

# The Role of curcumin extract in Ameliorating Cyclophosphamide-Induced Cardiotoxicity

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**Abstract.** Cyclophosphamide, a chemotherapeutic and immunosuppressive drug, has been linked to problems after bone marrow transplantation in the past. More recently, it has been demonstrated that cardiotoxicity limits the dose of cyclophosphamide, and cardiology is receiving more attention. Though the exact mechanism of cyclophosphamide-induced cardiotoxicity is unknown, oxidative and nitrative stress are suspected to play a role. As a result, the focus of this review is on antioxidants and how they can reduce or prevent cyclophosphamide-induced cardiotoxicity. It will place a special emphasis on the cardioprotective properties of naturally occurring, plant-derived antioxidants such as curcumin extract, which have recently attracted a lot of interest.

## 1 Introduction

Cyclophosphamide is an alkylating agent of the nitrogen mustard class, which is an oxazaphosphorine class with immunosuppressive and potent cytotoxic effects; it was first synthesized by Arnold and colleagues in 1958 [1, 2, 3]. It is widely used in the treatment of autoimmune diseases, including lupus and several types of vasculitides [4]. Cyclophosphamide is a biologically inactive prodrug that needs cytochrome-P450 mediated activation [5, [5,6]. The drug is first transformed into hydroxylated intermediates, which are subsequently broken down, resulting in the production of the active chemicals, phosphoramidate mustard and acrolein. Cyclophosphamide is associated with various toxicities, with the cumulative dose being the principal risk factor [7]. Short-term effects include alopecia, myelosuppression, gastrointestinal effects (nausea, vomiting, anorexia, and diarrhea), and hemorrhagic cystitis. Long-term risks include teratogenicity, pulmonary toxicity, gonadal toxicity, and cardiotoxicity [7]. One of the reasons for restricting or limiting the clinical application of the use of cyclophosphamide is multiple organ toxicities. (CP) is a potent anticancer agent with well-known cardiotoxicity that limits its clinical applications [8]. Cardiotoxicity associated with anticancer treatment ranges from endothelial damage to myocardial infarction, cardiomyopathy, ischemia, angina, and heart failure [9]. It is very helpful to understand the severity of the disease, the diagnosis, and the prognosis of the patient receiving chemotherapy in order to comprehend the molecular process which follows these unwanted side effects [10]. In this study Curcumin extract was used to minimize the

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side effects or diminish the pathological consequences brought on by cyclophosphamide especially Cardiotoxicity. *Curcuma longa*, which can grow up to 1 m tall and is upright and leafy, is a member of the Zingiberaceae family. Tropical and subtropical regions are widely scattered throughout the world's regions that are typically cultivated primarily in India in Asiatic nations plus China [11, 12]. Curcumin is the active form of turmeric and is found in 75% of its extracts. Curcumin can help with metabolic and oxidative disorders such as type 2 diabetes, Crohn's disease and thrombosis, and anti-inflammatory effect, with help of wound or tissue healing. [13].

The aim of study to study the toxicological efficacy (cardiotoxicity effect) of an appropriate dose of cyclophosphamide, an anticancer drug, and use *curcuma longa* extract to improve the side effects or to reduce the pathological effects resulting from cyclophosphamide, through study parameters, we were able to preserve the properties as an anticancer drug, and heart histological study.

## **2 Materials and methods**

### **2.1 Ethics Statement**

Rats were handled and euthanized according to Ethics and Guidelines for the Care and Use of Laboratory Animals; procedures were approved by the University of kufa [14].

### **2.2 Animals**

There were 30 Adalat albino strain rats (4 months old) used in this experiment, weighing between 200 and 250 g. The animals were kept in the Biology Department's and Faculty of Science's Animal House at the University of Kufa, where the environment included a moderate temperature and a 12-hour cycle of darkness and light.

### **2.3 Calculating the doses of cyclophosphamide**

It was determined that 50 mg/kg of body weight single dose was the optimum dose of cyclophosphamide to develop its therapeutic efficacy and adverse effects according to [14]. The oral dosage for curcumin extract was 200 mg/kg per day for 14 days depended on [15].

