"EVALUATION OF CYTOPROTECTIVE EFFICACY OF CURCUMA CAESIA AGAINST CYCLOPHOSPHAMIDE INDUCED CARDIOTOXICITY"

Ravindra Mishra*, Harsha Rathore#, Radharaman Tiwari#, Vikas Basant, Dr. Vinay Jain#

Abstract

The present study investigates the "EVALUATION OF CYTOPROTECTIVE EFFICACY OF CURCUMA CAESIA AGAINST CYCLOPHOSPHAMIDE (CPY) INDUCED CARDIOTOXICITY**"** in Swiss albino mice. In traditional medicinal system different parts of the plant have been mentioned to be useful in a variety of diseases**.** The rhizome of Curcuma caesia contains bioactive components such as curcuminoids which is responsible for anti-oxidative, anti-inflammatory properties, wound healing, hypoglycaemia, anti-coagulant and anti-microbial activities The objective of the present study was to evaluate the cytoprotective efficacy of methanolic extract of curcuma caesia (rhizome) against cyclophosphamide (oncolytic agent) induced cardiotoxicity in Swiss albino mice by various haematological (RBC, WBC & Hb) biochemical analysis (serum analysis).Animals were divided into four groups of six animals each, total 24 animals were taken for this performing this activity. Group-I (control) received normal saline 1 ml/kg for two days. Group-II (positive control) received only cyclophosphamide drug at the dose of 200mg/kg; i.p on 0^{th} days for 48 hours. Group-III & IV received 250mg/kg and 500mg /kg of methanolic extract of rhizome of curcuma caesia prior 30 minutes before the administration along with CYP (200mg/kg; i.p) respectively for two days. The results showed that the efficacy of methanolic extract of curcuma caesia is potential towards the cardiotoxicity produced by the CYP on the tested animals during experiment. After the evaluation of certain parameters such as organ body weight index, WBC, RBC and Hb levels, serum LDH and creatinine kinase enzyme levels etc. we can conclude that the curcuma caesia shows effective results against the CYP induced cardiotoxicity ($p<0.05$) as compared to control group at a regular interval of 2-hour readings for 2 days. There was dose dependent decrease in cardiotoxicity volume in ethanolic extract treated groups. The methanolic extract (250mg/kg and 500mg/kg) significantly ($p<0.05$) and dose-dependently inhibited the cardiotoxicity induced by CYP.

Keywords- Cyclophosphamide, Cardiotoxicity, Curcuma Caesia, Rhizome, Cardiovascular, Disease.

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1. INTRODUCTION

1.1 Heart

Human heart is a muscular organ "covered in a double walled sack called the pericardium" the major function of the heart is to pump blood. When the functioning of heart is affected by any of the causes, it will lead to severe cardiovascular disease.

1.2 Cardiotoxicity

Cardiotoxicity is defining by as the 'toxicity that affects the heart'. It includes direct effect of the drug on the endothelial wall of heart and leakage of plasma protein and erythrocytes**.** The histologic finding indicates acute pericarditis and haemorrhagic myocarditis with fibrin platelet microthrombi in capillaries and fibrin strands in the interstitium on ultra-structural assessment**.** Cardiotoxicity has increasing relevance in cancer treatments, which lead to enhanced survival and least adverse effects. The mechanism of antineoplastic drugs induced cardio toxicity focused on targeted therapies, as well as these strategies decrease and treats this spectrum of toxic effects.

The cardiotoxicity of anticancer agents can lead to considerable difficulties that can affect patients being treated for a variety of cancer tumours. The severity of such toxicity depends on many factors such as the molecular site of action, the instant and cumulative dose, the method of administration, the presence of any primary cardiac condition, and the demographics of the patients. Moreover, toxicity can be affected by current or previous treatment with other antineoplastic agents. Cardiotoxic effects can produce instantly during administration of the drug, or they may not obvious themselves until months or years after the patient has been treated.

1.3 Enzymes That Detect Cardiac Toxicity:

1.3.1. Creatine Kinase

Creatine kinase is an enzyme articulated in a no. of tissues. Main function of ck is that catalyses the exchange of creatine to phosphocreatine degrading ATP to ADP. Creatine kinase is an indicator of cardiac muscle damage. CK levels begin to enhance about 3 to 12 hours after a heart attack, peak within 18 to 24 hours and regularly return to normal within two to three days. This makes the enzyme useful in detecting another heart attack within seven days of an initial attack, when blood troponin levels are still present. Normal levels are between 5-35U/ml.

1.3.2. Lactate Dehydrogenase

Lactate dehydrogenase catalyses the exchange of pyruvate to lactate. LDH levels are also high in tissue breakdown or haemolysis. LDH-1 is a form of the enzyme lactate dehydrogenase, which is particular for the heart. Levels increase about 28 to 48 hours after a heart attack and may remain in the blood for 6 to 8 days. Another form, LDH-2, usually stays constant during a cardiac condition, therefore the LDH-1, LDH-2 ratio is also an important index of cardiac damage. LDH-1 levels may also increase in conditions that damage red blood cells. Normal levels are between 150-450U/ml. Cyclophosphamide is an alkylating agent that has both potent and immunosuppressive properties and antineoplastic action.

1.4 Specific Drug Cause Cardiotoxicity

1.4.1. Cyclophosphamide

Cyclophosphamide (CYP) is widely used as a high spectrum antineoplastic. It is used for the treatment of cancer such as leukaemia, multiple myeloma, lymphomas, rheumatic arthritis and in bone marrow transplantation. However, its clinical use is limited by seriously high incidence of systemic cardiotoxicity. A number of CYP induced biochemical changes have been identified that can damage reactive oxygen species, production of reactive nitrogen species, inhibition of cardiac muscle gene expression, induction of cardiac cell apoptosis.

Cyclophosphamide rapidly impairs cellular respiration and also damages myocardium of heart leading to the permeability of calcium ions mediated by oxidative stress. Cyclophosphamide induced cardiotoxicity has been implicated to increase the generation of superoxide radicals and hydrogen peroxide. These reactive oxygen species (ROS) damage the heart by increasing the oxygen radical detoxifying capacity of cardiac mitochondria. Therefore, the antioxidant therapy may be useful in the management of CYP induced cardiotoxicity.

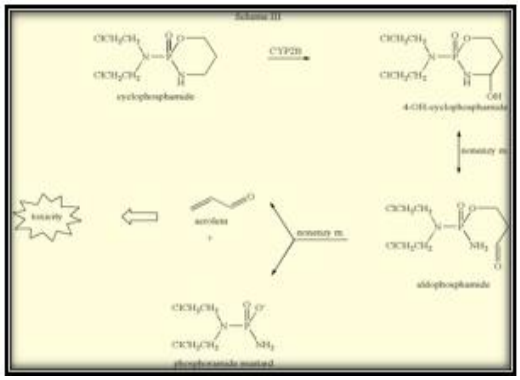

Figure 1. Cyclophosphamide Metabolites

Cyclophosphamide is an inactive prodrug (alkylating agent) that requires metabolic activation by the cytochrome P-450 enzyme system. The process of CYP activation produces hydroxylated active metabolites, e.g., acrolein, phosphoramide mustard, and nitrogen mustard, which is toxic agents, or the inactive compound carboxyphosphamide (Tew *et al*., 1996). CYP metabolites react with carboxyl (COOH), mercapto (-SH), amino (-NH_2), phosphate (-PO_3H_2), and hydroxyl (-OH) groups and can form cross-links with DNA and proteins (Slavin *et al*., 1975; Fleming, 1997; Nieto *et al*., 2000). CYP exert its cardiotoxic effects through produce toxicity in the endocardial capillary endothelium, resulting in increased permeability and microthromboses and extravasations of plasma & red blood cells in to the myocardium (Fleming, 1997; Slavin *et al.*, 1975).

