

Original Article

IMPACT OF GERANIOL ON CYCLOPHOSPHAMIDE PROVOKED CARDIOTOXIC PROPERTIES-  
AN *IN VIVO* STUDY

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ABSTRACT

**Objective:** Toxicity profiles of diverse anti-neoplastic drugs are extensively documented in past decades and the protective role of natural products against such toxic drugs plays a vital role in the evolution of chemoprevention. Globally, Cyclophosphamide (CYP) is used as an anticancer drug, but now it has been limited due to its acute and chronic cardio toxicity. Hence the present study was designed to investigate the protective role of Geraniol (GOH) against CYP induced cardio toxicity.

**Methods:** The activity of serum marker enzymes and lipoprotein fractions, cardiac marker enzymes and lipid profiles as well as antioxidant status was done using standard procedures *in vivo*. In addition, optimum dosage of GOH was determined as obtained by the levels of serum marker enzymes for tissue damage and the pathological changes were assessed by histology.

**Results:** Administration of CYP to rats results in hoisted levels of marker enzymes (in both serum and heart tissue) and reduced levels of antioxidants. GOH (200 mg/kg body weight) restrained the elevation of marker enzymes (in both serum and heart tissue) and augmented the levels of antioxidants. Moreover, histological observations added proofs to the protective role of GOH against cardiac damage.

**Conclusion:** CYP administered pathological changes were attenuated by the oral treatment of GOH at a dose of 200 mg/kg body weight of rat and suggests that the cardioprotective effects could be related to up regulation of antioxidant mechanism. Conclusive outcome from our findings suggests that GOH might be a potential chemo preventive agent in attenuating drug induced cardio toxicity.

**Keywords:** Cyclophosphamide, Geraniol, Cardiotoxicity.

INTRODUCTION

Most of antineoplastic chemotherapeutic agents result in cytotoxicity to one or more target tissues [1]. Now a day, most research on anticancer dosing strategies focusses on minimizing cytotoxicity rather than optimizing efficacy. In addition, most antineoplastic cytotoxic agents have diverse action modes [2]. Cardio toxicity can be induced by various chemotherapeutic agents, in particular by alkylating agents such as cyclophosphamide (CYP). Alkylating agents are a class of anticancer drugs that are awfully reactive and can bind covalently to a numerous biomolecules. CYP (N, N-Bis (2-chloroethyl) tetrahydro-2H-1, 3, 2-oxazaphosphorine-2-mine-2-oxide) (fig.1b) is an oxazaphosphorine alkylating agent, which is often used in chemotherapy and immunosuppressive treatment protocols [3]. It is widely used as a remedial platform for chronic and acute leukemias, lymphomas, multiple myeloma and rheumatic arthritis and also in the preparation for bone marrow transplantation [4-5]. The ability of CYP to interfere with normal cell division in all proliferating tissues provides the support for its therapeutic effects and many of toxic properties [3]. The major imperfection of CYP is the damage of normal tissue, end up in multiple organ toxicity. The critical factor for both therapeutic and toxic effects of CYP is the need of metabolic activation by hepatic microsomal cytochrome P450 mixed functional oxidase system [6-9]. It is already mentioned that high therapeutic doses of CYP could cause a noxious cardio toxicity that has an integration of symptoms and signs of myopericarditis leading to lethal complications like arrhythmias, congestive heart failure, myocardial depression and cardiac tamponade. The pathogenesis of CYP-induced acute cardio toxicity was ascribed to the elevation in free oxygen radicals and the destruction in the antioxidant defense mechanism. Impaired secretion of heart lipoprotein lipase, hypertriglyceridemia and hypercholesterolemia have been reported in CYP-treated rabbits [10-13]. Moreover, it has been reported that CYP induced its acute cardio toxicity by hoisting inner mitochondrial membrane permeability to calcium and reducing the activities of Krebs cycle enzymes with the subsequent uncoupling of mitochondrial-linked