### **2.4 The distribution of the experimental rats**

There were 30 healthy male albino rats used. Six groups were formed from the animals (10 rats for each group). 50 animals were separated into 5 groups, with 10 animals serving as the control group.

1. G1 (control group) contained ten male rats in good health. Throughout the duration of the experiment, the animals received 0.5 ml of normal saline solution (0.9%) orally each day.

2. G2 (cyclophosphamide group): included 10 rats. For the duration of the experiment, the animals

3. received intraperitoneal injections of cyclophosphamide at a dose of 50 mg/kg (single dose)

4. G3 (the protection group) included 10 male rats. The five animals in this group were dosed with a solution of curcumin extract (1 ml/animal) at a concentration of 200 mg/kg body weight per day for seven days and then injected with cyclophosphamide at a dose of 50 mg/kg (a single dose).

The other five animals are from the same group and were dosed with a solution of curcumin extract per day for fourteen days and then injected with cyclophosphamide. Animals were dosed with a solution of curcumin extract per day for fourteen, the rats were anesthetized with ketamine and xylazine and then sacrificed at the end of the experiment. After inserting a 5 ml needle into the heart to puncture it, 3–4 ml of blood was drawn for the blood sample. After being placed in gel-lined plastic tubes for 5 minutes, the blood underwent a 15-minute centrifugation procedure at 3000 rpm. For use in the investigation of various blood parameters, a serum in the quantity of 0.4 to 0.5 ml was obtained, put in a 1.5 ml Eppendorf tube, and stored in the freezer.

## 2.5 Measuring of serum biomarker

### TNT (Troponin)

Troponin levels in animal serum were determined using a technique created by the company.

#### FABP (Heart-Type Fatty Acid-Binding Protein)

Heart-Type Fatty Acid-Binding Protein The method was developed by the company to measure the protein levels in animal serum.

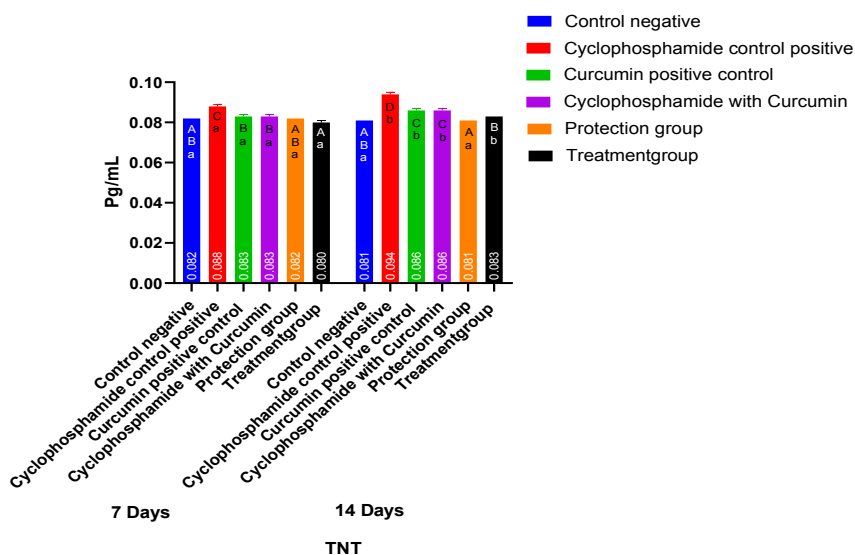
Organ tissue sections (heart) were deparaffinized twice with xylene for two minutes each and then rehydrated three times with ethanol diluted differently (100%, 90%, and 70%) for two minutes each. The tissue pieces were then further rinsed in running tap water, re-fixed in Bruin's solution for 1 hour at 56 C, and rinsed for 5–10 minutes to get rid of the yellow tint.

Organ sections were seen and studied under a light microscope at magnification of 40x, 100x, 200x, 400x, and 1000x.

