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Research Article

Cardiac Protective Activity of Ethanol Extract of White Curcumin (*Curcuma Zedoaria*), Against Cyclophosphamid Induced Cardiovascular Complications in Male Sprague Dewley Mice

Liaqat Ali[1]*, Saleha Akram Nizami[2] and Hafiza Sobia Ramzan[3]

[1]*Associate Professor, Anatomy, Swat Medical College, Swat, Pakistan*

[2]*Working as a Senior Demonstrator in Al-Aleem Medical College, Pakistan*

[3]*Institute of Molecular Biology and Biotechnology, University of Lahore, Pakistan*

***Corresponding Author:** Liaqat Ali, Associate Professor, Anatomy, Swat Medical College, Swat, Pakistan.

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Abstract

The common side effects associated with cyclophosphamide administration are bone marrow suppression, exposure to infections, amenorrhoea, toxic kidney poisoning and cystitis, as well as cardiovascular complications, including sinus bradycardia, pericarditis, myocarditis, and type 1 heart problems. A natural resource used as an alternative solution to reduce these unwanted effects, is *Curcuma zedoaria*, due to the presence of curcumin. This study therefore aims to determine the extent of protection of *Curcuma zedoaria* offers against cyclophosphamide in rat hearts, based on 4 indicators, CKMB enzymes, peripheral blood, troponin T and cardiac cell histopathology. The research was conducted using 30 rats divided into 6 groups, a normal group (without treatment), a negative control group (Na-CMC 0.5%), a positive control group (catechins), and 3 experimental groups administered white turmeric rhizome ethanol extract, in various doses. Each rats group was given 150 mg/kg of cyclophosphamide solution, 11 to 15 days before the experiment. The results showed the mice's CKMB and troponin T, and leukocyte levels decreased with increasing doses of *Curcuma zedoaria*. In addition, the erythrocyte levels were within the normal range in each group except the negative group, while the platelet levels were all within the normal range except for the negative group and the 200mg/kgbb turmeric dose group. Meanwhile, the lymphocyte levels in each group were within the normal range. The histopathological picture shows reduction in cariorexis and caryolysis features with increasing doses, especially in the group administered with 800 mg/kgbb dosage. However, all experimental groups were discovered to suffer from active inflammation. Therefore, *Curcuma Zedoaria* was concluded to exhibit partial protective activity against the effects of cyclophosphamide overexposure in mice.

Keywords: *Curcuma Zedoaria*; Cyclophosphamide; Curcumin; CKMB; Troponin T; Blood Examination and Histopathology

Introduction

Cyclophosphamide is able to inhibit fat cardiac disease, as well as protein and carnitine polymiotransferae I gen expression in the heart [2]. This pathway decreases adenosine triphospate, facilitates the accumulation of toxic metabolites from fatty acid oxidation, and causes cardiomyopathy. Fat heart disease binds protein, for instance, in chemotherapy-induced cardiac toxicity, while carnitin supplementation induced by numerous cyclophosphamid leads to cardiac toxicity [3], and damages the mitochondria used to accumulate calcium in in heart. The export of calcium to the sarcoplasmic reticulum through calcium overdensity, decreases ATP and increases ROS [4], while increasing mitochondrial function through lupelol and ester supplementation, helps to protect the heart from cyclophosphamide toxicity.

Generally, cyclophosphamide undergoes mechanisms inducing heart toxicity as well as oxidative and nitrative stress. Meanwhile, proteins undergo mechanisms leading to cardiomyocyte inflammation, calcium imbalance, necrosis, overdistended cardiomyocytes, cell cleavage, vacuolization, and biochemical pathway disruption. These cause heart failure and lead to death, in the absence of proper treatment [5].

Previous studies have shown peritoneal injection of curcumin C3 nanoparticle acts as an anticonvulsant for pentylenetrazole induced convulsion in mice. Curcumin is an orange pigmented plant polyphenol able to provide chemical protection against cell damage by toxic exogenous and endogenous stimulants, and to treat renal, miocardiac and body metabolism disorder. Therefore, the aim of this study is to evaluate the cardio-protection effect of curcuma against cyclophosphamide induced cardiovascular complications.