ATP synthesis [14, 15]. In 1972, cardiotoxicity was first influenced by CYP as a complication of bone marrow transplantation [16]. In the procedures of bone marrow transplantation and peripheral blood stem cell transplantation (PBSCT), high-dose chemotherapy including CYP, is enormously used. After one week administration of CYP results in acute heart failure secondary to cardio toxicity and the incidence rate is about 20% and mortality about 8% after bone marrow transplantation [17-18]. In addition, cardio toxicity associated with high-dose CYP have been reported as an impediment of various therapeutic regimens [19-20]. Therefore, there is an indeed requirement for the novel therapeutic agents, which helps in the protection of normal tissue from drug induced toxicity with the absence of toxic properties.

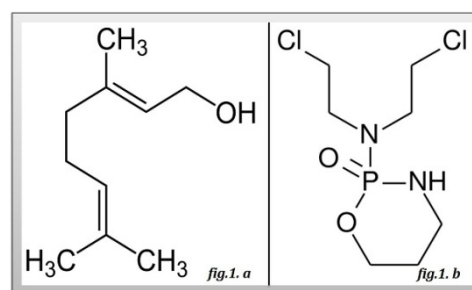


Fig. 1: a) Structure of Geraniol [GOH] and b) Cyclophosphamide [CYP]

Essential oils and their constituents have been used widely in traditional medicine for their diverse pharmaceutical actions. Recent days, plant-derived products to be of great focus due to their multiple pharmacological activities [21-25]. Monoterpenes are one such plant derivatives which possess diverse medicinal properties

and these components are considered as a new class of chemo preventive agents. Geraniol (GOH; 3, 7-dimethyl-2, 6-octadien-1-ol) (fig.1a), an acyclic monoterpene is predominantly found in citrus fruits, lemongrass and aromatic herb oils. GOH is a colorless oily liquid soluble only in solvents. It is a primary part of palmarosa oil, rose oil and citronella oil [26]. Experimental studies have demonstrated numerous biological effects of GOH including antibacterial [27], antifungal [28], anti-inflammatory [29], antioxidant [30], anti-cancer [30] and anti-ulcer activity [31]. In addition, recent studies reveal that GOH have significant neuroprotective and hepatoprotective activity [32-33]. But still, there is no relevant data available on the cardioprotective effect of GOH. Hence this study is designed in such a way to elucidate the cardioprotective role of GOH on experimentally induced cardiotoxicity. We analyzed the profiles of serum and tissue marker enzymes as well as serum and tissue lipids status. In addition, we studied the alteration on antioxidant enzymes and histopathological evaluation was done to assess the pathological change that occurs in rats during tissue damage.

## MATERIALS AND METHODS

### Source of chemicals and drugs

Cyclophosphamide (Cytosan®) was purchased from Hi Media laboratories, India. GOH was purchased from Sigma-Aldrich, Bangalore. All other chemicals used were of analytical grade obtained from SRL/TCI/HIMEDIA laboratories, India.

### Experimental animals

Male albino Wistar rats weighing about 150–155 g were procured from TANUVAS, Chennai, India. The animals were house caged under suitable environmental conditions ( $25 \pm 2$  °C) and were acclimatized to 12-h light: 12-h dark cycles. Animals were fed with commercially available standard rat pelleted feed (M/S Hindustan Foods Ltd, Bangalore, India) throughout the experimental period. The animals had free access to water. All the experiments were designed and conducted according to the ethical norms approved by Institutional Animal Ethics Committee guidelines (IAEC No. 17/01/2014).

### Experimental design (fig. 2)

**Group I** Normal control rats received corn oil which served as a vehicle (0.25 ml/100g body weight) throughout the experimental period along with standard diet and drinking water.

**Group II** Rats received the standard diet and drinking water throughout the experimental period and on the first day of experiment, a single dose of CYP (200 mg/kg body weight, intraperitoneally using saline) was given [34] and maintained for 10 days.

