthine to xanthine and can further catalyze the oxidation of xanthine to uric acid. The XOD activity was significantly decreased in both heart and muscle tissues in cadmium treated animals when compared to their respective controls. This inhibition of these antioxidant enzymes will make such tissues prone to damage by free radicals and in part would have contributed to the observed increase in MDA levels of heart and muscle.

CONCLUSION:

In conclusion that myocardial tissue injury refers to irreversible cellular injury (necrosis) induced by detrimental scavenger system due to elevated ROS with continuous exposure of free radicals to the cell leads to necrosis. Despite being essential for the ultimate survival of the myocardial cell, the sudden reintroduction of molecular oxygen can be unfavourable to the ischaemic myocyte. The cadmium toxicity could have induced oxidative damage in both heart and muscle by enhancing peroxidation of membrane lipids by inducing inhibition of the antioxidant enzymes.There is a complex interrelationship among the metal induced toxicity that contribute to free radical exposure leads to tissue injury due to imbalanced antioxidant system leads to ROS are produced by the injured endothelium, and the ischaemic myocardium itself Free radical scavengers, including SOD, catalase and SOD mimetics, decrease the contribution of superoxide (O^2) and hydroxyl radicals (OH^{2-}) leads to myocardial cell injury.

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