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Cytoprotective effect of *Ocimum Gratissimum in* the Attenuation of Myocardial Infarction Induced by *Musa Acuminate*

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ABSTRACT

Treatment of myocardial infarction (MI) has undergone major advances in recent years, including reductions in mortality and hospital stays. Cardioprotective effects *Ocimum gratissimum* fresh leaves were evaluated in rat model having acute MI induced by *Musa acuminata*. Eating bananas on an empty stomach is not good to the health, because when stomach is empty, there is nearly no food in the stomach that can be digested. If this movement eats bananas, it will speed up the stomach's movement and the promotion of blood circulation, to increase the heart load. It is very easy to induce the myocardial infarction. Adult male rats were divided into 4 groups. 3 groups receive *Musa acuminata* (banana) and 1 group Test drug. Cardio toxicity, evident from increased activities of Serum Creatine phosphokinase, Lactate Dehydrogenase, Aspartate Transaminase and Alanine Transaminase in *Musa acuminata* administered rats, and it was reversed by *Ocimum gratissimum* treatment. *Musa acuminata* administered rat show abnormal levels of SOD, Catalase along with high Malondialdehyde levels. The results of biochemical observation of serum and heart tissue were supplemented by histopathological examinations of rat heart sections to confirm the myocardial injury. These findings highlight the efficacy of *Ocimum gratissimum* as a Cytoprotectant in *Musa acuminata* induced cardio toxicity.

Keywords: *Musa acuminate*, *Ocimum gratissimum*, myocardial infarction

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INTRODUCTION

Treatment of myocardial infarction (MI) has undergone major advances in recent years, including reductions in mortality and hospital stays[1,2]. It is used for treatment of chronic and acute leukemia's, multiple myeloma, lymphomas, and in preparation for bone marrow transplantation although it has tumor selectivity, it also possesses a wide spectrum of toxicities [3]. The cardiotoxic effect of *Musa acuminate* consists of acute, dose dependent cardiac damage. Reactive oxygen species have been implicated in the development of cardiac toxicity after administration of *Musa acuminata*[4]. Cardio toxicity of *Musa acuminate* may be controlled by pharmacological interventions that reduce oxidative stress. *Ocimum gratissimum* is a unique, effective and safe substance that displays the best for scenario for natural antioxidant. It acts on both membrane phase and aqueous phase[5]. We have also identified it to be cardio protective. The aim of present study is to assess the oxidative stress and cardiac damage in *Musa acuminate* administered rats. Cytoprotective effects *Ocimum gratissimum* fresh leaves were evaluated in rat model having acute MI induced by *Musa acuminate*.

MATERIALS AND METHODS

Experimental Animals

Wistar Albino rats of either sex, weighing 150–200 g, were used in the study. The study protocol was approved by the Institutional Ethics Committee and conducted according to the Indian National Science Academy Guidelines for the Use and Care of Experimental Animals. They were kept in standard laboratory conditions under natural light and dark cycle, and are housed at ambient temperature (22±1°C), relative humidity (55±5%). Animals had access to standard pellet diet and water given *ad libitum*.

Induction of Myocardial Infraction

Eating bananas on an empty stomach is not good to the health, because when stomach is empty, there is nearly no food in the stomach that can be digested. If this movement eats bananas, it will speed up the stomach's movement and the promotion of blood circulation, to increase the heart load. It is very easy to induce the myocardial infarction.

Experimental Method

Wistar albino rats of either sex (150–200 g), administer extract daily for 10 days and were divided into four main groups. Group I animals Served as vehicle treated controls. Group II animals were injected Intraperitoneally with a single dose of *Musa acuminate*, on the first day of the experimental period. Group III & IV animals were administered *Musa acuminate* and

immediately followed by administration of 100mg/kg and 200mg/kg of *Ocimum gratissimum* daily for 10 days. After 10 days, all the animals were sacrificed. Hearts were removed and processed for histopathological and biochemical studies Heart tissues were immediately rinsed in the ice old physiological saline. The tissues were homogenized in 0.01 M Tris buffer. At the end of the study the biological parameters are evaluated in the blood serum.