1.4.2. Metabolism of Cyclophosphamide

Cyclophosphamide (CY) is metabolized to 4-hydroxycyclophosphamide (HCY) in the hepatic cytochrome P-450 enzyme (CYP) system (CYP2B6 and/or CYP2C19). HCY enters cells as tautomer aldocyclophosphamide (AldoCY). Through β-elimination, AldoCY can be converted to phosphoramide mustard (PM) & acrolein. Alternatively, AldoCY can be also oxidized to the inactive metabolite o-carboxyethylphosphoramide mustard (CEPM) by aldehyde dehydrogenase 1 (ALDH1). Other metabolites include chloroacetaldehyde (CAA), deschloroethyl-cyclophosphamide (DCCY),4-keto-cyclophosphamide (KetoCY), hydroxypropyl-phosphoramide mustard (HPPM), iminocyclophosphamide (IminoCY), and glutathionyl-cyclophosphamide (GSCY).

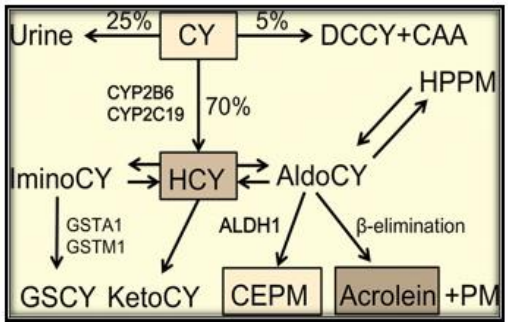

Figure 2. Cyclophosphamide Metabolic Pathway

1.5 Cytoprotective Herbal Drugs

Now –a-day natural products are an essential part of human health care system, because there is now accepted concern over toxicity and side effect of modern drugs. There is also a understanding that natural medicines are safer and allopathic drugs are often ineffective. Heart disease and drug induced cardiac toxicity remain as one of the serious health problems. However, we do not have reasonable heart protective drugs in allopathic medical practice for serious heart disorders. Herbal drugs play a role in the organization of various heart disorders and adverse outcome most of which speed up the natural curative processes of the heart. Number of medicinal plants and their formulations are used for heart cytoprotecion in india. More than fifteen of these plants are evaluated for their cardioprotective action in glow of modern medicine *(A. Subramoniam et al., 1998).*

Scientists and some industrialists deliberate on various potential plant remedies for ailment of heart disorder and adverse effect organization. In the decade 70s, the world scientific community concentrated on a herbal plant *vinca rosea*. Then in 80s the attention was focused on *panics ginseng*. Now, the news of diverse activities of the neem tree indicates that it may become centre for research in 90s. Indian Council of Medical Research, New Delhi, in its revived research on traditional medicine, had adopted heart disease as one among six thrust areas and for multidisciplinary study. Screening of active constituents from hawthorn (*crataegus oxycanthus*), garlic (*allium sativum*) have shown marked protection against heart failure. For example, extracts of amla (*emblica officinalis*), Danshen *(salvia miltiorrhiza*), the disorder of heart may be acute coronary syndrome (ACS), ischemic heart disease, myocardial infarction. Heart enzymes act as a catalogue of sub-clinical heart damage. Creatine kinase (CK), lactate dehydrogenase (LDH), superoxide dimustase (SOD), serum glutamic oxaloacetic pyruvic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) is reported as a cardiac injury.

2. PLANT PROFILE

2.1 Curcuma Caesia

The genus "Curcuma" is a well-known spice of India. It is called as haldi and more than 200 species and subspecies of it is found all crossways the world. One of which is *Curcuma caesia* Family Zingiberaceae. It is also known as "Kali Haldi". It is a vertical rhizomatous herb with large leaves. Fresh rhizomes are aromatic with strong camphoraceous odour and are functional externally to sprain and bruises *(The Wealth of India: A Dictionary of India Raw Materials & Industrial Products. Vol. II, 2001)***.** Black Turmeric (*Curcuma caesia*) is resident to North-East and Central India. It is also sparingly found in papi hills of East Godavari, the root hills of the Himalayas and North

hill forest of Sikkim. The rhizomes of Black Turmeric have a high reasonable importance owing to its reputed medicinal properties. The rhizomes are used in the treatment of haemorrhoids, leprosy, asthma, cancer, epilepsy, fever, wound, vomiting, menstral disorder, smooth muscle relaxant activity *(Singh V et al; 2003)*, anthelmentic, aphrodisiac, inflammation, gonorrhoeal discharges, etc. *(Singh V et al; 2003, Arulmozhi DK et al; 2006*). Almost all species of *Curcuma* contains antioxidant activity and the pharmacological effects and diagnosis for future clinical use had been tried so far *(Miquel J et al; 2002).* Its rhizomes have been usually is used in treating leucoderma, asthma, tumours, piles, epilepsy, toothache, fever, vomiting, bronchitis etc *(Chopra et al; 1958; Amalraj et al; 1989; Khare, 2007).* The rhizome paste is applied on bruises, contusions & rheumatic pains. The rhizome is also used in dysentery, diarrhoea and cough as aromatic and as a source of d-camphor *(Craker and Simon, 1996; Sarangthem and Haokip, 2010).* Earlier workers reported the essential oil composition form *C. Caesia* rhizome and its antifungal *(Banerjee and Nigam, 1976)*, anti-asthmatic and smooth muscle relaxant effects in guinea pig trachea *(Arulmozhi et al; 2006)***,** antimicrobial activity *(Krishnaraj et al; 2008)*, phenolics contents and in vitro antioxidant activity of *C. caesia* rhizomes *(Krishnaraj et al; 2010, Mangla et al; 2010).* occurrence of curcuminoids, phenolics, flavonoids, volatile oil, protein, amino acids and alkaloids were reported in the rhizomes of Indian *C. caesia (Sarangthem and Haokip, 2010)*, and CNS depressant activities some in vitro antioxidant activity (Effect of MECC on ROS and RNS) *(Indrajit Kaemakar et al; 2011).*

(a) Plant (b) Rhizomes (c) Fresh Rhizomes (d) Dried Rhizome

Figure 3. Different Parts of Curcuma Caesia

Kingdom	***Plantae***
Subkingdom	*Viridaeplantae*
Phylum	*Tracheophyta sinnott*
Class	*Magnoliopsida*
Order	*Zingeberales*
Family	*Zingiberaceae*
Subfamily	*Zingiberoideae*
Tribe	*Hedychieae*
Genus	*Curcuma*
Species	*Curcuma caesia*