**Group III** Rats were treated with GOH (dissolved in corn oil) alone by orally at a dose of 200 mg/kg body weight (based on effective dose fixation studies) on all the days of the experimental period (10 days).

**Note:** Various doses of GOH (100, 200, 300, 400 mg/kg body weight) were administered orally to the CYP intoxicated animals to optimize the GOH dose for its maximum efficacy in the minimum dose, determined by the levels of serum marker enzymes for tissue damage (data shown in fig. 3). It was found that 200 mg/kg body weight of GOH has the maximum protective efficacy in the minimum dose.

**Group IV** Rats were treated with GOH (200 mg/kg body weight dissolved in corn oil 0.25 ml/100g body weight) to the CYP induced group of rats from the day 1 till the end of the experimental period.

At the end of the experimental period, the animals were anesthetized with Ketamine (90 mg/kg) and Xylazine (10 mg/kg) and sacrificed by cervical decapitation. Blood was collected and allowed to coagulate at room temperature for 30 min. Serum was separated by centrifugation at 3000 rpm for 15 min at 4 °C. The heart was immediately separated and washed in ice-cold saline. The tissues were sliced and homogenized in 0.1 M Tris-HCL buffer (pH 7.4). The homogenates were centrifuged at 1000 rpm for 10 min at

4 °C in a cooling centrifuge. The supernatants were separated and used for analyzing various parameters.

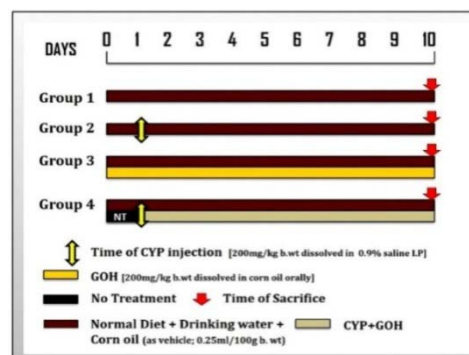


Fig. 2: Schematic representation of experimental design carried out for this study

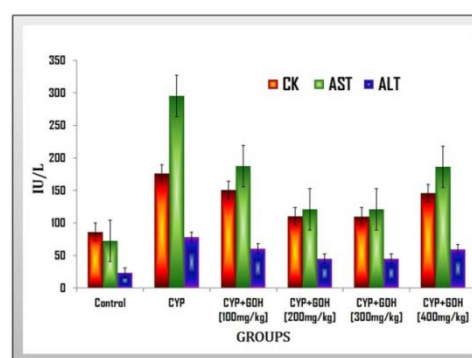


Fig. 3: Effect of different doses of GOH on control and CYP induced experimental groups of rats. Results are expressed as mean $\pm$ SD for six rats in each group

## Biochemical parameters

### Estimation of marker enzymes

Activities of creatinine kinase [35], lactate dehydrogenase (LDH) [36], aspartate transaminase (AST) [37], alanine transaminase (ALT) [37] and alkaline phosphatase (ALP) [38] were estimated in serum and tissue samples.

### Estimation of enzymic and non-enzymic antioxidants

Enzymatic antioxidant such as superoxide dismutase (SOD) [39], catalase (CAT) [40], glutathione peroxidase (Gpx) [41], glutathione reductase (GR), glutathione S-transferase (GST) [42] and non-enzymic antioxidant such as glucose-6-phosphate dehydrogenase (G6PD) [43], vitamin C (VIT C) [44], vitamin E (VIT E) [45], vitamin A (VIT A) [46] and glutathione (GSH) [47] content were assayed in tissue homogenate.

### Estimation of Serum lipoprotein fractions

Serum lipoproteins such as LDL, HDL and VLDL [48] were estimated in serum samples.

### Estimation of Cardiac lipid status

Cardiac lipids such as free cholesterol [49], esterified cholesterol [49], phospholipids [50-51], free fatty acids [52] and triglycerides [53] content were assayed in tissue homogenate.