Biological Parameters

Estimation of Serum Aspartate Amino Transferase (AST) By Kinetic Method

Normal range of AST is 8 – 40 IU/L at 37^{o}C.Label reagent tubes as Blank, control and test. Incubate reagent tubes at 37^{o}C for 5 minutes and add 0.8 ml of AST reagent to each tube. Add 100 µl of normal, control serum to the control tube. Cap the tube and mix well by inversion and add 100 µl of test serum sample to test tube. Cap the tube and mix well by inversion. Incubate reagent tubes at 37^{o}C for 60 minutes. Add 0.5 ml of color developer A to each reagent tube. Cap the tubes and mix well by inversion. Let the reagent tubes stand at room temperature for 20 minutes. Add 2 ml of color developer B to each reagent tube. Cap the tubes and mix well by inversion. Let the tubes stand at room temperature for 5 minutes. Wipe the reagent tubes clean with a lint free tissue paper. Place the blank tube in the test well and adjust the photometer to zero absorbance. Place the control and test tubes in the test well and record the absorbance of the control and test samples[6].

Estimation of Serum Alanine Transaminase (ALT)

Normal range of ALT is 5 – 30 U/L at 37^{o}C. Label reagent tubes as Blank, control and test. Incubate reagent tubes at 37^{o}C for 5 minutes. Add 0.8 ml of ALT reagent to each tube. Add 100 µl of normal, control serum to the control tube. Cap the tube and mix well by inversion. Add 100 µl of test serum sample to test tube. Cap the tube and mix well by inversion. Incubate reagent tubes at 37^{o}C for 30 minutes. Add 0.5 ml of color developer A to each reagent tube. Cap the tubes and mix well by inversion. The reagent tubes stand at room temperature for 20 minutes. Add 2 ml of color developer B to each reagent tube. Cap the tubes and mix well by inversion. Let the tubes stand at room temperature for 5 minutes. Wipe the reagent tubes clean with a lint free tissue paper. Place the blank tube in the test well and adjust the photometer to zero absorbance. Place the control and test tubes in the test well and record the absorbance of the control and test samples[7].

Estimation of Superoxide Dismutase (SOD)

Superoxide dismutase was assayed according to Mark Lund method. To 0.5 ml of tissue homogenate, 0.5 ml of distilled water was added to dilute the sample. To this 0.25 ml of ice-cold

ethanol and 0.15 ml of chloroform were added. The mixture was shaken for a minute at 4^0 C and then centrifuged. The enzyme activity in the supernatant was determined. Adrenochrome produced in the reaction mixture containing 0.2 ml of EDTA, 0.4 ml of Sodium carbonate and 0.2 ml of epinephrine in a final volume of 2.5 ml was followed at 470 nm. Transition of epinephrine to Adrenochrome was inhibited by the addition of the required quality of enzyme. The amount of enzyme required to produce 50% inhibition of epinephrine to Adrenochrome transition was taken as one enzyme unit. Activity of the enzyme was expressed as units/min/mg protein[8].

Estimation of Lipid Peroxidation (LPO)

The lipid Peroxidation products (as Malonaldehyde) were determined by the Thiobarbituric acid reaction as described by the following method**.** 0.1 ml of the heart homogenate, 2.0 ml of 20% TCA was added. The contents were mixed well and centrifuged at 4000 rpm for 20 minutes. 2.0 ml of the supernatant was mixed with 2.0 ml of Thiobarbituric acid reagent. Reagent blank standards (5-20 n moles) were also treated similarly. The contents were heated for 20 minutes in a boiling water bath. The tubes were cooled to room temperature and the absorbance was read at 532 nm. The lipid peroxide content was expressed as moles MDA (Malonaldehyde) per 100 mg protein[9].

