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Cardio Protective Effect of *Asteracantha longifolia* Leaves in the Attenuation of Cardiac Injury Induced by Cyclophosphamide

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ABSTRACT

Treatment of myocardial infarction (MI) has undergone major advances in recent years, including reductions in mortality and hospital stays. The size of a MI and the concurrent effect on left ventricular (LV) function are essential for decisions regarding patient care and treatment. In the present study, cardio protective effects of *Asteracantha longifolia* leaves extract were evaluated in rat model having acute MI induced by Cyclophosphamide. Cyclophosphamide is one of the most prescribed Antineoplastic drug to cause lethal cardio toxicity. Adult male rats were divided into 4 groups. 3 groups receive single intraperitoneal injection of Cyclophosphamide (200 mg/kg) and *Asteracantha longifolia* was also included. Cardio toxicity, evident from increased activities of Serum Creatine phosphokinase, Lactate Dehydrogenase, Aspartate Transaminase and Alanine Transaminase in Cyclophosphamide administered rats, was reversed by *Asteracantha longifolia* treatment. Cyclophosphamide administered rat show abnormal levels of SOD, Catalase along with high Malondialdehyde levels. However, normalized Lipid peroxidation and antioxidant defences were reported in *Asteracantha longifolia* treated rats. 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 *Asteracantha longifolia* as a Cytoprotectant in Cyclophosphamide induced cardio toxicity.

Keywords: Cyclophosphamide; Cardio toxicity, *Asteracantha longifolia*, Antioxidants.

1. INTRODUCTION

Cyclophosphamide (CP) is widely used as an anticancer and immunosuppressant drug. It is used for treatment of chronic and acute leukemia's, multiple myeloma, lymphomas, and in preparation for bone marrow transplantation [1, 2] although it has tumor selectivity, it also possesses a wide spectrum of toxicities [3]. The crucial factor for therapeutic and toxic effects of CP is the requirement for metabolic activation by hepatic microsomal cytochrome P450 mixed functional oxidase system. Administration of high dose of CP could cause the lethal cardiotoxicity [4]. The cardiotoxic effect of CP consists of acute, dose dependent cardiac damage. Reactive oxygen species have been implicated in the development of cardiac toxicity after administration of CP [5]. Cardio toxicity of CP may be controlled by pharmacological interventions that reduce oxidative stress. *Asteracantha longifolia* 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. We have also identified it to be cardio protective. The aim of present study is to assess the oxidative stress and cardiac damage in CP administered rats.

2. MATERIALS AND METHODS

2.1. Drugs and Chemicals

Cyclophosphamide, 1,1,3,3-tetraethoxypropane, bovine, ferrous sulphate.

2.2. 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*.

2.3. Experimental Models of Myocardial Infraction

Cyclophosphamide induce cardio toxicity in rats.

2.4. Cyclophosphamide Induce Myocardial Infraction

Wistar albino rats of either sex (150–200 g) 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 Cyclophosphamide (200mg/kg) dissolved in saline, on the first day of the experimental period. Group III & IV animals were administered Cyclophosphamide and immediately followed by administration of 100mg/kg and 200mg/kg of *Asteracantha longifolia* 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.

2.5. Biological Parameters

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

Normal range of AST is 8 – 40 U/L at 37°C.Label reagent tubes as Blank, control and test. Incubate reagent tubes at 37°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°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].

2.5.2. Estimation of Serum Alanine Transaminase (ALT)

Normal range of ALT is 5 – 30 U/L at 37°C. Label reagent tubes as Blank, control and test. Incubate reagent tubes at 37°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°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].

2.5.3. Estimation of Superoxide Dismutase (SOD)

Superoxide dismutase was assayed according to Mark Lund method [87] 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° 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].

2.5.4. 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 per 100 mg protein [9].

2.5.5. 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].

3. RESULTS AND DISCUSSION

3.1. Effect of HAAL on mda

In the present study, the levels of MDA are decreased when compared to that of CP treated group. The levels of MDA are more in serum when compared to that of tissue.

Table .1. Effect of HAAL on mda

TREAMENT	MDA (TISSUE)	MDA (SERUM)
CONTROL	1.75±0.05	3.58±0.45
CYCLOPHOSPHAMIDE	3.9±0.06	5.87±0.56
HAAL 100 mg/kg	2.81±0.06	3.94±0.73
HAAL 200 mg/kg	1.81±0.09	3.26±0.26

Values were expressed as mean ± S.E.M. (n=6). Value comparisons were made between Cyclophosphamide Vs Normal, HAAL 100mg/kg, HAAL 200mg/kg.(*p<0.05). *-value is less significant, **-value is significant, ***-value is highly significant.

3.2. Effect of HAAL on SOD

In the present study, the levels of SOD are increased when compared to that of CP treated group. The levels of SOD are more in tissue when compared to that of serum.

Table .2. Effect of haal on SOD

TREAMENT	SOD (TISSUE)	SOD (SERUM)
CONTROL	5.05±0.09	2.87±0.16
CYCLOPHOSPHAMIDE	2.45±0.08	1.48±0.15
HAAL 100 mg/kg	4.87±0.06	1.83±0.17
HAAL 200 mg/kg	4.82±0.08	2.62±0.2

Values were expressed as mean ± S.E.M. (n=6). Value comparisons were made between Cyclophosphamide Vs Normal, HAAL 100mg/kg, HAAL 200mg/kg.(*p<0.05). *-value is less significant, **-value is significant, ***-value is highly significant.

