

Cardioprotective effect of curcumin and piperine combination against cyclophosphamide-induced cardiotoxicity

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Abstract

Cyclophosphamide (CP) is a very popular antineoplastic and immunosuppressant agent which comes under the class of oxazaphosphorine-alkylating agent. Its ability to kill tumor cell is mainly due to its DNA alkylation. Although it has tumor selectivity and a wide spectrum of clinical uses, CP is responsible to cause multiple organ toxicity. CP causes generation of two active metabolites such as phosphoramidate mustard and acrolein which can react with carboxyl (-C[O] OH), mercapto (-SH), amino (-NH₂), phosphate (-PO₃H₂), and hydroxyl (-OH) groups, and can form cross-links with DNA and proteins. CP predominantly in high dose causes lethal cardiotoxicity such as congestive heart failure, arrhythmias, cardiac tamponade, and myocardial depression. The precise mechanism by which CP causes toxicity is still under investigation. Excessive generation of free oxygen radicals and decrease in the antioxidant defense mechanism by CP may be the prime reason of cardiotoxicity. Moreover, CP is associated with hypercholesterolemia, hypertriglyceridemia, and impaired secretion of heart lipoprotein lipase. CP-induced alterations of lipid metabolism pathways in various conditions lead to myocardial lipid accumulation and lipotoxic cardiomyopathy.[1,2] The concept of using phytochemicals has ushered in a new revolution in pharmaceuticals. Naturally occurring polyphenols have gained importance because of their minimal side effects, low cost, and abundance. Curcumin, a natural pigment with antioxidant activity and a major active component of turmeric, is extracted from the powdered dry rhizome of *Curcuma longa* Linn (Zingiberaceae) and it has been used for centuries in indigenous medicine.[3]

Curcumin possess various pharmacological effects such as antioxidant, anti-inflammatory, anti-thrombotic, anti-apoptotic, and hepatoprotective activities. It has been reported that curcumin plays a pivotal role against skin, oral, lung, fore stomach, colon, and prostate cancers. In some of the recent studies, it has been revealed that curcumin was found to be beneficial against isoproterenol, ischemia reperfusion, and doxorubicin-induced cardiotoxicity.[4,5]

The major problem associated with curcumin is its poor bioavailability due to its rapid metabolism in the liver and intestinal wall. Curcumin, therefore, could be a therapeutic option for the treatment of different diseases, provided limitations in its oral bioavailability can be overcome. In an interesting study, it has been noted that concomitant administration of curcumin 2 g/kg and piperine 20 mg/kg in rats and curcumin 2 g and piperine 20 mg in humans resulted in 154% and 2000% increase in curcumin bioavailability, respectively.[6]

Piperine is a major alkaloid component of black pepper (*Piper nigrum* Linn), which is a widely consumed spice. In the previous studies, piperine has been reported for antitumor, antioxidant, anti-inflammatory, antimycobacterial, and insecticidal activities.[7]

This novel combination of curcumin and piperine has reported better hypocholesterolemic and neuroprotective activities.[8,9]

However, in a contra-indicatory study, it has been reported that curcumin and piperine combination did not bring any advantage to the curcumin effects on antidiabetic and antioxidant activities.[10]

Till now, no study has been performed to evaluate the effect of curcumin and piperine combination against CP-induced cardiotoxicity. In this present study, the effect of curcumin alone (200 mg/kg, p.o.) and the combination of three subtherapeutic doses of curcumin (100, 50, and 25 mg/kg, p.o.) with piperine (20 mg/kg, p.o.) was investigated against CP-induced cardiotoxicity. The objective of the present study was to address the poor bioavailability of curcumin by combining with piperine and witness incremental therapeutic potential against CP-induced cardiotoxicity.

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Materials and Methods

Chemicals

All chemicals used were of analytical grade and purchased from standard companies such as R L Fine Chem, Bengaluru, and Rankem, Mumbai. Biochemical kits were procured from Crest Biosystems (Goa, India).