Table 1: Taxonomical Classification

2.4 Phytochemical Studies

Various physicochemical constants, such as moisture content, ash value, acid-insoluble ash, LOD, water and alcohol soluble extractive values are depicted in *Table 2.* The percentages of successive Soxhlet solvent extractives calculated and the results are depicted in *Table 3.* Preliminary phytochemical studies showed the presence of

alkaloids, steroids, phenolics, and tannins as the major constituents in the successive solvent extraction *(Table 4).*

Parameters	***Value (%) w/w***
Ash content	*9.028*
Acid insoluble ash	*4.31*
Loss on drying	*9.944*
Moisture contents	*8.8*
Water soluble extractives	*13.68*
Alcohol soluble extractives	*6.17*
Volatile oil	*1.8*

Table 2: Physicochemical constants rhizomes of *Curcuma caesia*

Parameters	***Value (%) w/w***
n-Hexane	*2.23*
Petroleum ether	*0.79*
Benzene	*0.92*
Chloroform	*0.36*
Ethyl acetate	*0.67*
Methanol	*4.68*
Water	*1.14*

Table 3: Successive value of *Curcuma caesia* rhizomes.

Class of compounds	*n-Hexane extract*	*Pet. Ether extract*	*Benzene extract*	*Chloroform extract*	*Eth.acetate extract*	*Methanol extract*	*Water*
Alkaloids (Dragendroff's)	-	-	-	-	-	+	+
Carbohydrates (Molish's)	-	-	-	-	-	+	+
Steroids/Terpenoid (Liberman)	+	+	-	+	+	-	-
Protein/aminoacid (Millon's)	-	-	-	-	-	-	-
Saponin (foam)	-	-	-	-	-	-	-
Fixedoil/fat (tincturealkana)	+	-	-	-	-	+	-
Flavonoids (shinoda)	-	-	-	-	-	+	-
Phenolics(ferricchlo r)	-	-	-	-	+	+	+
Tannins (brominewater)	-	-	-	-	-	+	+

Table 4: Phytochemical screening of *curcuma caesia*, rhizome extracts.

2.5 Bioactive Components in *Curcuma Caesia*

Curcuma Caesia contain maximum curcuminoids, oil content, flavonoids, phenolics, different important amino acids, protein and high alkaloid content which reveals that the presence of these bioactive secondary metabolites correlates with the medicinal uses of *C. Caesia* as fragrances, flavouring and many important useful pharmaceutical products *(Kananbala Sarangthem et al; 2010).*

The research on the volatile oil of *C. caesia* rhizomes resulted in the identification of 30 components, representing 97.48% of the oil, with camphor (28.3%), ar-turmerone (12.3%), (*Z*)-ocimene (8.2%), ar-curcumene (6.8%), 1, 8-cineole (5.3%), elemene (4.8%), borneol (4.4%), bornyl acetate (3.3%) and curcumene (2.82%) as the major constituents. *(Panday and Ashim, 2003)*

Parameters	***Contents in rhizomes***
Total curcuminoid (mg/g dry wt.)	*78.4± 0.06*
Volatile oil content (% v/w)	*6.75± 1.12*
Total phenols (mg/g dry wt.)	*60± 0.03*
Flavonoids (mg/g dry wt.)	*30± 0.06*

Alkaloids (mg/g dry wt.)	*04.25± 1.66*
Soluble protein (mg/g fresh wt.)	*47.5± 1.9*

Table 5: Content of volatile oil, total curcuminoids and other bioactive components in Curcuma caesia.

2.6 Ethnobotanical Claims

The *Curcuma caesia* rhizome is pungent, bitter, fragrant, heating, appetizer, vulnerary, anthelmintic, antipyretic, alexiteric, destroys foulness of the breadth, useful in leucoderma, piles, bronchitis, asthma, tumours, tuberculous, glands of the neck, enlargement of the spleen, epileptic seizure. The rhizome has a bitter, sharp, pungent taste and a good odour, laxative, tonic to the brain and the heart, aphrodisiac, alexipharmic, emetic, emmenagogue, expectorant, carminative; useful in gripping of children, pains, inflammation and toothache.

2.7 Pharmacological Activity

Medicinal uses of the *Curcuma caesia* rhizome arise from the bioactive components. Bioactive components such as curcuminoids are responsible for anti-oxidative and anti-inflammatory properties, wound healing, hypoglycaemia, anti-coagulant, anti-microbial activities *(chatopadhyay et al; 2004).* Curcuminoids exhibit free radical scavenging property and antioxidant activity *(Jayaprakasha et al; 2006).* Main bioactive substances in the rhizomes are due to curcumin and two related dimethoxy compounds, demethoxy-curcumin and bisdemethoxy curcumin. Flavonoids & phenolic compounds which are widely distributed in different parts of plants have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc. *(Miller et al; 1996).*

3. MATERIAL AND METHODS

3.1 Drug: - Cyclophosphamide (manufacturing company: Zydus oncoscience, Ahmedabad)

3.2 Chemicals and Reagents

Chemicals	***Manufacture***
Curcuma caesia	*Aayush Ayurvedic lab, Gwalior.*
Potassium Chloride	*Johnson laboratories INC.*
Methyl alcohol	*Glaxo smith pharmaceutical, Mumbai.*
Sodium chloride	*Central drug house, New Delhi*
EDTA	*Central drug house, New Delhi*
Ether	*Avantor performance, India*
Glutathione	*Merck Ltd. India*
Thio barbituric acid	*Qualikems finisher, Vadodara*
Tri's buffer	*Himedia lab, Mumbai*
Mayer's reagent	*Central drug house, New Delhi*
Molisch's reagent	*Avantor Performance Material, India*
Dragendorff's reagent	*Central drug house, New Delhi*
Hager's reagent	*GlaxoSmithKline, Mumbai*
Million's reagent	*Jyoti chemicals, Modinagar*

Table 6: List of Chemicals and Reagents

3.3 Instruments and Equipment's

Electronic balance	*Wensar ltd, India*
UV spectrometer	*Systronics ltd, India*
Micropipette	*Jyoti scientific laboratories*
pH meter	*Jyoti scientific laboratories*
Heating mantle	*Jyoti scientific laboratories*
Digital microscope	*Jyoti scientific laboratories*
Soxhlet apparatus	*Borosil ltd, India*
Test tubes/ beakers	*Borosil ltd, India*
Petri disk/ glass rod	*Borosil ltd, India*
Oral feeding needles	*Jyoti scientific laboratories*

Polypropylene rat and mice cages	*Jyoti scientific laboratories*

Table 7: List of Instruments and Equipment

3.4 Plant Material

The rhizome of C. caesia was collected from the Dandori district, M.P, India plant was identified and authenticated by Dr. Vinay Jain, HOD, Pharmacognosy, Shriram college of pharmacy Banmore, Morena (M.P)

3.5 Preparation of Extracts

Rhizome are cut into small pieces (5cm), shade dried and ground to fine powder. The powdered plant material (450gm), was extracted with methanol (750ml), using a Soxhlet apparatus at a temperature of 55 to 60°C for a period of 16 hours. The crude extracts were filtered and evaporated to dryness in vacuum at 35°c and 0.8° m.p in a buchi evaporator. The residues were weighed and stored at 4° C until use.