3.3. Effect of HAAL on LDH

In the present study, the levels of LDH in tissue are increased when compared to that of CP treated group. The levels of SOD are less in tissue when compared to that of serum.

Table .3. Effect of HAAL on LDH

TREAMENT	LDH (TISSUE)	LDH (SERUM)
CONTROL	38.7±0.16	121.7±0.63
CYCLOPHOSPHAMIDE	18.93±1.12	326.9±0.72
HAAL 100 mg/kg	26.54±1.1	26.54±1.1
HAAL 200 mg/kg	31.07±1.7	31.07±1.7

Values were expressed as mean ± S.E.M. (n=6). Value comparisons were made between Cyclophosphamide Vs Normal, HAAL 100mg/kg, HAAL 200mg/kg.(*p<0.05). *-value is less Significant, **-value is significant, ***-value is highly significant.

3.4. Effect of haal on ALT and AST (serum)

In the present study, the levels of ALT and AST in serum are decreased when compared to that of CP treated group.

Table.4. Effect of haal on ALT and AST (serum)

TREAMENT	ALT (SERUM)	AST (SERUM)
CONTROL	26.5±0.72	39.5±0.64
CYCLOPHOSPHAMIDE	135±6.8	175.7±6.4
HAAL 100 mg/kg	115±4.9	153.3±4.7
HAAL 200 mg/kg	95±5.6	125.2±5.2

Values were expressed as mean ± S.E.M. (n=6). Value comparisons were made between Cyclophosphamide Vs Normal, HAAL 100mg/kg, HAAL 200mg/kg.(*p<0.05). *-value is less significant, **-value is significant, ***-value is highly significant.

3.5. Effect of HAAL ON ALT and AST (Heart)

In the present study, the levels of ALT and AST in heart tissue are increased when compared to that of CP treated group.

Table.5. Effect of HAAL ON ALT and AST (Heart)

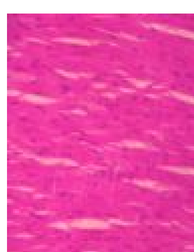
TREAMENT	ALT (HEART)	AST (HEART)
CONTROL	21.83±0.64	335±0.64
CYCLOPHOSPHAMIDE	6.03±0.9	8.25±0.49
HAAL 100 mg/kg	9.12±0.48	10.57±0.72
HAAL 200 mg/kg	14.83±0.52	17.94±0.94

Values (IU/L) were expressed as mean ± S.E.M. (n=6). Value comparisons were made between Cyclophosphamide Vs Normal, HAAL

100mg/kg, HAAL 200mg/kg.(*p<0.05). *-value is less significant, **-value is significant, ***-value is highly significant

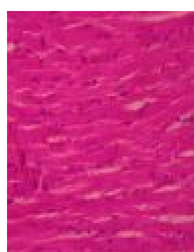
3.6. Histopathological studies

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.



Group 1

Group 2



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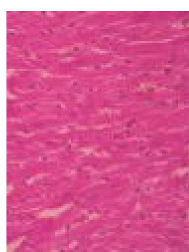

Group 4

High dose of CP can cause an acute form of Cardio toxicity within 10 days of its administration. Administration of intermittent massive dosage of CP has to be advantageous in chemotherapy. 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 through intra cellular phosphoramide mustard, the principal alkylating metabolite of CP which affects endothelium and ion transport mechanisms. Cardiac pathology from CP is by direct endothelial damage with extravasation of proteinaceous fluid, high concentrations of CP and erythrocytes into myocardial interstitium and muscle cells. The determination of antioxidant status of heart after CP administration in experimental models is important to develop strategies to reduce cardio toxicity.

In the present study, CP 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. *Asteracantha longifolia* restored the activities of these enzymes of these marker enzymes to near normalcy. This suggests the cardio protective role of *Rosa alba,* which is in line with a recent report where Asteracantha longifolia 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 cp 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 *Asteracantha longifolia* 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 CP 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. *Asteracantha longifolia* prevents the free radical mediated inactivation of enzymes, restoring them to normal level. Besides, another possible reason for the lowered antioxidant activities in the CP 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 CP induces fibrosis and protein effusion into the heart, it further exaggrevates the already down regulated antioxidant system.

From these observations it is possible to conclude that CP administration results in pronounced oxidative stress and myocardial damage. *Asteracantha longifolia* 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 *Asteracantha longifolia* as a cardio protective agent.

4. CONCLUSION

In the present study *Asteracantha longifolia* in low to moderate doses possess cardio protective effect. Pre and concurrent treatment of mild and moderate doses of HAAL (100 mg/kg) and HAAL (200 mg/kg) respectively offers protection from myocardial injury in CP induced myocardial damage. However, high dose of HAAL 500 mg/kg was found to increase the oxidative stress that could aggravate the pathological complications and was failed to prevent CP induced myocardial injury.

This can be proven by decreased concentration of enzymes like SOD, CAT, LDH, AST and ALT upon administration of *Asteracantha longifolia* to the CP induced cardio toxic rats. The levels of LPO got reduced upon administration of *Rosa alba,* by decreasing the oxidative stress caused by CP.

Therefore, *Asteracantha longifolia* flowers could provide beneficial effects to the heart and at moderate doses. In conclusion, *Asteracantha longifolia* was found to be effective in normalizing the antioxidants as well as the cardiac markers.

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