Experimental Animals

Healthy male adult Wistar albino rats, weighing 150–200 g, were housed in polypropylene cages, maintained under standardized condition (12 h L: D cycles, 25°C ± 5°C) with paddy husk bedding at the Central Animal House of the institute and were provided with standard pellet food and had free access to purified drinking water. The guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India, were followed, and prior permission was sought from the Institutional Animal Ethics Committee for conducting the study (SDCP/IAEC-03/2013-14).

Phytochemicals

Curcumin and piperine were obtained from Yucca Enterprises, Mumbai, India.

Dose Selection

From earlier literature review, the cardioprotective dose of curcumin was found to be 200 mg/kg, p.o. and the same dose was selected for the present study.[5]

In high-dose combination group, half of the therapeutic dose of curcumin, i.e., 100 mg/kg, p.o. was selected, and for moderate- and low-dose combination groups, further reducing the dose to 50 mg/kg, p.o. and 25 mg/kg, p.o. was selected.

In case of piperine, based on the earlier literature survey, the dose at which it is able to increase the serum concentration of curcumin, i.e., 20 mg/kg, p.o. was selected.[6]

Experimental Protocol

The animals were divided into six different treatment groups of eight animals each. Group I and Group II received saline and termed as normal control and CP control, respectively; Group III received curcumin 200 mg/kg, p.o. (Cur-200); Groups IV, V, and VI received curcumin 100 mg/kg, p.o. (Cur-100), curcumin 50 mg/kg, p.o. (Cur-50), and curcumin 25 mg/kg, p.o. (Cur-25), respectively. Groups IV, V, and VI with different doses of curcumin received piperine 20 mg/kg, p.o. (PIP-20). All treatments were continued for 10 days by oral route. Except Group I, all the other groups were subjected to CP toxicity with the dose of (200 mg/kg i.p.) on day 1.[2]

Electrocardiographic Studies

Forty-eight hours after the last treatment, the animals were anesthetized with the combination of ketamine (75 mg/kg, i.p.) and xylazine (8 mg/kg, i.p.). Leads were attached to the dermal layer of both the front paws and hind legs and recordings were made with the help of a digital physiograph (model no-DI-2, INCO, Ambala City, India). The changes in heart rate, QRS interval, QT interval, and RR interval were noted.[2]

Oxidative Marker Enzyme Assay

Forty-eight hours after the last treatment, blood was collected and separated by centrifugation and analyzed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP), creatine kinase-MB (CK-MB), creatine kinase-NAC (CK-NAC), and lactate dehydrogenase (LDH). The levels of marker enzymes were analyzed using commercial kits (Crest Biosystems) with the help of a semi-autoanalyzer (model: Prietest Touch, Robonik India Pvt. Ltd.).

Then, the animals were sacrificed under mild ether anesthesia. Four hearts from each group were homogenized with sucrose solution (0.25 M) for the preparation of heart tissue homogenate (HTH). The levels of superoxide dismutase (SOD), catalase, and thiobarbituric acid-reactive species (TBARS) were investigated in HTH.[2,11]

Lipid Profile Assay

Serum cholesterol and triglyceride levels were measured by commercial kits (Crest Biosystems) with the help of a semi-autoanalyzer.[2]

Histological Analysis

Heart sections were prepared from the remaining four hearts in each group, stained with hematoxylin and eosin, and changes in histology were observed. The myocardial damage was indicated by scoring method depending on the severity as follows, no change = 0 score; mild = 1 score (focal myocytes damage or small multifocal degeneration with slight degree of inflammation); moderate = 2 score (extensive myofibrillar degeneration); and marked = 3 score (necrosis with diffuse inflammation).[11]

Statistical Analysis

Results are expressed as mean \pm standard error. Statistical significance was assessed using one-way analysis of variance followed by Tukey–Kramer multiple comparison tests. $P < 0.05$ was considered statistically significant.