Materials and Methods

The materials utilized in this study include *Curcuma zedoria*, Camellia sinensis Cycloid, Aquades, alpha-naphthol, HNO_3, $RC(O)_2O$,H_2SO_4, Ethanol (distilled), mercury (II) chloride, calcium chloride, calcium iodide, iodium, bismuth (III) nitrate, HCl, $Pb(C_2H_3O_2)2$, $FeCl_3$, 10% formalin buffer, buffer $Na_3C_6H_5O_7$, isopropanol, hexane, 0.5% Na CMC, zinc powder, toluene, xylene, and dye (hematoxilin and eosin).

Sampling collection

The Curcuma rhizome obtained from Lahore, cleaned and dried. Subsequently, the rhizomes were macerated with 96% ethanol, poured into a glass tube, and 75 pieces of polarised liquid were added. The tube was then sealed and left to stand 5 day in the absence of sunlight. This was followed by cleaning, pressing, and maceration, with 96% ethanol. The rhizomes were then cut into 100 pieces, sealed, and left to stand for 2 days in the absence of sunlight. This was followed by filtration, evaporation using a rotary evaporator and drying at 40°C, to obtain thick extract.

Meanwhile, cathecin leaves were washed and ground with a grinder. Subsequently, 50 grams of the powder obtained was then extracted for 3 hours with 250 ml water use using soxlet and reflux apparatus with hot (45°C) water. The extract was then placed in a rotary evaporator, frozen at -20°C, and crushed to obtain powder. This powder was then combined with saline liquid (15 w/v), and intraperitoneally injected into mice at a dosage of 6 kg/bb.

Experimental method

Only male spragler dewey mice were used for this study. The mice were grouped into 6 different groups and placed in separate cages.

Group	Treatment
Normal sample	Distilled water and food
Negative population control	Distilled water and food Intrapertioneally Na CMC 0,5% once a day in 10 day Cyclophosphamid injection 150 mg/kgbb day 11 to day 15
Positive population sample	Distilled water and food Intrapertioneally Catechin 200 mg/kgbb once a day in 10 day Cyclophosphamid injection 150 mg/kgbb day 11 to day 15
Ethanol curcuma extract control 200 mg/kg bb	Distilled water and food Intrapertioneally curcumnin ethanol extract 200 mg/kgbb once a day in 10 day Cyclophosphamid injection 150 mg/kgbb day 11 to day
Ethanol curcuma extract control 400 mg/kg bb	Distilled water and food Intrapertioneally ethanol exract 400 mg/kg bb once a day in 10 day Cyclophosphamid injection 150 mg/kgbb day 11 to day 15
Ethanol curcuma extract control 400 mg/kg bb	Distilled water and food Intrapertioneally ethanol exract 800 mg/kg bb once a day in 10 day Cyclophosphamid injection 150 mg/kgbb day 11 to day 15

Table 1: Experimental Method.

CKMB and troponin T analysis

The CKMB and Troponin T serum were analysed in the Clinical laboratory, Faculty of Pharmacy. North Sumatra University. CKMB analysis was performed using the IFCC method, while Troponin T was analysed through ELISA test (Sardini, 2007).

Hematology analysis

The mice were first administered with cyclophospamid, and curcumin extract was administered 2 hours later. Using ether as anesthesia, incisions were made on the organs, and blood samples were collected in a glass tube containing liquid EDTA, to ascertain the lymphocyte, erythrocyte, thrombocyte and leucocyte count, and determine blood morphology using wright giemsa staining.

Histopathology analysing

The mice hearts were harvested after mice were pronounced clinically dead, cleaned with 0.9% Nacl injection, and a part of each heart tissue was cut (0.5 x 0.5 x 0.5cm dimension for each part). Subsequently, the tissues were treated with 10% formalin, immersed in paraffin, cut into about 4µm per slide, and subjected to histopathology analysis using hematoxilin-eosin dye, in the Laboratory.