### Histological evaluation

Histological evaluation was performed on a portion of the heart tissue after fixation with 10% formalin, embedded in paraffin wax, sectioned and was stained with hematoxylin and eosin to assess the pathological changes.

### Statistical analysis

All data obtained were analyzed by Students-*t*-test using MS-Excel, represented as mean±SD, for six animals ( $n=6$ ) in each group. The results were computed statistically (SPSS/10 software package; SPSS Inc., Chicago, IL, USA) using one-way ANOVA. Post hoc testing was performed for intercomparisons using the LSD. In all tests, the level of statistical significance was set at  $p<0.05$ .

### RESULTS

#### Effect of GOH on body weight, heart weight and relative heart weight

The cardioprotective effect of GOH against CYP induced tissue damage was elucidated in male Wistar albino rats. During the experimental period, animals were administered with CYP (200 mg/kg body weight, intraperitoneally using saline) and GOH (200 mg/kg body weight dissolved in corn oil 0.25 ml/100g body weight) and all rats showed greater tolerance to treatment with GOH. There were no death records in experimental groups. Fig. 4 shows Initial body weight and final body weight of the control and experimental group of animals. In CYP administered animals, there is a significant ( $p<0.05$ ) reduction in the final body weight whereas GOH treated animals showed significant ( $p<0.05$ ) gain in the final body weight when compared to CYP administered group II rats. In addition, there was no significant difference in the body weight of GOH alone treated rats compared to control rats.

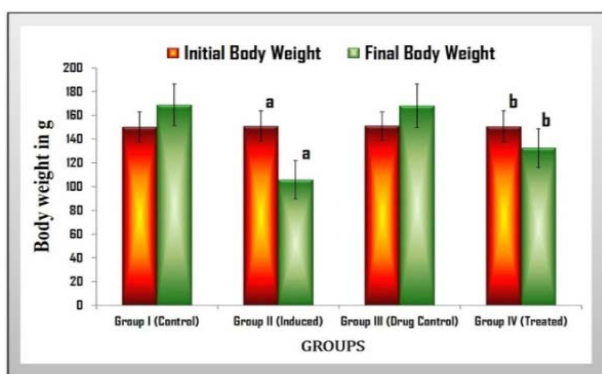


Fig. 4: Effect of GOH on Initial body weight and final body weight of control and experimental groups of rats

Results are expressed as mean±SD for six rats in each group. Statistical significance at  $p<0.05$  compared with <sup>a</sup>Compared with control and <sup>b</sup>Compared with CYP administered group.

Fig. 5 depicts the heart weight and relative heart weight of control and experimental group of animals. CYP induced rats distinctly augmented the heart weight and relative heart weight compared to group I control animals.

The GOH treated group IV animals exhibited obvious decrease in the heart weight and relative heart weight when compared with CYP administered animals. No distinct difference found between the control and GOH alone treated animals which clearly represent the nontoxic property of GOH.

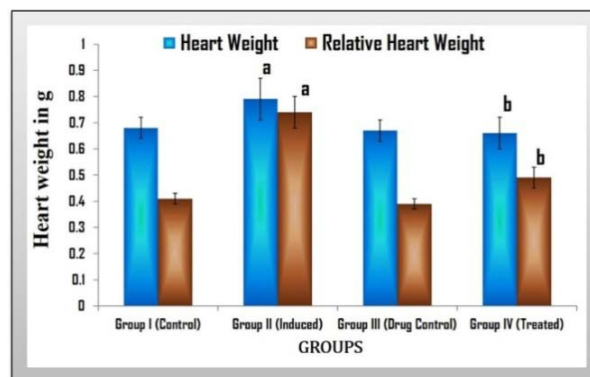


Fig. 5: Effect of GOH on heart weight and relative heart weight of control and experimental groups of rats

Results are expressed as mean±SD for six rats in each group. Statistical significance at  $p<0.05$  compared with <sup>a</sup>Compared with control and <sup>b</sup>Compared with CYP administered group.