3.6 Animals

Male Swiss albino mice weighing (23-28 g) were obtained from the central animals of Shriram College of Pharmacy, Banmore and were maintained in polypropylene cages on rodent pellet condition of controlled temperature (22±2°C) and acclimatized to 12/12 h light/dark cycle. Free access to food and water were allowed until 2 hours before the experiment. The care and maintenance of the animals were as per approved guidelines of the "Committee for the purpose of control and supervision of experiment on animals (CPCSEA)". Food and water were provided 2 hours after the experiment. All experiment on animals were conducted according to the guidelines of establishment's ethical committee on animal experimentation.

3.6.1. Experimental Design

Male Swiss albino mice are divided into 4 groups, the first group(control) received normal saline 1ml/kg for two days. The second group (positive control) received only cyclophosphamide drug 200mg/kg; i.p on 0th day for 48 hours. Both 3rd and 4th groups are treated received 250mg/kg and 500mg/ kg of methanolic extract of rhizomes C. caesia prior 30 minutes before the administration along with CYP (200mg/kg; i.p) for two days.

3.6.2. Grouping And Dosing of Animals

Total no. of animals = 24, divided into 4 groups,6 in each

Group I: Normal control (vehicle only i.e., 0.9 % NaCl).

Group II: Cyclophosphamide (200mg/kg; i.p).

Group III: C. caesia (250mg/kg; orally) + CYP (200mg/kg; i.p)

Group IV: C. caesia (500 mg/kg; orally) + CYP (200mg/kg; i.p)

The body weight of the animals was also recorded for two days. After 48 hours animals were anaesthetized with ether for the collection of blood from retro orbital plexus, and then the sacrificed by cervical dislocation for the removal of heart. Various haematological & biochemical analysis were carried out.

3.7 Haematological parameters

The haematological variables viz. RBC, WBC and Hb were measured.

3.7.1. Biochemical Evaluation in Serum.

Serum MDA Assay: - The reaction mixture containing 1ml 0.67% thiobarbituric acid (TBA), 1ml of 2 ml 20% trichloroacetic acid (TCA), and 100 micro litre serum was incubated at 100° Celsius for 20 min and centrifuged at 12,000 rpm for 5 min. the absorbance of the supernatant was read at 532 nm and MDA concentration was determined by using a molar extinction coefficient of 1.56× 105/M/cm and the values was expressed as mm.

3.7.2. Biochemical Evaluation in Heart Tissue

MDA Assay: - A portion of the heart was used for the biochemical estimation. Heart lipid peroxidation was determined by measuring the level of MDA according to the method of *(ohkawa et.al.,1979).* 2ml of suspension medium was taken from the supernatant of the 10 % tissue homogenate in 1.5 % KCl and centrifuge at 5000 rpm for 10 min.1 ml of 30% TCA followed by 1 ml of 0.8% TBA was added to it. the tubes were covered with aluminium foil and kept in shaking water bath for 30 min at 80°C for 30 min. After 30 min. tubes was taken out and kept in ice cold water for 10 minutes. They were than centrifuge at 3000 rpm for 3 minutes. The absorbance of supernatant was read at 540 nm at room temperature against blank (2 ml distilled water 1ml TBA and 1 ml TCA).

GSH Assay: - In this assay Tissue GSH was determined by the method of *Sedlak and Lindsay,1968*. A portion of the re-perfused heart tissue 250-300 mg homogenized in 5-8 ml of 0.02 M EDTA and then 4 ml cold distilled was added to it. After mixing of 1ml of 50% TCA was added to it and shaken for 10 minutes and centrifuged at 6000 rpm for 15 min. 4 ml of 0.4 M tris buffer was mixed with supernatant and 0.01 M DTNB. The

absorbance of this resulting mixture was read at 410nm at room temperature against reagent blank.

3.7.3. Histopathology of Heart

Tissue Fixation: - Heart tissue of mice was removed and washed with normal saline.
Formaldehyde, as 4% buffered formaldehyde (10% buffered formalin), is the most widely employed universal fixative particularly for routine paraffin embedded section. The cleared tissue in 10% natural buffered formalin solution (Ph 7.0-7.2).

3.8 Stages of Processing

1. Dehydration
2. Clearing
3. Embedding
4. Staining.

3.9 Statistical Analysis

Statistical evaluation was made using one way ANOVA followed by Dunnett's test. A probability of 0.05 and less was taken as statistically significant. The analysis was carried out using SPSS 16.0.

4 RESULT AND OBSERVATION

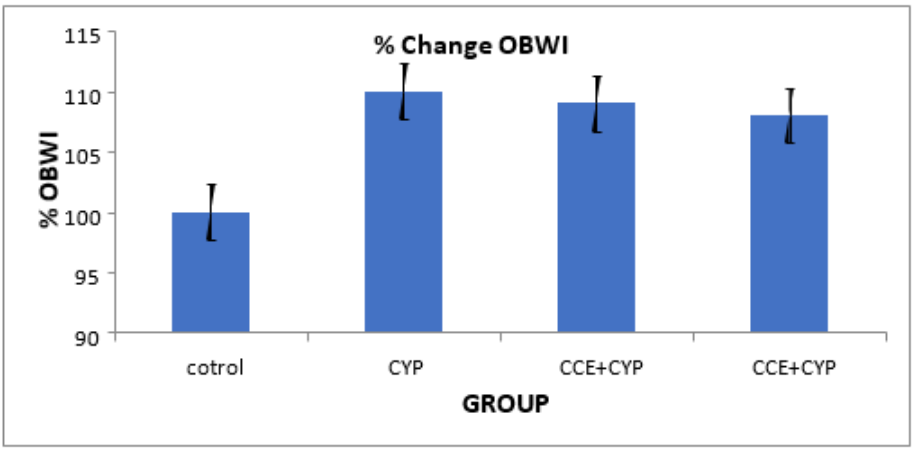

Figure 4: Effect of pre-treatment of CCE on heart organ body weight indices (OBWI).

Particulars	***Description***
Plant name	*Curcuma caesia*
Part used	*Rhizomes*
Solvent used	*95% Methanol*
Weight of dried Fruit	*450 gm*
Practical yield	*30gm*
Percentage yield	*6.7*

Table 8: Percent yield of extract of shade dried powered rhizomes of *Curcuma caesia*

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P<0.05, compared to control group (One way ANOVA followed by Dunnett's test).

P<0.05, compared to positive control group (One way ANOVA followed by Dunnett's test).
Control value for heart OBWI: 2.98± 0.18.