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Results

Effect on Electrocardiographic Parameters

CP control group reported a significant increase in QRS, RR, PR interval, and QT segment and a significant decrease in heart rate compared to normal control. All treatment groups such as Cur-200, Cur-100+Pie20, Cur-50+Pie20, and Cur-25+Pie20 demonstrated a significant improvement in electrocardiographic (ECG) parameters compared to CP control group. Cur-50+Pie20 and Cur-25+Pie20 treatment groups rectified all ECG parameters significantly compared to Cur-200 alone-treated group [Table 1].

Table 1

Groups	Heart rate (beats/min)	QRS interval (ms)	QT segment (ms)	R
NC	183.52±4.27	120.28±3.52	121.90±5.95	
CP control	88.12±4.02***	267.78±4.64***	244.05±5.91***	3
Cur-200	142.76±1.74***,###	214.76±3.17***,###	196.43±2.26***,###	27
Cur-100+Pie20	148.55±1.96***,###	210.64±3.35***,###	155.04±2.29***,###	27
Cur-50+Pie20	173.76±2.93***,###,†††	171.82±3.35***,###,†††	133.08±2.29***,###,†††	23
Cur-25+Pie20	162.81±1.82***,###,††	190.60±3.24†	157.82±3.41***,###,††	25

All the values are in mean±SEM, n=8. ***P<0.001 when compared to normal control, ###P<0.001 when compared to CP control, †P<0.05 when compared to Cur-200. SEM=Standard error of mean, NC=Normal control, CP=Cyclophosphamide, Cur=Curcumin

Effect on electrocardiographic patterns against cyclophosphamide-induced myocardial toxicity

Serum Enzyme Biomarkers

CP control group demonstrated a significant increase in serum AST, ALT, ALP, CK-MB, CK-NAC, and LDH values compared to normal control. Treatment groups such as Cur-200, Cur-100+Pie20, Cur-50+Pie20, and Cur-25+Pie20 showed a significant decrease in AST, ALT, ALP, CK-MB, CK-NAC, and LDH values compared to CP control. Cur-50+Pie20 and Cur-25+Pie20 treatment groups showed a significant decrease in AST, ALT, ALP, CK-MB, CK-NAC, and LDH values compared to Cur-200 alone-treated group [Table 2].

Table 2

Groups	Blood serum level		
	AST	ALT	ALP
NC	47.98±1.06	35.92±1.03	45.92±1.04
CP control	214.01±1.22***	81.03±1.35***	166.77±1.12***
Cur-200	176.08±1.51***,###	66.11±1.42***,###	136.506±1.30***,###
Cur-100+Pie20	169.70±2.01***,###	59.40±2.13***,###	128.69±2.11***,###
Cur-50+Pie20	125.76±2.02***,###,†††	42.34±2.00***,###,†††	125.69±2.11***,###,†††
Cur-25+Pie20	138.71±2.58***,###,†	55.18±2.85***,###,†	131.57±2.44***,###,†

All the values are in mean±SEM, n=8. ***P<0.001 when compared to normal control, ###P<0.001 when compared to CP control, †P<0.05 when compared to Cur-200. NC=Normal control, CP=Cyclophosphamide, Cur=Curcumin, Pie=Piperine, AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, ALP=Alkaline phosphatase, CK-MB=Creatine kinase-MB, CK-NAC=Creatine kinase-N-acetyl-L-cysteine, LDH=Lactate dehydrogenase

Effect on serum marker enzymes against cyclophosphamide-induced myocardial toxicity

Effect on Lipid Profile

CP control group showed a significant increase in serum cholesterol and triglyceride levels compared to normal control group. All the treatment groups such as Cur-200, Cur-100+Pie20, Cur-50+Pie20, and Cur-25+Pie20 evidenced a significant decrease in cholesterol and triglyceride levels compared to CP control group. Cur-50+Pie20 and Cur-25+Pie20 treatment groups demonstrated a significant decrease in cholesterol and triglyceride levels, respectively, compared to Cur-200 alone-treated group [Table 3].