Data analysis

Each blood cell type was measured in Mean ± SD with percentage (%) and analyzed with One Way ANOVA, followed by post hoc test, with coefficient interval of 95% ($\alpha = 0,05$).

Results

Results should have correlation with the purpose of the study (research question/s). Tables/figures in the results are important and relevant with the results. The text/narration does not repeat the results in the tables/figures. The text/narration provides clarifying information for the results in the tables/figures and placed before the tables/figures.

Tables 1, 2, and 3 show the results of troponin T assay, cmb analysis, and blood cell type evaluation, respective.

Interpretation of laboratory analysis

According to the tables 2 and 3, the CKMB and Troponin T levels are highest in the negative group, while the positive and the normal groups have almost the same values. Therefore, CKMB and Troponin T levels were concluded to decrease with increasing dose of *curcumin zedoaria* extract.

The table 4 shows the leucocyte count decreases with increasing dose of *Curcuma zedoaria* and is highest in the negative group. Meanwhile, the 0.5% Na CMC group's erythrocyte count was discovered to be the highest and to differ significantly from the, normal, and 400 mg/kgbb ethanol extract and Catechin groups ($p < 0.05$). In addition, the thrombocyte count for the catechin group differs significantly from the Na CMC 0.5% group ($p < 0.05$), while the lymphocyte count was discovered to differ significantly between each group ($p > 0.05$).

Group	Result	Mean
Normal Group	0.065 pg/ml 0.07 pg/ml 0.062 pg/ml 0.067 pg/ml	0.066 ± 0.003 pg/ml
Catechin or positive group	0.075 pg/ml 0.07 pg/ml 0.064 pg/ml 0.061 pg/ml	0.0675 ± 0.006 pg/ml
Negative group	0.848 pg/ml 0.449 pg/ml 1.036 pg/ml 0.533 pg/ml	0.7615 ± 0.273 pg/ml
Ethanol extract of 200 mg/kgbb	0.174 pg/ml 0.197 pg/ml 0.135 pg/ml 0.151 pg/ml	0.1642 ± 0.027 pg/ml
Ethanol extract of 400 mg/kgbb	0.083 pg/ml 0.113 pg/ml 0.117 pg/ml 0.118 pg/ml	0.1077 ± 0.0166 pg/ml
Ethanol extract of 800 mg/kgbb	0.091 pg/ml 0.107 pg/ml 0.114 pg/ml 0.083 pg/ml	0.0987 ± 0.0142 pg/ml

Table 2: Interpretation of the Results of Troponin T Analysis with Cyclophosphamide Administration.

No	Group	CKMB Mean ± SD
1	Normal Group	354.0 ± 81.0 kg/bb
2	positive group	389.0 ± 102.5 kg/bb
3	Negative group	843.0 ± 140.0 kg/bb
4	Ethanol extract of 200 mg/kgbb	600.0 ± 37.0 kg/bb
5	Ethanol extract of 400 mg/kgbb	595.0 ± 157. kg/bb
6	Ethanol extract of 800 mg/kgbb	572.0 ± 61.0 kg/bb

Table 3: Interpretation of the results of CKMB analysis with cyclophosphamide administration.

No	Keterangan	Leukocyte Mean ± SD	Erythrocyte Mean ± SD	Thrombocyte Mean± SD	Lymphocyte Mean ± SD
1	Normal Group	0.98 ± 026#c	4.90 ± 0.26#c	5.00 ± 1.00#c	58.67 ± 23.46#“
2	positive group	1.17 ± 0.,54*c	5.43 ± 0.03*c	52.33 ± 3.51*c	39.00 ± 2.00*“
3	group 0.5% CMC Na	9.91 ± 1.39ab	7.60 ± 0.24ab	660.27 ± 294.27ab	46.33 ± 2.52*#
4	Ethanol extract of 200 mg/kgbb	5.83 ± 1.02abc	6.32 ± 0.24*#“	349.67 ± 157.39*#“	58.33 ± 15.82*#“
5	Ethanol extract of 400 mg/kgbb	2.71 ± 0.91*#c	6.58 ± 0.28a#“	16.00 ± 2.00*#c	32.67 ± 5.13*#“
6	Kelompok EEKP 800 mg/kgbb	1.40 ± 0.23*#c	6.27 ± 0.24*#“	28.50 ± 2.78*#c	49.33 ± 1.53*#“

Table 4: Tukey Analysis HSD for Each Blood Cell Type.