## 3 Results

### 3.1 TNT (Troponin)

The groups treated with cyclophosphamide and curcumin chemicals and sample collection on 7th and 14th days after the experimental time were different from the control group considerably ( $P \leq 0.05$ ), according to the antioxidant biomarker data. The groups are statistically diverse, as indicated by various capital letters. On the 7th day the cyclophosphamide-treated group showed a significant ( $P \leq 0.05$ ) increase in troponin levels. When compared with the control group and cyclophosphamide with curcumin extract, the protection group had a significant ( $P \leq 0.05$ ) decrease when compared with the cyclophosphamide-treated group. On the 14th day, when compared to the group that received cyclophosphamide treatment, the protective group showed a significant ( $P \leq 0.05$ ) drop because of the influence of curcumin, which decreased levels of troponin. When compared to the control group, the cyclophosphamide-treated group exhibits a significant ( $P \leq 0.05$ ) rise in troponin levels. but showed a significant ( $P \leq 0.05$ ) increase in troponin levels clearly the on the 7th day, better than on the 14th day. Also showed the productive group showed a significant ( $P \leq 0.05$ ) decreased levels of troponin When compared to the cyclophosphamide-treated group clearly the on the 7th day, better than on the 14th day.



**Fig. 1.** Level of Troponin (Pg/mL) in the serum of rat animal groups

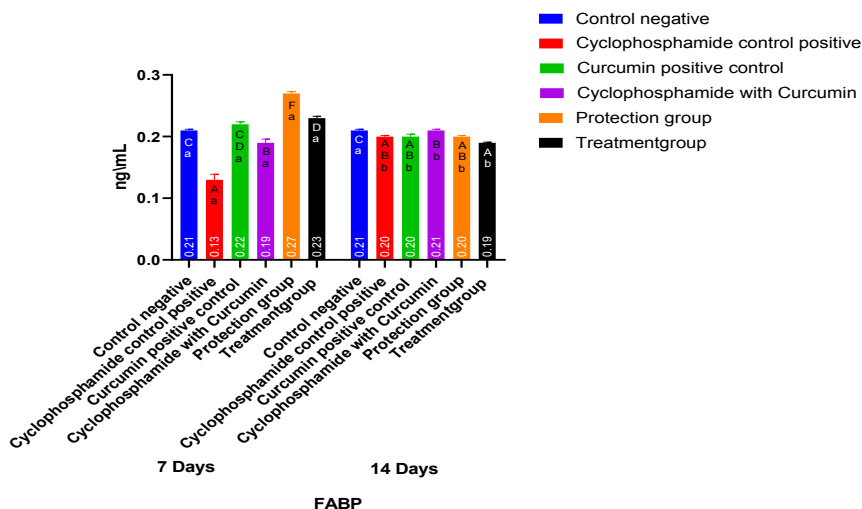
**Table 1.** The Level of Troponin (Pg/mL) in the serum of rat animal groups, comparative between groups and between (7) day and (14) day

	(7) day	(14) day
Control	0.08 ± 0 A B a	0.08 ± 0 A a
Cyclophosphamide	0.09 ± 0.001 C a	0.09 ± 0 B b
Protection group	0.08 ± 0 A B a	0.08 ± 0 A b

The capital letters indicate the difference between different groups at the same time, while small letters indicate similar groups at two different times.

### 3.2 FABP (Heart-Type Fatty Acid-Binding Protein)

The cyclophosphamide-treated group on the 7th day showed a significant ( $P < 0.05$ ) decrease in heart-type fatty acid-binding protein levels when compared with the control group. The protection group showed improvement in heart-type fatty acid-binding protein levels. It showed a significant ( $P < 0.05$ ) increase when compared with the cyclophosphamide-treated group. The cyclophosphamide-treated group showed a significant ( $P > 0.05$ ) increase on the 14th day when compared to the 7th day in heart-type fatty acid-binding protein levels, but less than the control group. On the other hand, the protection group showed a significant ( $P < 0.05$ ) increase on the 7th day when compared with the same group on the 14th day.