Table 3

Groups	Blood serum level mg/dl		Heart tissue homogenate (units)	
	Cholesterol	Triglyceride	SOD	Catalase
NC	34.45±0.57	75.46±0.34	46.43±0.32	45.49±0.32
CP control	95.44±0.32 ^{***}	118.39±0.28 ^{***}	23.34±0.71 ^{***}	21.44±0.46 ^{***}
Cur-200	49.52±0.24 ^{*****}	86.63±0.73 ^{*.**}	34.82±0.92 ^{**.**}	32.67±0.53 ^{**.**}
Cur-100+Pie20	47.98±0.34 ^{*.***}	84.54±0.61 ^{*.**}	36.73±0.46 ^{**.**}	34.23±0.65 ^{**.**}
Cur-50+Pie20	36.53±0.72 ^{***.††}	77.68±0.84 ^{***.††}	43.63±0.27 ^{***.††}	43.38±0.72 ^{***.††}
Cur-25+Pie20	41.13±0.29 ^{***.†}	79.51±0.21 ^{***.†}	41.54±0.83 ^{***.†}	41.64±0.50 ^{***.†}

All values are mean±SEM, n=8, *P<0.05, **P<0.01, and ***P<0.001, when compared to normal control, **P<0.01, ***P<0.001, and ††P<0.01, and †††P<0.001 when compared to Cur-200. NG=Normal control, CP=Cyclophosphamide, Cur=Curcumin, TBARS=Thiobarbituric acid reactive species, SEM=Standard error of mean

Effect on serum lipid profile, antioxidants in heart tissue homogenate, and histological score against cyclophosphamide-induced myocardial toxicity

Effect on Superoxide Dismutase and Catalase

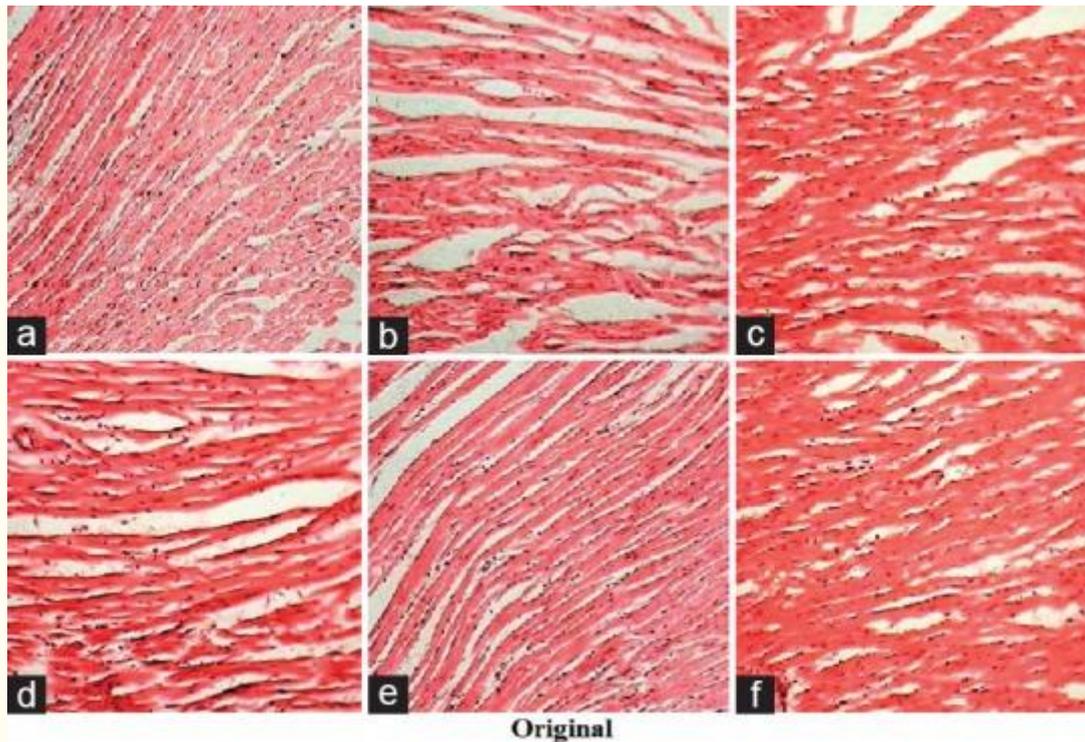
CP control group documented a significant reduction in SOD and catalase levels compared to normal control group. Cur-200, Cur-100+Pie20, Cur-50+Pie20, and Cur-25+Pie20 treatment groups witnessed a significant increment in SOD and catalase values compared to CP control group. Cur-50+Pie20 and Cur-25+Pie20 treatment groups showed a significant increase in SOD and catalase levels, respectively, compared to Cur-200 alone-treated group [Table 3].