Estimation of Lactate Dehydrogenase (LDH)

Total LDH estimation also lacks specificity since these enzymes are present in various tissues besides myocardium such as in skeletal muscle, kidneys, liver, lungs and blood cells. However, like CK, LDH too has two isoforms of which LDH_1 is myocardial specific. Estimation of ratio of LDH_1: LDH_2 above 1 is reasonably helpful in making a diagnosis. LDH levels begin to rise after 24 hours, reach peak in 3-6 days and return to normal in 14 days[10].

RESULTS AND DISCUSSION

High dose of *Musa acuminate* can cause an acute form of Cardio toxicity within 10 days of its administration. In the present study, *Musa acuminate* administration significantly increased the activities of serum MDA, SOD, LDH AST, ALT. The values are tabulated in table 1 and 2. Increased activities of these enzymes in serum are well known diagnostic indicators of cardiac injury. During myocardial necrosis these enzymes are released from heart to blood serum. *Ocimum gratissimum* restored the activities of these enzymes of these marker enzymes to near normally. This suggests the *Ocimum gratissimum* has been shown to be protective against cardiac injury elicited by reperfusion.

Table 1 Effect of *Ocimum gratissimum* on MDA, SOD and LDH (Serum & Tissue)

Treatment	MDA (Tissue)	MDA (Serum)	SOD (Tissue)	SOD (Serum)	LDH (Tissue)	LDH (Serum)
Control	1.49±0.05	2.98±0.45	5.05±0.09	2.87±0.16	38.7±0.16	121.7±0.63
Musa acuminate	3.9±0.06	5.87±0.56	2.45±0.08	1.38±0.15	18.93±1.1	326.9±0.72
Ocimum gratissimum (100 mg/kg)	2.45±0.06	3.78±0.73	4.67±0.06	1.78±0.17	26.54±1.1	26.54±1.1
Ocimum gratissimum (200 mg/kg)	1.98±0.09	3.13±0.26	4.98±0.08	2.45±0.2	31.07±1.7	31.07±1.7

All Values were expressed as mean ± S.E.M. (n=6).
Value comparisons were made between *Musa acuminate* Vs control,

Table 2 Effect of *Ocimum gratissimum* on ALT & AST (Serum) and ALT & AST (Heart)

Treatment	ALT (Serum)	AST (Serum)	ALT (Heart)	AST (Heart)
Control	26.5±0.72	39.5±0.64	21.83±0.64	335±0.64
Musa acuminate	135±6.8	175.7±6.4	6.03±0.9	8.25±0.49
Ocimum gratissimum 100 mg/kg	115±4.9	153.3±4.7	9.12±0.48	10.57±0.72
Ocimum gratissimum 200 mg/kg	95±5.6	125.2±5.2	14.83±0.52	17.94±0.94

All Values were expressed as mean ± S.E.M. (n=6)
Value comparisons were made between *Musa acuminate* Vs control,

Histopathological Studies

At the end of the experiment, myocardial tissues from all the groups were subjected to histopathological studies. This evaluation was performed on lower portion of the heart tissue. Fresh heart tissues were excised and the fixed in 10 % Formalin for 24 hours. The fixative was removed by washing through running tap water. After dehydration through a graded series of alcohol, the tissues were cleaned in methyl benzoate, embedded in paraffin wax. Sections were cut into 5 µm thickness and stained with Haematoxylin and Eosin. After repeated dehydration and cleaning, the sections were mounted and observed under light microscope with magnification of 100x for histological changes (figure 1).