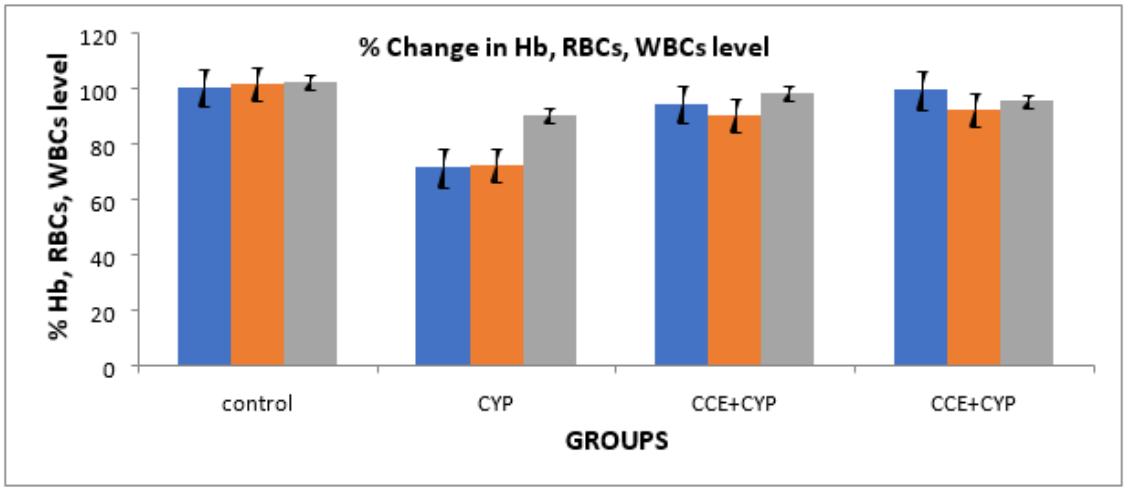

Figure 5: Effect of pre-treatment of CCE on % Hb, RBCs, and WBCs level in CYP induced cardiotoxicity in mice.

P<0.05, compared to control group (One way ANOVA followed by Dunnett's test).
P<0.05, compared to positive control group (One way ANOVA followed by Dunnett's test).

Control value for Hb: 14± 0.6gm%, RBCs: 4.5± 0.14 million/cms, WBCs: 6200± 315/cms.

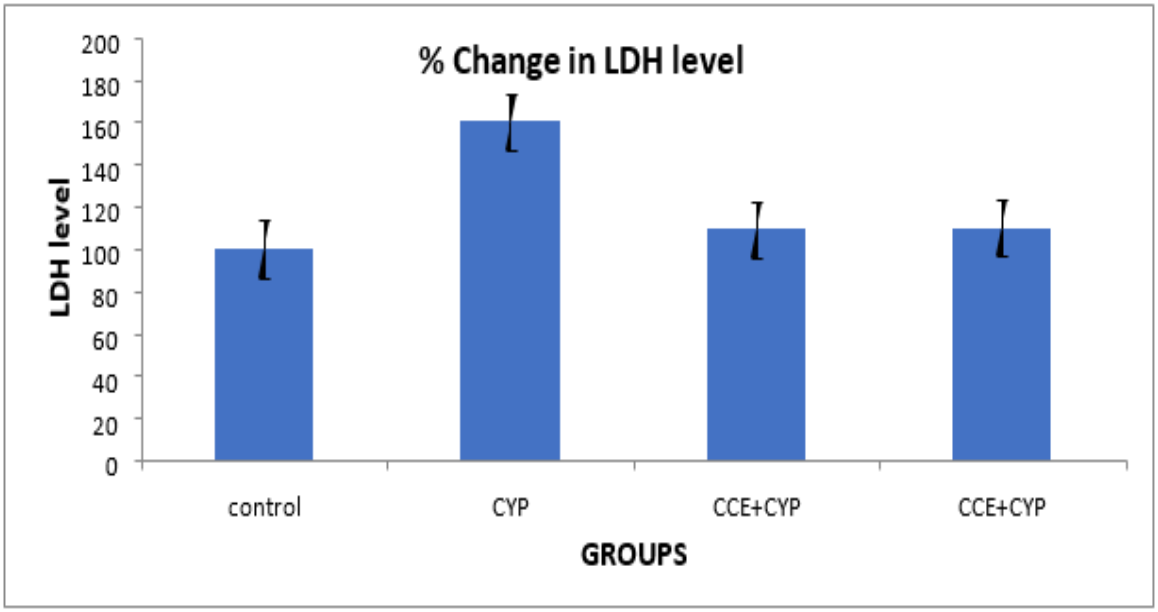

Figure 6: Effect of pre-treatment of CCE on % LDH level in CYP induced cardiotoxicity in mice.

P<0.01, compared to control group (One way ANOVA followed by Dunnett's test).

P<0.05, compared to positive control group (One way ANOVA followed by Dunnett's test).
Control value for LDH: 123± 5.6 U/L

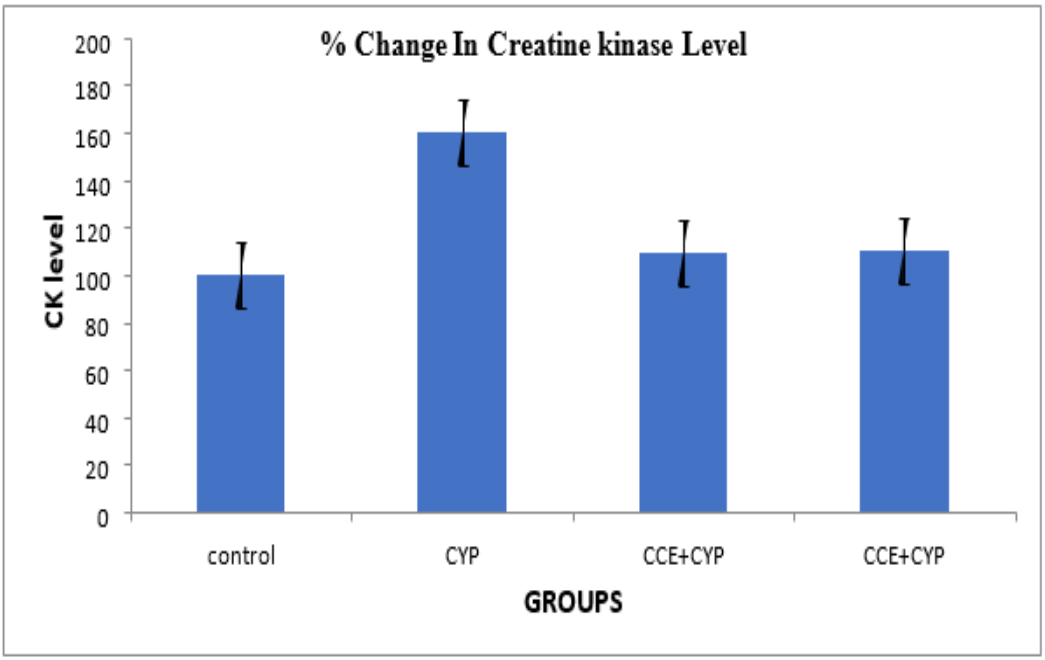

Figure 7: Effect of pre-treatment of CCE on % CK level in CYP induced cardiotoxicity in mice.

P<0.05, compared to control group (One way ANOVA followed by Dunnett's test).