Effect on Thiobarbituric Acid-reactive Species

A significant rise in TBARS was observed for CP control group compared to normal control group. Cur-200, Cur-100+Pie20, Cur-50+Pie20, and Cur-25+Pie20 treatment groups caused a significant reduction in TBARS level compared to CP control group. Cur-50+Pie20 and Cur-25+Pie20 treatment groups resulted in a significant reduction in TBARS values compared to Cur-200 alone-treated group [Table 3].

Effect on Histological Score

CP treatment caused severe damage in myocardial tissue integrity, which is evidenced by a significant increase in histological score compared to normal control. Extensive myofibrillar degeneration, marked diffuse inflammation, and increased interstitial space were caused by CP administration. Cur-200, Cur-100+Pie20, Cur-50+Pie20, and Cur-25+Pie20 treatment groups resulted in a significant restoration of histological score compared to CP control group. Cur-50+Pie20 and Cur-25+Pie20 treatment groups caused a significant improvement in histological score compared to Cur-200 alone-treated group [Table 3 and Figure 1].



[Figure 1](#)

(H and E, $\times 400$) stained microscopic section of rat heart (a) normal control group (normal texture of cell); (b) cyclophosphamide control group (necrotic cells with degeneration of myofibril, increased interstitial space, and diffuse inflammation); (c) Cur-200 (small multifocal degeneration, slight inflammation, and fall in interstitial space); (d) Cur-100+Pie20 (less interstitial space, myofibrillar degeneration); (e) Cur-50+Pie20 (less interstitial space); (f) Cur-25+Pie20 (slight inflammation, fall in interstitial space). Cur=Curcumin, Pie=Piperine

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Discussion

The current research was designed to evaluate the effect of curcumin alone and curcumin and Piperine (Pie) combination against CP-induced myocardial toxicity.

The study revealed that curcumin alone and its combination with piperine showed a significant protection against CP-induced myocardial toxicity. Moreover, the present study also reflected that piperine was able to synergize the effect of curcumin.

Curcumin is the main active constituent of *C. longa* belonging to the family Zingiberaceae. Curcumin is a polyphenol having potent antioxidant activity. It neutralizes the free radical and prevents the myocardium from the free radical stress. Curcumin is blessed with cardioprotective, anti-ischemic, anti-inflammatory, hepatoprotective, and antidiabetic activities. But from the beginning, curcumin is cursed with poor bioavailability, and many efforts are under investigation which can improve the bioavailability of curcumin.[5,6]

Another potential phytoconstituent, piperine, is isolated from the fruits of *P. nigrum*, belonging to family Piperaceae, which possesses anti-convulsant, anti-depressant, anti-malarial, anti-inflammatory, anti-arthritis, immunomodulatory, antioxidant, anti-asthmatic, anti-carcinogenic, anti-ulcer, and anti-amebic properties.[12,13]

Piperine is a natural bio-enhancer which is responsible for increasing absorption from gastrointestinal tract, inhibiting drug efflux pump, and inhibiting drug-metabolizing enzyme. Piperine by virtue of these effects increases the potency of co-administered drugs.[14]