#### Effect of GOH on the levels of serum and cardiac marker enzymes

Table 1 and 2 depicts the effect of GOH on the levels of marker enzymes CK, LDH, AST, ALT and ALP in the serum and heart of control and experimental group of rats. The levels of marker enzymes are significantly ( $p<0.05$ ) elevated in CYP-induced animals when compared with normal control animals. GOH-treated rats showed a significantly ( $p<0.05$ ) curtailed levels of these enzymes when compared with CYP-induced animals.

Moreover, there is no significant difference between the control and GOH alone treated animals. This may clearly indicates that GOH possess better restoration potential of cardiac tissue.

Table 1: Effect of GOH on the activities of marker enzymes in the serum of control and experimental groups of rats

Groups	CK	LDH	AST	ALT	ALP
Control	86.25±7.40	1006±110	72.32±6.55	23.20±2.17	10.63±1.18
CYP	176.34±16.32 <sup>a</sup>	1543±146 <sup>a</sup>	295.2±23.04 <sup>a</sup>	78.23±6.25 <sup>a</sup>	26.32±3.09 <sup>a</sup>
GOH	86.74±9.74	1087±124	72.84±5.88	22.98±3.11	10.78±0.96
CYP+GOH	110.24±12.27 <sup>b</sup>	1323±139 <sup>b</sup>	121.2±20.33 <sup>b</sup>	45.16±5.27 <sup>b</sup>	13.26±1.79 <sup>b</sup>

Results are expressed as mean±SD for six rats in each group. Statistical significance at  $p<0.05$  compared with <sup>a</sup>Compared with control and <sup>b</sup>Compared with CYP. Units — CK:  $\mu\text{mol}$  of phosphorus/h/mg protein; LDH, AST and ALT:  $\mu\text{mol}$  of pyruvate/h/mg protein; ALP: KA U/l.

Table 2: Effect of GOH on the activities of Cardiac marker enzymes in the tissue of control and experimental groups of rats

Groups	CK	LDH	AST	ALT
Control	28.7±2.32	10.06±0.99	7.23±0.41	11.50±1.17
CYP	8.76±1.04 <sup>a</sup>	6.48±0.92 <sup>a</sup>	3.96±0.27 <sup>a</sup>	4.62±0.30 <sup>a</sup>
GOH	28.5±2.26	10.87±0.96	7.10±0.47	11.68±1.16
CYP+GOH	19.76±2.30 <sup>b</sup>	8.76±1.01 <sup>b</sup>	5.28±0.76 <sup>b</sup>	8.96±0.96 <sup>b</sup>

Results are expressed as mean±SD for six rats in each group. Statistical significance at  $p<0.05$  compared with <sup>a</sup>Compared with control and <sup>b</sup>Compared with CYP. Units — CK:  $\mu\text{mol}$  of phosphorus liberated/h/mg protein; LDH, AST and ALT:  $\mu\text{mol}$  of pyruvate liberated/h/mg protein.

**Effect of GOH on antioxidant status in heart**

Table 3 and 4 represents the enzymic and non-enzymic antioxidant status in the heart of the control and experimental group of animals. CYP induced animals exhibited a significant ( $p<0.05$ ) destruction in the activities of enzymic antioxidants such as SOD, CAT, GPx, GR and GST when compared with normal control animals, GOH supplemented animals showed a significant ( $p<0.05$ ) elevation in the activities of these enzymes when compared with group 2 CYP-

induced animals. In addition, the activities of non-enzymic antioxidants such as GSH, G6PD, VIT A, VIT C and VIT E are also significantly declined in CYP induced animals when compared with control animals. In GOH treated animals, there is a significant ( $p<0.05$ ) raise in the activities of GSH, G6PD, VIT A, VIT C and VIT E when compared with CYP induced animals. No adverse effect was observed in GOH alone treated animals. This clearly depicts the antioxidant potential of GOH due to its protective levels of enzymic and non-enzymic antioxidant status.