P<0.05, compared to positive control group (One way ANOVA followed by Dunnett's test).
Control value for CK: 78.57± 5.4 U/L

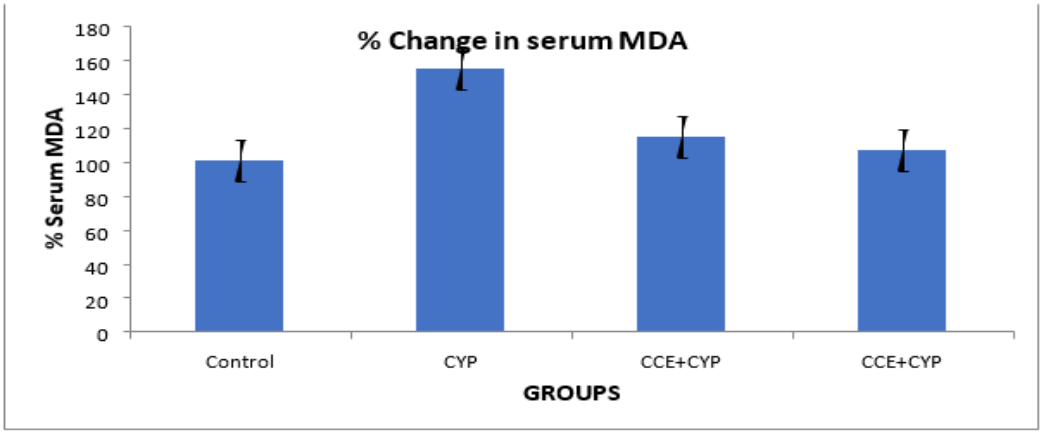

Figure 8: Effect of pre-treatment of CCE on % serum MDA level in CYP induced cardiotoxicity in mice.

P<0.05, compared to control group (One way ANOVA followed by Dunnett's test).
P<0.05, compared to positive control group (One way ANOVA followed by Dunnett's test).

Control value for Serum MDA: 0.22± 0.07x10 $^{-3}$ mM.

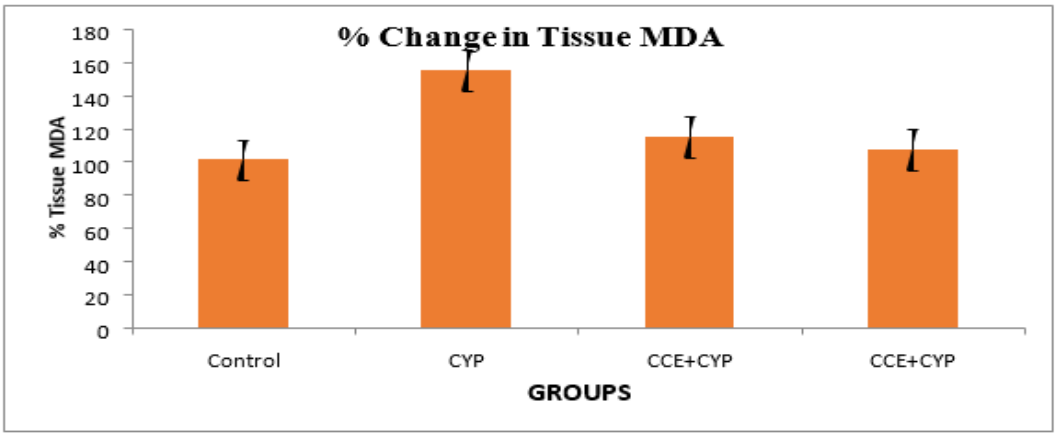

Figure 9: Effect of pre-treatment of CCE on % Tissue MDA level in CYP induced cardiotoxicity in mice.

P<0.05, compared to control group (One way ANOVA followed by Dunnett's test).
P<0.05, compared to positive control group (One way ANOVA followed by Dunnett's test).
Control value for Tissue MDA: 93.2± 0.2 nmol/gm of tissue.

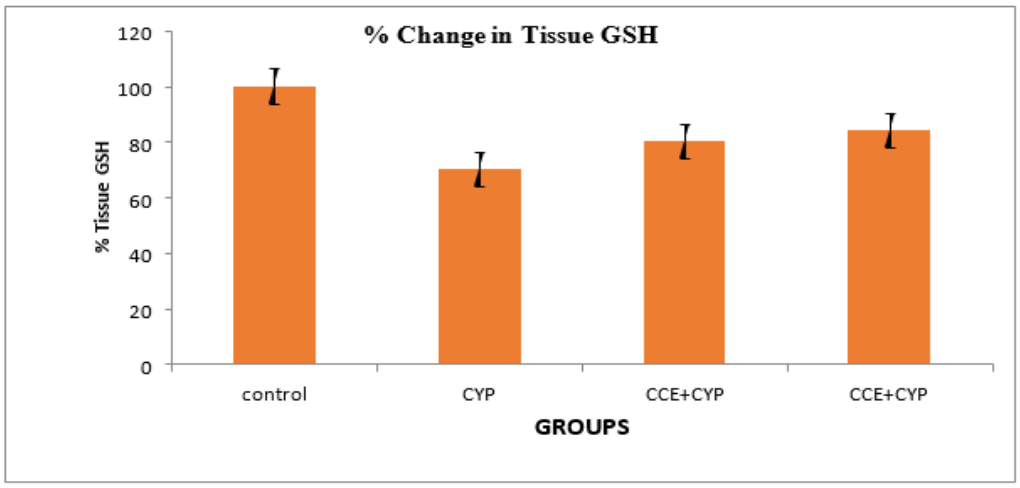

Figure 10: Effect of pre-treatment of CCE on % Tissue GSH level in CYP induced CP; Cardiotoxicity in mice.

P<0.05, compared to control group (One way ANOVA followed by Dunnett's test).
P<0.01, compared to positive control group (One way ANOVA followed by Dunnett's test).
Control value for Tissue GSH: 7.8± 0.8micro moles/gm of tissue.