CP has a destructive effect on myocardial cells. CP mediates the production of xanthine oxidase which catalyzes the oxidation of hypoxanthine to xanthine and generates superoxide and uric acid. Superoxide is known to generate reactive oxygen species. Thus, it can be understood that CP causes increased production of free radicals and decreased levels of antioxidant enzymes. Apart from that, it initiates and propagates lipid peroxidation.[2]

In our present study also, CP administration was responsible for significant decrease in SOD and catalase and increase in TBARS levels, which confirms the induction of myocardial toxicity. The groups treated with curcumin alone and combination of curcumin and PIP showed myocardial protection by increase in SOD and catalase and decrease in TBARS levels. Moderate (Cur-50)- and low (Cur-25)-dose combinations of curcumin and PIP demonstrated better protection compared to curcumin alone-treated group.

Due to generation of free radicals and decrease in antioxidant defense system, CP destroys myocardial cell integrity. Myocardial cell damage is responsible for leakage of different biomarkers such as CK-MB, LDH, ALT, AST, and ALP from the myocytes to the blood. Estimation of these marker enzymes serves as a diagnostic tool to detect myocardial necrosis.[2,15]

In the present study, animals treated with only CP showed a remarkable increase in the levels of serum marker enzymes such as LDH, CK-MB, CK-NAC, AST, ALT, and ALP that confirms myocardial toxicity. Curcumin alone and its combination with piperine restored these marker enzyme levels near to normal. Among the combination groups, moderate- and low-dose combinations of curcumin and PIP were found to be the most effective combinations.

In this present study, CP-induced myocardial toxicity is evidenced by abnormal ECG changes such as decrease in heart rate, RR interval and prolongation of QT interval, PR interval, and QRS interval.

Decrease in heart rate may be due to release of significant amount of acetylcholine, which is also linked with the genesis of myocardial damage.[16] CP administration causes increase in the cellular Na⁺ content and decrease in K⁺ content which may be responsible for the prolongation of QT interval. Loss of potassium may be the reason for QT interval elongation. Atrioventricular (AV) block caused by CP may be the prime reason for the prolongation of PR interval. Change in parasympathetic tone and conduction system deformation are the reasons for AV block.[17]

Curcumin alone and the combination groups of curcumin and PIP showed significant restoration of ECG parameters. Among all the treatment groups, moderate- and low-dose combination of curcumin and PIP showed best results to bring the ECG readings near to normal.

Histological study in CP-induced cardiotoxicity supported the findings of other parameters analyzed in different treatment groups. For the normal heart, myocardial fibers were found to be of uniform size, shape, and configurations, with no inflammatory cell infiltrates.[2,11] CP caused enormous changes in the myocardial cell associated with degeneration of myocardial tissue, vacuolization of the cardiomyocytes, infiltration of inflammatory cells, and myofibril loss. Treatment with curcumin alone and the combination of curcumin and PIP showed inhibition of cardiac damage by decreasing the fragmentation of myofibrils and inflammation. Among the combination groups, low- and moderate-dose combinations of curcumin and PIP were found to be most effective to retrieve the pathological changes associated with CP in myocardial cell.

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Conclusion

Curcumin alone and different doses of curcumin when combined with piperine show a significant beneficial effect against CP-induced cardiotoxicity. Combination groups reflect a substantial amount of synergistic activity compared to curcumin alone-treated group. Among the combination groups, moderate-dose combination, that is, curcumin (50 mg/kg, p.o.) combined with piperine (20 mg/kg, p.o.) shows the maximum cardioprotection.

The efficacy of curcumin could be attributed to its potential antioxidant activity and nitric oxide release-enhancing property. In the combination groups, bioavailability-enhancing effect of piperine further enhances the efficacy of curcumin. Moreover, antioxidant property of piperine synergizes cardioprotection by curcumin.

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Nil.

Conflicts of Interest

There are no conflicts of interest.

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