**Table 3: Effect of GOH on the activities of enzymic antioxidants in the Heart of control and experimental groups of rats**

Groups	SOD	CAT	GPx	GR	GST
Control	2.17±0.17	75.06±5.39	20.23±1.41	1.36±0.10	1.09±0.12
CYP	0.99±0.07 <sup>a</sup>	35.48±3.92 <sup>a</sup>	13.96±1.27 <sup>a</sup>	0.63±0.05 <sup>a</sup>	0.68±0.06 <sup>a</sup>
GOH	2.22±0.16	76.87±4.96	20.10±1.47	1.27±0.08	1.03±0.14
CYP+GOH	1.88±0.09 <sup>b</sup>	67.76±6.23 <sup>b</sup>	17.28±1.76 <sup>b</sup>	1.01±0.11 <sup>b</sup>	0.87±0.07 <sup>b</sup>

Results are expressed as mean±SD for six rats in each group. Statistical significance at  $p<0.05$  compared with <sup>a</sup>Compared with control and <sup>b</sup> Compared with CYP.

Units: SOD in units/mg protein, CAT in  $\mu\text{mol}$  of  $\text{H}_2\text{O}_2$  decomposed/min/mg protein, GPx in  $\mu\text{mol}$  of GSH utilized/min/mg protein, GR in  $\mu\text{mol}$  of NADPH oxidized/min/mg protein and GST in  $\mu\text{mole}$  of CDNB-GSH conjugate formed/min/mg protein.

**Table 4: Effect of GOH on the activities of non-enzymic antioxidants in the Heart of control and experimental groups of rats**

Groups	GSH	G6PD	VITAMIN C	VITAMIN E	VITAMIN A
Control	5.76±0.57	1.26±0.13	1.20±0.13	1.83±0.25	1.17±0.10
CYP	3.39±0.37 <sup>a</sup>	0.79±0.06 <sup>a</sup>	0.79±0.09 <sup>a</sup>	1.36±0.07 <sup>a</sup>	0.76±0.02 <sup>a</sup>
GOH	5.92±0.56	1.27±0.12	1.22±0.11	1.81±0.27	1.16±0.09
CYP+GOH	4.88±0.59 <sup>b</sup>	1.06±0.10 <sup>b</sup>	1.01±0.10 <sup>b</sup>	1.50±0.16 <sup>b</sup>	1.06±0.07 <sup>b</sup>

Results are expressed as mean±SD for six rats in each group. Statistical significance at  $p<0.05$  compared with <sup>a</sup>Compared with control and <sup>b</sup> Compared with CYP.

Units: GSH glutathione in g/mg protein, G6PD in  $\mu\text{mol}$  of NADPH oxidized/min/mg protein, Vit C in mg/g of wet tissue, Vit E in mg/g of wet tissue and Vit A in mg/g of wet tissue and GSH in  $\mu\text{g}$ /mg protein.

**Effect of GOH on serum lipoprotein-cholesterol profile**

Table 5 shows the serum lipoprotein fractions such as LDL, HDL and VLDL of control and experimental groups of rats. The level of HDL was significantly ( $p<0.05$ ) reduced in CYP induced group when

compared with control animals. The levels of VLDL and LDL were significantly ( $p<0.05$ ) increased in CYP induced group when compared with control animals. However, significantly decreased levels of VLDL and LDL and elevated levels of HDL were observed in GOH treated animals when compared to CYP induced animals.