**Fig. 2.** Level Heart-Type Fatty Acid-Binding Protein (ng/mL) in the serum of rat animal groups

**Table 2.** The Level Heart-Type Fatty Acid-Binding Protein (ng/mL) in the serum of rat animal groups, comparative between groups and between (7) day and (14) day

	(7) day	(14) day
Control	0.21 ± 0.002 C a	0.21 ± 0 A a
Cyclophosphamide control positive	0.13 ± 0.009 A a	0.2 ± 0 A B b
Protection group	0.27 ± 0.003 F a	0.2 ± 0 A b

The capital letters indicate the difference between different groups at the same time, while small letters indicate similar groups at two different times.

## 4 Discussion

Cyclophosphamide-induced heart injury is poorly known [16]. Endothelial cell degeneration causes direct injury to the heart and capillary blood vessels in the presence of toxic metabolites, leading to edema, interstitial bleeding, and the development of microthrombosis [17]. Nitric oxide bioavailability may be decreased as a result of cyclophosphamide-induced production of reactive oxygen species, which could affect endothelial function [18]. FABP can be used as early diagnostic markers for chemotherapy-induced cardiac toxicity [19]. Our study showed inhibition of heart-type fatty acid-binding protein in animals given cyclophosphamide [20] in 7 days more than 14 days, but noted the high improvement of heart-type fatty acid-binding protein in animals protected against cyclophosphamide by using curcumin extract.

The preferred biomarker for identifying heart damage is troponin on the actin filament so cyclophosphamide causes cardiac toxicity but curcumin extract related to anti-toxicity.

## 5 Conclusions & recommendations

Different natural, plant-derived antioxidants (curcumin extract) showed significant cardioprotective effects in vivo preclinical protection against cyclophosphamide.

A present study shows Curcumin's protective effects on myocardial remodeling. Curcumin has a beneficial impact on the numerous alterations that myocardial infarction causes in the infarcted heart. This nutritional supplement reduces the negative change in the levels of the apoptotic regulators BCL2 and BAX (After apoptotic stimulation, life or death depends on the balance between the proteins Bax, an inducer of apoptosis, and Bcl-2, an inhibitor of apoptosis. We hypothesized that ischemia induces bcl-2 or Bax expression, which may be connected to myocyte death in human hearts).

Found and protects cardiomyocytes by activating the JAK2/STAT (Janus kinase 2/signal transducer and activator of transcription Nrf2-ARE pathway is an intrinsic mechanism of defense against oxidative stress. Nrf2 (is a transcription factor that induces the expression of a great number of cytoprotective and detoxificant genes Pathway). Additionally, it lessens inflammation by downregulating cytokines like TNF, IL-6, and IL-1, and oxidative stress by increasing NRF2. It also restricts injuries, which call for Pathway regulation of SIRT1 in toxicological damage, SIRT1 can catalyze the deacetylation of acetyl lysine of histone substrate and some non-histone substrates to regulate gene expression) SIRT1 (By lowering collagen and decreasing myofibroblast overactivation, it also has a favorable impact on remodeling following infarction, resulting in a stable scar and avoiding fibrosis. either increased or decreased. Curcumin's protective effects on myocardial [21]

One of the main mediators of inflammation is TNF. However, it wasn't until our team isolated two distinct molecules from macrophages and lymphocytes that we were able to determine the exact chemical composition of TNF, there is a study showing Suppression of TNF by curcumin in vivo that curcumin can suppress TNF-induced NF- $\kappa$ B activation and the expression of pro-inflammatory genes Since then, numerous mechanisms by which curcumin can exhibit anti-inflammatory activity have been proposed [22].