Mean ± standard deviation, with a significance of P < 0,05.

Interpretation of histopathology analysis in mice heart

Figure 1: Normal Group.

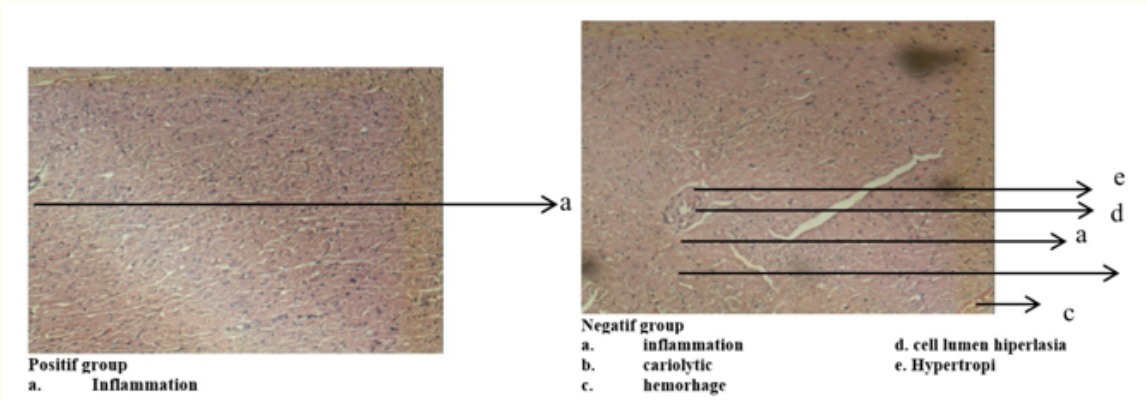

Figure 2: Positive Group.

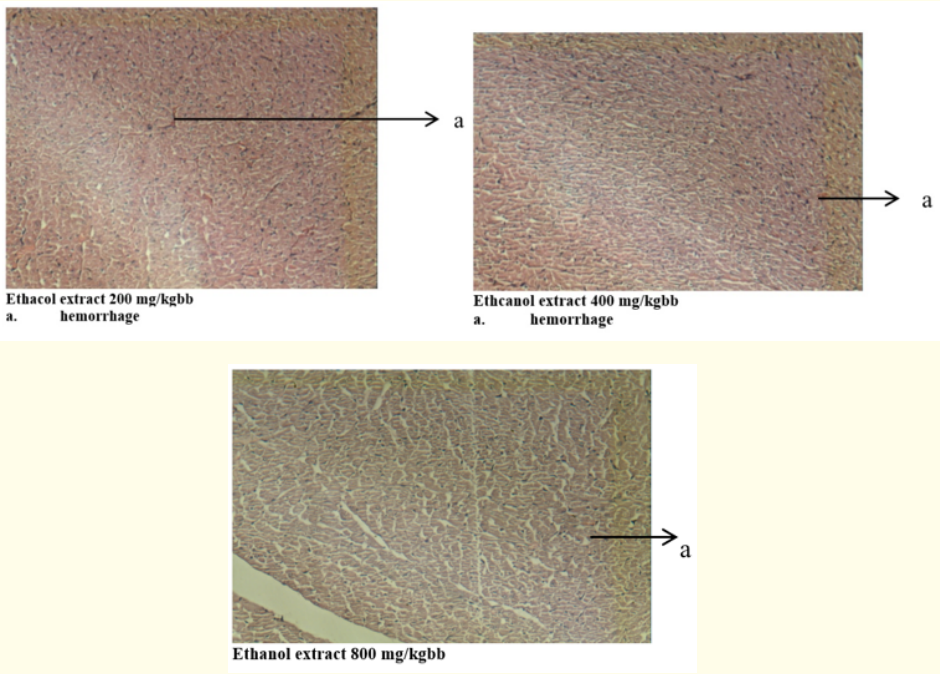

Figure 3: Ethanol Extract 800mg/kgbb.