Histopathology of Heart Tissue

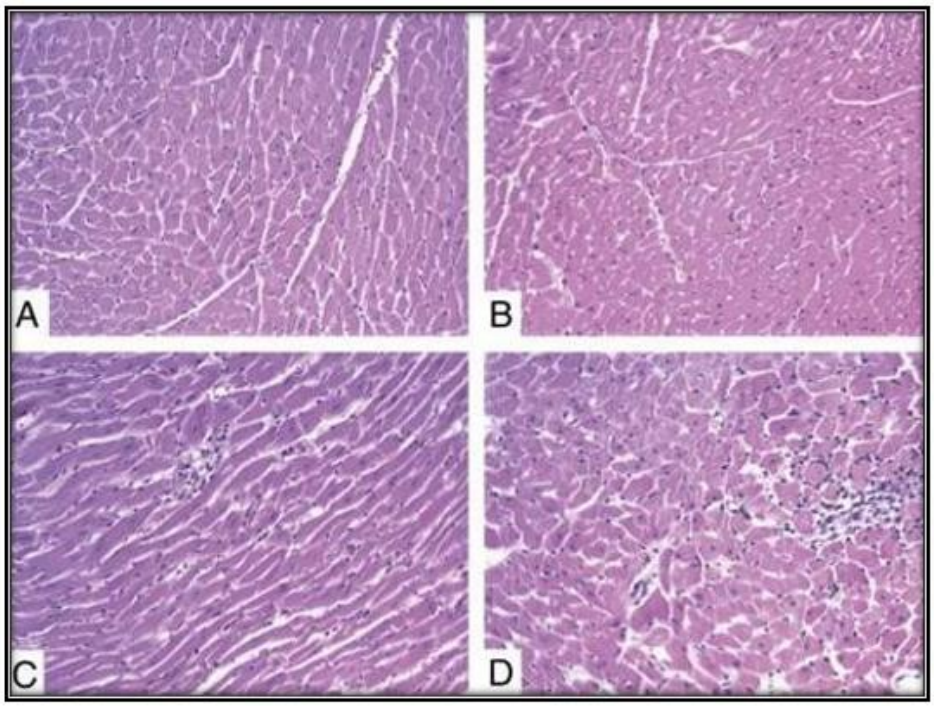

Figure 11: Photomicrographs of heart tissue of mice treated with CYP, CCE.

Fig.11 (A) showing the histology of normal heart tissue in *Group-I*, treated with vehicle exhibited normal myocardial cells each with well define mycoplasma, prominent nucleus and nucleolus.

Fig.11 (B) showing the histology of heart section in *Group-II*, treated with cyclophosphamide (200mg/kg), shows damage of myocardial architecture with myocardial necrosis, fatty changes and inflammation.

Fig.11 (C) showing the histology of heart tissue in *Group-III*, Curcuma caesia (250mg/kg) + CYP clearly show potential recovery of normal myocyte compared to cyclophosphamide treated group.

Fig.11 (D) showing the histology of heart tissue in *Group-IV*, treated with Curcuma caesia(500mg/kg) plus CYP returned the injury heart to quite normal when compared to cyclophosphamide group.

Table 9: Effect of pre-treatment of CCE on histopathology of heart in Cyclophosphamide induced cardiotoxicity in mice

5.RESULTS

The cyclophosphamide induce cardiac injury was treated with *Curcuma caesia* for two days continuously. The following observations were obtained.

5.1 OBWI and Body Weight

Figure 4 shows OBWI were changed extensively by pre-treatment with *Curcuma caesia* III, IV group evaluate to cyclophosphamide treated group. Initial & final body weights of all groups. CYP treated group show change in body weight which was not significant as compare to control group. pre-treatment with *Curcuma caesia* and III and IV group show little changes in body weight which were not significant as compare to CYP treated group.

5.2 Haematological Parameters

Figure 5 shows, WBC, RBC count and Hb level. CYP treated group show significant ($P<0.05$) decrease in WBC count., depletion of Hb and RBCs count were not changed significantly as compared to control group. pre-treatment with CCE in test group was significantly changed ($p<0.05$) WBC count and Hb as compared to CYP treated group, Where RBCs count were not changed significantly as compared to CYP treated group.

5.3 Biochemical Evaluation in Serum

Figure 6,7 shows the activities of serum LDH and creatine kinase (CK) enzymes in all treatment groups. There was a significant increase ($p<0.05$) was observed in CYP treated group compared to control group. Pre-treatment with CCE show

significant changes in LDH and CK activities compared to CYP treated mice.

5.4 Serum MDA

Figure 8 shows Serum MDA level. Serum MDA was significantly increase ($p<0.05$) in CYP treated group, compared to the Control group. Pre-treatment with CCE was significantly changed ($p<0.05$) serum MDA as compared to CYP group. decrease of serum MDA levels in CCE treated groups was in dose dependent manner.

5.5 Tissue MDA

In order to evaluate the effect of treatments on lipid peroxidation. MDA levels were assay in tissue (Figure 9). Lipid peroxidation was increased significantly in CYP treated group compare to the control group ($p<0.05$). Pre-treatment with CCE significantly ($p<0.05$) reduced CYP induced increase of TBARS content as compare to CYP treated mice. Tissue MDA levels in CCE treated groups were reduced in dose dependent manner.

5.6 Tissue GSH

Figure 10 summaries tissue GSH levels. Tissue GSH levels was decreased significantly ($p<0.05$) in CYP treated mice as compared to control group. The data showed that CCE significantly ($p<0.05$) improved the decreased levels of tissue GSH as compared to CYP treated group. Effectiveness of CCE at both doses were in dose dependent manner.

5.7 Histological Studies

The result of histological study has existing in Figure 11 exposure of mice cardiac myocytes to Cyclophosphamide lead to histological changes including increased cardiac injury scores simultaneous with the presence of muscle necrosis, edema and inflammation in comparison with control.

6. DISCUSSION

Cyclophosphamide is an anticancer drug that is very effective in the treatment of various human cancers particularly lymphomas and some types of leukaemia and autoimmune diseases. The clinical value of the oncolytic agent has been compared by the dose that most common obstacle myocarditis in heart *(Hu et al., effects of curcumin, 2008).* Due to the pleiotropic effect of dose-limiting toxicities; one complication is that a suitable candidate to be tested in the present work for cardiotoxicity of any possible protective effects *(Arafa, 2009).* Cyclophosphamide is challenge to encourage cardiotoxicity that was well considered morpho-logically and biochemically. Cardiotoxicity was manifested by obvious heart congestion, oedema, cardiac inflammation and extravasation in the cardiac tissues, as well as a marked leucocytic in filtration as resolute by macroscopic and histopathological examination *(Lieber et al., 1984; Malley and Vizzard, 2002).*

In case of toxic heart, heart weight and heart volume are enlarged. In this case water is retained in cytoplasm of cardiocytes leading to enlargement of heart cells, consequential in increased total heart mass and volume. In present study body weight was increased in CYP treated groups but not significantly as compared to control group. Body weight was not extensively changed by pre-treatment with methanolic extract of *Curcuma caesia.*

Cyclophosphamide is quickly decreased the WBCs, RBCs and Hb, Similar results were earlier documented. Though the exact pathogenesis of CYP where by cyclophosphamide induces toxicity is mediated through its toxic metabolite, acrolein and mustered the molecular events underlying such toxicity are yet to come. The pathogenetic pathways may include oxidative species damage, discharge of some inflammatory molecules such as cytokines and nitric oxide as well as poly (adenosine- di- phosphate ribose) polymerase activation. In present study treatment with *Curcuma caesia* the haematological parameters are enhance in small amount.