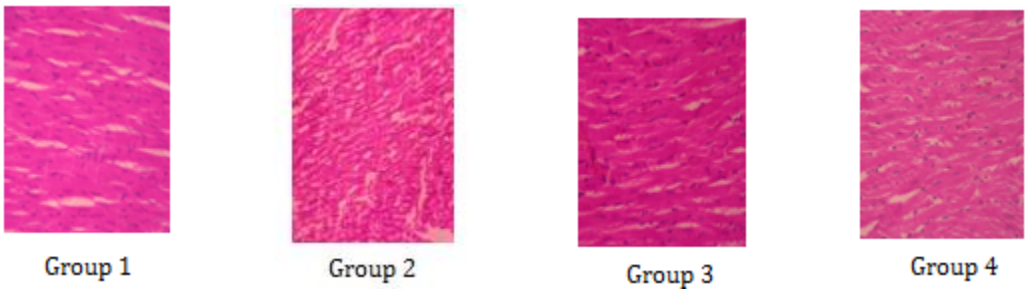

Figure 1 Histopathological study of cardiac muscle

High dose of *Musa acuminate* can cause an acute form of Cardio toxicity within 10 days of its administration. Evidence of cardiomyopathy with vascular involvement in rates has been

reported. Cellular mechanisms of cardiotoxicity are thought to be mediated by an increase in free radicals which affects endothelium and ion transport mechanisms. Cardiac pathology from *Musa acuminate* is by direct endothelial damage with extravasation of proteinaceous fluid, high concentrations of *Musa acuminate* and erythrocytes into myocardial interstitium and muscle cells. The determination of antioxidant status of heart after *Musa acuminate* administration in experimental models is important to develop strategies to reduce cardio toxicity.

In the present study, *Musa acuminate* administration significantly increased the activities of serum LDH, AST, ALT. These Observations are consistent with previous reports. Increased activities of these enzymes in serum are well known diagnostic indicators of cardiac injury. During myocardial necrosis these enzymes are released from heart to blood serum. *Ocimum gratissimum* restored the activities of these enzymes of these marker enzymes to near normalcy. This suggests the cardio protective role of *Ocimum gratissimum* which is in line with a recent report where *Ocimum gratissimum* has been shown to be protective against cardiac injury elicited by reperfusion. Free radicals cause membrane injury by initiating LPO which results in loss of function and integrity of myocardial membranes. The present data reveal that *Musa acuminate* exposure produced a marked oxidative impact as evidenced by increased LPO. This might results from increased production of free radicals and decreased in antioxidant status. As *Ocimum gratissimum* is soluble in both membranes and aqueous phases, it effectively prevents the damage of cell membranes by lipidperoxidase. The myocardium has a variety of endogenous antioxidants. The major antioxidant enzymes SOD, CAT act in coordination and provide cellular defense against reactive oxygen species (ROS) decline in the activities of these enzymes for *Musa acuminate* administration might be due to inactivation of these enzymes by ROS. This decline further aggravates the levels of free radicals in heart. CAT protects SOD against inactivation by Hydrogen peroxide. Reciprocally SOD may protect CAT from inhibition by Super oxide radicals. The low levels of enzymic antioxidants in heart make it vulnerable to free radical damage. *Ocimum gratissimum* prevents the free radical mediated inactivation of enzymes, restoring them to normal level. Besides, another possible reason for the lowered antioxidant activities in the *Musa acuminate* challenged tissues may be unit expression of enzyme activity. The specific activity of enzymes is expressed as its activity relative to the total protein content. Since *Musa acuminate* induces fibrosis and protein effusion into the heart, it further exaggerates the already down regulated antioxidant system. From these observations it is possible to conclude that *Musa acuminate* administration results in pronounced oxidative stress and myocardial damage. *Ocimum gratissimum* was found to be effective in normalizing the

antioxidants as well as cardiac markers. Further studies are to be conducted to understand the mechanism of action of *Ocimum gratissimum* as a cardio protective agent.

CONCLUSION

In the present study *Ocimum gratissimum* in low to moderate doses possess cardio protective effect. *Ocimum gratissimum* could provide beneficial effects to the heart and at moderate doses. In conclusion, *Ocimum gratissimum* was found to be effective in normalizing the antioxidants as well as the cardiac markers. This can be proven by decreased concentration of enzymes like SOD, CAT, LDH, AST and ALT upon administration of *Ocimum gratissimum* to the *Musa acuminate* induced cardio toxic rats. The levels of LPO got reduced upon administration of *Ocimum gratissimum*, by decreasing the oxidative stress caused by *Musa acuminate*.

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