The results of histopathology analysis indicate the occurrence of carioltytic, hemorrhage, inflammation, cell lumen dysplacia and hipertrophy in only the negatif group ($p < 0.05$). Meanwhile hemorrhage did not occur in only the normal group ($p < 0.05$).

Discussion

Cyclophosphamide is always used as an alkylating agent in treating cancer, including breast cancer, as well as multiple myeloma [6], renal disease, and rheumatic ailments, including phempigus, rhematoid artritis, cystemic sclerosis, interstitial lung disease, vasculopathy lupus and lupus with thrombocytophenia prupura [7]. Measuring the level of creatinine kinase isoenzyme MB (CKMB) is a standard method for diagnosing heart attack (infark miokard), with counting comparism ratio of 2.5% (from comparing the level of this enzyme in the heart with the levels in different organs) [8]. CKMB is usually detected about 3-8 hours after cardiac cell damage or sudden heart attack, peaks after about 24 hours after symptom onset and begins to decrease after 48-72 hours. These fluctuations are referred to as pathophysiology of the heart, however, fluctuation of this enzyme is not a standard diagnosis for cardiac disease [8]. Troponin and CKMB levels present challenges as cardiac complication diagnoses due to fluctuation, therefore, there is a need for reassessment and to re-run tests after 3-6 hours in emergency clinical units.

The mice in the negative control group (Na CMC 0.5%) were discovered to have the highest CKMB values compared to other groups, and this enzyme decreased in each group, with increasing dosage of *Curcuma zedoaria*. However, the group with the highest dosage, 800 mg/kg bb had lower CKMB level, compared to the normal group. This study therefore shows *Curcuma zedoaria* offers cardiac protection against cyclophosphamide induced cardiac complication in mice. Curcumin possesses activated biomechanisms, including nuclear factor erythroid-derived 2 (Nrf2) members of the NF-E2 family of nuclear basic leucine zipper transcription factors, and regulates the gene expression several enzymes able to detoxify pro-oxidative stressors. A study by Li., *et al*. (2015) reported minute dose of curcumin (10mg/kgbb) provided cardiac protection through the activation of nerf 2 signaling pathway, and promoted antioxidative activity.

The main causes of cariovascular diseases, including increased level of oxidative stress and greater intensity of inflammatory response are diminished by suppplementation of curcumin. This reduces inflammatory response by reducing NF-kB activation and suppressing the gene expression of inflammatory cytokines, including TNF-α, IL-1and IL-6. Zein shaban., *et al*. stated curcumin lowered NO production and down regulation of iNOS mRNA expression, and Consequently, increased plasma SOD production and upregulation of EPO mRNA expression. The Curcumin upregulated mRNA expression of the VEGF isoforms and receptor is possibly a pathway used by curcumin to protect cardiac muscles from the combined oxidative stress exerted by diabetes and nicotine.

This study shows administering curcumin at dosage of 800 mg/kg bb or higher possibly balances the blood cell count, as well as CKMB and Troponin T level, reduce local Inflammation in mammalian blood cell, core cell and facilitate protein self healing and remodelling in the human cell. Meanwhile, chemical agents are able to imboost the inflamamtory process and adjust membrane permeablity, osmotic homestatis and enzyme integrity. Leucocytes migrate from blood cell to tissue through chemotaxis, and are grouped into 2 cell types, granulocyte or polymorphonuclear and agranulocyte or mononuclear. Furthermore, inflammatory neutrophils consist of phosphatase acid, α-mannosidase, arylsulfatase, β-galactosidase, β-glucoronidase, cathepsin, 5'-nucleotidase, elastase, colagenase, myeloperoxidase, lisozim. Lymphocytes are classified based on size, morpholgy and function. Immunty mediated are lymphocytes T, Lympcyt B and cell NK. Lymphocytes T play are significant for cell-mediated immunity, B lymphocytes are crucial for humoral immunity, while NK cells destroy malignant and virus-infected cells. Conversely, monocytes turn into macrophages at the site of inflammation, leading to bacterial phagocytes, other cells, and tissue debris. Furthermore, monocytes function as antigen-presenting cells (APCs), and these are significant for cellular immunity.