Cyclophosphamide is quickly increased the serum level of LDH and CK enzyme activities. related results were previously accepted *(Wong et al., 2000; Linares Fernández and Alfieri, 2007).* Though the accurate pathogenesis of CYP where by cyclophosphamide induces toxicity is mediate through its toxic metabolite, acrolein and mustered the molecular proceedings underlying such toxicity are yet to come. The pathogenetic pathways may comprise oxidative species break, discharge of some inflammatory molecules such as cytokines and nitric oxide as well as poly (adenosine- di-phosphate ribose) polymerase activation *(Dang et al.,2008*). In the present study there was inhibition of those enzymes when treated with *Curcuma caesia.*

Cyclophosphamide is rapidly increased the serum level of MDA enzyme activities. Comparable results were earlier recognized though the exact pathogenesis of CYP where by cyclophosphamide induce toxicity is mediate through its toxic metabolite, acrolein and mustered the molecular actions underlying such toxicity are yet to come. The pathogenetic pathways may comprise oxidative species break, release of some

inflammatory molecules such as cytokines and nitric oxide as well as poly (adenosine- di-phosphate ribose) polymerase activation. In the present study there was inhibition of those enzymes when treated with *Curcuma caesia.*

Cyclophosphamide is fast increased the tissue MDA enzyme activities. like results were previously recognized though the exact pathogenesis of CYP where by cyclophosphamide induce toxicity is mediate through its toxic metabolite, acrolein and mustered the molecular events underlying such toxicity are yet to come. The pathogenetic pathways may include oxidative species damage, discharge of some inflammatory molecules such as cytokines and nitric oxide as well as poly (adenosine- di- phosphate ribose) polymerase activation. In the present study there was inhibition of those enzymes when treated with *Curcuma caesia.* Cyclophosphamide is fast increased the tissue GSH enzyme activities. like results were earlier documented though the exact pathogenesis of CYP where by cyclophosphamide induce toxicity is mediate through its toxic metabolite, acrolein and mustered the molecular actions underlying such toxicity are yet to come. The pathogenetic pathways may include oxidative species damage, discharge of some inflammatory molecules such as cytokines and nitric oxide as well as poly (adenosine- di- phosphate ribose) polymerase activation. In the present study there was increased level of this enzymes when treated with *Curcuma caesia.*

Curcuma caesia has proven cytoprotective efficacy when administered prior to cyclophosphamide confront as shown morphologically and biochemically. The *Curcuma c.* pigment prohibited the severe inflammation and congestion. Only minute extravasation and leucocytic permeation were observed *(Arafa, 2009)* though the accurate pathogenesis whereby cyclophosphamide induce toxicity is mediate through its toxic metabolite acrolein, the molecular events fundamental such toxicity are yet to come. The pathogenetic pathways may include oxidative damage, discharge of some inflammatory molecules such as cytokines and nitric oxide as well as poly (adenosine-di-phosphate-ribose) polymerase activation (*Dang et al., 2008).* comparable findings were reported for *Curcuma caesia* in other inflammatory situation including acute pericreatitis *(Xu et al., 2001)* and acute heart damage *(Mahesh and Kuttan, 2005).* Curcumin regulated both the hypocalcaemia and hyponitraemia induce by cyclophosphamide. Likewise, Babu and Srinivasan *(Sheeja and Kuttan, 2006)* have established that finding curcumin prohibited diabetic mice and the urinary loss of potassium and sodium and as a result corrected hypocalcaemia and hyponatraemia. *Curcuma caesia* is a well-known inducible nitric oxide synthase inhibitor *(Takahashi et al., 2011).* The function of nitric oxide in CYP induced cardiotoxicity has been recently measured. Nitric oxide has been reported to be implicated in diverse physiological and pathophysiological process including immune defensive mechanism.

Based on this large explanation, it could be concluded that *curcuma caesia* has demonstrated cytoprotective efficacy in the cyclophosphamide-induced cardiotoxicity. Such defence is maybe mediate through modulation of the discharge of some inflammatory molecules, such as TNF-α and nitric oxide, improving the energy grade and variable the oxidative and anti-oxidative balance. Glutathione, (l-g-glutamyl-l-cysteinyl glycine), which is present in all mammalian tissue, provide reducing capacity for several reaction and plays an important role in detoxification of hydrogen peroxide (H_2O_2), other peroxides and oxygen free radicals. The production and degradation of glutathione are inhibited by reaction of the g-glutamyl cycle; the level reduce in blood, glutathione (GSH) has been reported in patents affect by deficiency of the enzymes implicated in the synthesis of glutathione. In cells, total glutathione can be free or bound to proteins; measurement of free glutathione in blood samples is essential for evaluation of the redox and detoxification status of cell in relation to its protective role against oxidative and free radical mediated cell damage; moreover, GSH measurement is important for the diagnosis of g-glutamyl cycle disorders (A.Meister *et al.,*1989). Purpose of tissue GSH, concomitant to its reduced, is a key factor to show the amount antioxidant reserve in the organism *(Lu et al., 1999; Odukoya et al., 2007; Balouchzadeh et al., 2011)*. In the present study show that, the content of GSH in the cyclophosphamide treated group was significantly decreased. *((Handa and Sharma, 1990).* In present study GSH depletion occur in CYP treated group but in CCE group GSH level increases.

Histological outline of control animals shows normal myocytes *[Fig 11(a)].* The section of the heart of the toxic control group of animals exhibited severe strong necrosis *[Fig 11(b)]*. The heart section of the CCE treated animals show normal cardiac architecture with few fatty globules and lack of severe cardiac necrosis and inflammation *[Fig 11(c, d)].* In present study

Cytoprotecting of myocytes by *Curcuma caesia* against cyclophosphamide induce cardiotoxicity.

7. CONCLUSION

The methanolic extract of *Curcuma caesia* Rhizome has shown the capability to maintain the normal functional status of the heart. The cardioprotective effect of methanolic extract of *Curcuma caesia* was confirmed by the following parameters:

7.1 Physical Parameters:

The isolated heart from the toxicant (CYP) treated animals exhibited increase in their physical parameters like Body weight and OBWI. Indeed, animals treated *Curcuma caesia* extract showed decrease in the value of above physical parameters which is an indication of cardio protection

7.2 Biochemical Parameters:

In case of toxicant treated groups there will be rise in serum marker enzymes such as LDH, CK and protein. The same is observed in cardiac toxicity in clinical practice and hence are having diagnostic importance in the assessment of heart function. In the present study, pre-treatment with *curcuma caesia* extract significantly reduced the toxicant elevated levels of abovementioned serum marker enzymes and increase in the level of protein. Hence, at this point it is concluded that the extract of *Curcuma caesia* rhizomes possess cardioprotective activity.

7.3 Antioxidant Property:

Higher dose of cyclophosphamide result in the depletion of heart GSH pool subsequently, leads to increased lipid peroxidation and heart damage. In the present study, pre-treatment with *Curcuma caesia* extract significantly enhance the decrease levels of tissue GSH and decrease tissue MDA level. pre-treatment with *Curcuma caesia* extract significantly reduced the toxicant elevated levels of thiobarbituric acid reactive substances (TBARS) like malondehyde. Hence, it is concluded that the extract of *Curcuma caesia* rhizomes possess cardioprotective activity.

7.4 Histopathological Study

In toxicant treated animals there will be severe histopathological disorder in the cytoarchitecture of the heart. The same is observed in case of humans who are suffering from major heart disorders. In the present study, animal treated with extract under study exhibited minimal cardiac toxicity and intact cytoarchitecture of the heart was maintained, indicating cardio protection. Based on development in serum marker enzyme levels, physical parameters, Antioxidant parameters and histopathological studies, it is concluded that the methanolic extract of *Curcuma caesia* rhizomes possess cardioprotective activity.

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