**Table 5: Effect of GOH on serum lipoprotein fractions of control and experimental groups of rats**

Groups	LDL	HDL	VLDL
Control	11.17±0.87	41.26±4.43	29.20±3.13
CYP	34.99±2.87 <sup>a</sup>	27.79±3.06 <sup>a</sup>	46.79±5.39 <sup>a</sup>
GOH	10.89±1.16	41.77±5.12	28.97±3.11
CYP+GOH	20.88±2.29 <sup>b</sup>	36.06±3.70 <sup>b</sup>	35.01±4.10 <sup>b</sup>

Results are expressed as mean±SD for six rats in each group. Statistical significance at  $p<0.05$  compared with <sup>a</sup>Compared with control and <sup>b</sup> compared with CYP, Units: mg/dL

**Effect of GOH on cardiac lipid status**

Table 6 portrays the cardiac lipid fractions such as free cholesterol, esterified cholesterol, phospholipids, free fatty acids and triglycerides. CYP administered animals depict significant increase in the content of free cholesterol, esterified cholesterol and

triglycerides and there was a significant reduction in the content of phospholipids and free fatty acids. Supplementation of GOH brought about a significant ( $p<0.05$ ) increase in the content phospholipids and free fatty acids and a significant ( $p<0.05$ ) decline in the free cholesterol, esterified cholesterol and triglycerides content of heart, relative to CYP alone induced animals.

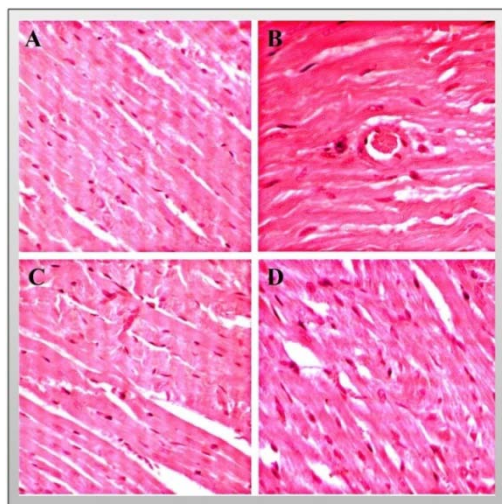
**Table 6: Effect of GOH on cardiac lipids of control and experimental groups of rats**

Groups	Free cholesterol	Esterified cholesterol	Phospholipids	Free fatty acids	Triglycerides
Control	3.57±0.47	1.46±0.13	15.20±1.33	3.28±0.33	3.36±0.29
CYP	4.99±0.27 <sup>a</sup>	2.79±0.36 <sup>a</sup>	10.79±0.59 <sup>a</sup>	2.49±0.26 <sup>a</sup>	7.79±0.59 <sup>a</sup>
GOH	3.59±0.46	1.41±0.12	14.97±1.31	3.27±0.31	3.39±0.41
CYP+GOH	4.08±0.38 <sup>b</sup>	1.94±0.17 <sup>b</sup>	13.01±0.80 <sup>b</sup>	2.91±0.22 <sup>b</sup>	5.01±0.60 <sup>b</sup>

Results are expressed as mean±SD for six rats in each group. Statistical significance at  $p<0.05$  compared with <sup>a</sup>Compared with control and <sup>b</sup> Compared with CYP, Units: mg/dL

### Effect of GOH on histological features of heart

The histology of the heart tissue sections, stained with hematoxylin and eosin (H&E) was assessed under a light microscope. Control animals (fig. 6A) revealed the normal architecture of the heart (appearance of enlarged, swollen mitochondria and vacuoles) and GOH alone supplemented animals (fig. 6C) also showed the normal histological appearance as compared to normal control animals indicating the nontoxic nature of GOH. The CYP induced animals (fig. 6B) showed myocyte swelling and hyalinization at focal, whereas Cyclophosphamide-administered and GOH-treated heart (fig. 6D) showing almost normal myocytes and the abnormal pathological findings are reduced. These restorations may be due to the protective effect of GOH against oxidative stress and tissue damage induced by CYP.