In this study, the leukocytes count was discovered to reduce with increasing dose of *Curcuma zedoaria*, and this value was highest in the negative control group (Na-CMC 0.5%). Meanwhile, there was no significant difference among the lymphocytes count of each group, indicating the occurrence of acute inflammation of the liver due to elevated leukocytes. However, this was not accompanied by elevated lymphocytes, as a marker of chronic active inflammation. The groups administered with the ethanol extract of *Curcuma zedoaria* had similar leukocyte counts, but this value reduces with increasing dose. This is in accordance with the results of study conducted by Wardhan,i *at al.,* (2019), stating *Curcuma zedoaria* had a partial protective effect on the white blood cells of mice exposed to $CuSO_4$ [10].

In addition, this study recorded a significant increase in the platelets count for each group, with the highest increase in the negative control group. The administration of *Curcuma zedoaria* caused a reduction in platelet levels, however, this was lower compared to the normal group. Secondary thrombocytosis can occur due to increased levels of thyrombopoetin, interleuctin-6, and other cytokines or catecholamines caused by inflammation, infection, cancer, or stress. This is in accordance with the results of the study by Garmana., *et al*. (2015), stating secondary thrombocytosis side effects occur as an adverse drug reaction (ROM) to chemotherapy [11]. In this study, a rise in erythrocyte count was recorded in all groups, compared to the normal group, and the highest increase was observed in the negative control group. Meanwhile, there were no significant differences in the final erythrocyte counts of the groups administered with *Curcuma zedoaria*. A previous study also reported injection of isoproterenol to increase percentage weight gain in mice due to decreased food and water intake [12]. The increase in body weight in mice administered with isproterenol is possibly due to increased water specific gravity, edematous intramuscular space, extensive cardiac fiber necrosis, and infiltrated inflammation in the injured cell tissue. Therefore, the initial administration of curcumin before isoproterenol administration, directly reduces the percentage increase in body mass and heart weight, and prevents injury to heart cells.

The results of this study also showed an improvement in the histopathology of the ethanolic extract of white turmeric at a dose of 800 mg/kgbb, matching the results of the catechin extract, and the normal group. Several studies have reported the pro-oxidant and oxidative stress properties of cyclophosphamide, leading to antioxidant activity and increased lipid peroxidation in several mice tissues. The histology assay results above show cyclophosphamide leads to heart poisoning. The figure above shows myocardial fibers of a normal heart is uniform in size, shape and configuration, without infiltration of the inflamed cells. Therefore, cyclophosphamide causes remarkable changes in heart cells correlated with heart cell degeneration, cardiomyocyte vacuolization, cell infiltration and loss of myofibrils. This is in accordance with line with a previous

study [9]. The treatment with only curcumin resulted in decreased myobril fragmentation as well as inflammation, and also reversed the pathological changes caused by cyclophosphamide in heart cells.

Summary and Conclusion

In the study regarding the protection of *Curcuma zedoaria* against cyclophosphamide induced cardiac complication in male sprawley mice, the following points were correlated.

- Cyclophosphamide administered to mice causes some pathological change, including rise in the levels of value heart enzymes like CKMB, leucocyte cell and thyrombocyte, as well as cardiac cell damage including karyolysis, hemorrhage and inflammation.
- A dosage of *Curcuma zedoaria* above 800 mg/kg bb lowers the levels of CKMB and blood cell count, except lymphocyte count in mice treated with 800 mg/kg of the extract, and this was similar to the value of the normal group. According to the histopathology analysis, the cardiac damage in mice treated with the extract was lower compared to the negative group.
- Furthermore, C*urcuma zedoaria* is assumed to effectively protect cardiac cells when administered at a dosage of 800 mg/kg bb dosage or higher, however, this requires further study to be proven.

A curcumin dosage above 800mg kg/bb is therefore suggested to provide improved protection for the heart and other organs.

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