The aforementioned study supports the results of our study that the groups of animals treated with curcumin extract showed a decrease in the level of TNF, IL-6 and the improvement was very clear in the protection group compared to the group of animals that received the cyclophosphamide alone. Also, our result show decreases the levels of TNF, IL-6 in (treatment group with curcumin extract post-cyclophosphamide) when compared to (control group), on the other hand also show (cyclophosphamide with curcumin extract), the contrary, our study showed an increase in the level of TNF, IL-6 in the blood plasma of rats treated with cyclophosphamide alone comparative to control group and other our experimental groups because cyclophosphamide induced cardiotoxicity and caused myocyte damage agrees with [23]. Important note: the effect of cyclophosphamide is strong in groups of animals that collected blood samples for 7 days or more than 14 days.

The preferred biomarker for identifying heart damage is troponin. On the actin filament, the 3-unit troponin complex (troponin I, T, and C) together with tropomyosin is crucial for the calcium-mediated control of skeletal and cardiac muscle contraction [24]. present study show cyclophosphamide -induced cardiotoxicity lead to myocardial damage by necrosis and finally lead to release of troponin in blood stream result increase levels of troponin in plasma[25]. as well as the development of protein adducts that cause inflammation of the cardiomyocytes According to our statistical findings, the rats who received cyclophosphamide injections had higher levels of NFATC1 measured than the other experimental animal groups. However, in the protective group and treatment group, curcumin extract showed decreased NFATC1 levels as a result of the extract's mechanism of action, which is consistent with [26]. antioxidative substances due to phenolic compounds, antiinflammatory compounds, and cell signaling pathways, as well as the latter two of which have previously been described. In this study, heart tissue was examined under a microscope using a special stain called masson trichrome stain,

Histologically, the heart is mostly composed of cardiomyocytes and connective tissue. The fibrous and thick connective tissue that makes up the cardiac skeleton is elastic. Specific

stains, such as Masson's elastic trichrome stains, can be used to enhance the visibility of these components. Our results showed that in histological sections of the hearts, the heart tissue of the animals in the control group has a typical cardiac architecture. Take note of the positioning of the cardiac myofibers and the presence of fibrous sheaths that protect the myofibers but, on the other hand, it showed that the heart of the group of rats that had received cyclophosphamide treatment on the 7 days had a number of pathological alterations, including spaces in the cardiac myofibers caused by necrosis and necrosis of the cardiomyocytes due to the toxicity of activated cyclophosphamide medication, which resulted in the loss of the cardiac myofiber arrangement. There is a study showing the animals' bodies weighed less after receiving CP therapy, but their hearts weighed more. Additionally, the mortality rate increased significantly. Due to CP toxicity, this suggests that the animals' overall health was abnormal. An increase in heart weight could be caused by significant cardiac muscle necrosis and inflammatory cells invading the injured area. There could also have been tissue edema [27]. Our study also shows, deposition of collagen fibers was observed to fill the spaces formed by necrotic cardiomyocytes. Cyclophosphamide induces apoptosis by adding an alkylene group to the DNA at the nucleotide 7 location, as we previously discussed. As a result, the tissue of the heart develops holes and necrosis in the place of the dead cells. In seven days, this was seen in the hearts of animals given this medication, and led to the loss of cardiac myofiber arrangement. However, the histological analysis of the heart tissue on day 14 revealed greater areas of damage and necrosis than it did on day 7, as well as the presence of inflammatory cells deposited at the sites of damage and precipitated collagen fibers [28]. The results of the current study show that endogenous antioxidant enzymes such as GSH, SOD, CAT, and G-PX decrease, which is consistent with these histological changes. MDA concentrations were found to be higher, while MOP, TNF, IL6, GAL3, and FABP protein concentrations were also higher. These enzymes and proteins have a role in cellular process mechanisms and in organ histopathology. Additionally [29], our work demonstrates the role of curcumin in reducing the necrosis caused by cyclophosphamide. The hearts of rats in the post-cyclophosphamide treatment protection group exhibited a number of pathological alterations, including cardiomyocyte necrosis in cardiac myofibers. Within the affected myofibers, a very small quantity of collagen fiber deposition was also seen. The hearts of the treatment group receiving curcumin showed normal cardiac architecture on day 14 as a result of the extract's ability to minimize the toxicity of cyclophosphamide [30].

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