**Fig. 6: Photomicrographs (100x) obtained from 6. A. Control group, 6. B. CYP group, 6. C. GOH alone group and 6. D. CYP+GOH group**

### DISCUSSION

Cyclophosphamide (CYP), a commonly used oxazaphosphorine alkylating agent, has been extended from neoplastic diseases to organ transplantation and diverse disorders and as an immunosuppressive agent. The major limitation of CYP is the injury of normal tissue, leading to multiple organ toxicity. It is well documented that high therapeutic doses of CYP could cause a lethal cardiotoxicity that has a combination of symptoms and signs of myopericarditis leading to fatal complications such as congestive heart failure, arrhythmias, cardiac tamponade and myocardial depression. Cellular mechanisms of CYP-induced cardiotoxicity are thought to be mediated by an increase in free oxygen radicals and the decrease in the antioxidant defense mechanism [54]. This study has been initiated to investigate the possible mechanisms whereby GOH could prevent the development of CYP-induced cardiotoxicity.

In this current study, administration of CYP (200 mg/kg body weight in single dose) noticeably reduced body weight significantly increased heart weight to overall body weight in comparison with normal control rats. In the present investigation, serum cardiotoxicity enzymatic indices, CK, LDH, AST and ALP which occurred as a result of damaged myocytes and these increase in the serum levels are the predominant indicators of cardiac damage. It is most likely to be the result of toxic effects of CYP, as elevated levels of these enzymes in serum are associated with cardiac injury such as myocardial infarction, myocarditis and heart failure. CYP induced cardiotoxicity may be due to direct myocardial endothelial damage, end up in extravasation of blood containing high levels of the drug and resulting in the release of CK and LDH from necrotic cells. GOH (200 mg/kg body weight) co-treatment brought back these enzymes to near routine level by possibly preserving the functional integrity of the myocytes, showing its defense action against CYP induced

cardiotoxicity. These results are consistent with the data presented by several authors, who demonstrated a marked elevation of CK, LDH, ALT, and AST, 10 days after administration of a single dose of CYP (200 mg/kg) [3, 15, 34, 55]. This protective effect of GOH could be due to stabilization of cardiac membranes with the consequent decrease in the release of cardiac enzymes. Increased activities of cardiac enzymes in serum are well-known diagnostic indicators of cardiac injury [55].

Previous studies by Madan *et al.*, showed 200 mg/kg body weight of GOH as a maximum protective dose for oral carcinoma [30]. Hence, we tried the same in cardiotoxicity conditions by treating various doses of GOH (100, 200, 300, 400 mg/kg body weight) were administered orally to the CYP intoxicated animals. It was found that 200 mg/kg body weight of GOH has the maximum protective efficacy in the minimum dose as determined by the levels of serum marker enzymes for tissue damage (data shown in fig. 3). Hence, we used this concentration as the standard concentration for further experimental studies.

Pathological condition that leads to hoisted production and/or abortive scavenging of reactive oxygen species (ROS) may play a central role in determining tissue injury. Over production of ROS during CYP therapy causes membrane injury by initiating lipid peroxidation that result in loss of function and integrity of myocardial membranes. Cellular mechanism of toxicity is provoked by an increase in the free radicals through intracellular phosphoramidate mustard and acrolein, the primary alkylating metabolites of CYP and it plays a vital role in the occurrence of cardiac injury. Several studies revealed the protective nature of enzymic antioxidants and non-enzymic antioxidants against oxidative damage [22, 34, 55-56]. Destruction in the levels of these antioxidant enzymes in the myocytes of CYP administered rats occurs as, a result of inactivation of these enzymes by ROS. This causes further elevation in the levels of ROS which severely decrease the activities of antioxidant enzymes. Reduced levels of the antioxidants in myocytes make it more exposed to the free radical damage. Mythili *et al.* reported that CYP (200 mg/kg) induced acute cardiotoxicity was attributed to the increase in ROS and the decrease in the antioxidant defense mechanisms in the heart and that antioxidant compounds attenuated CYP-induced cardiotoxicity [15]. In line with this text, in the present study, CYP significantly decreased the antioxidant mechanisms (table 3), thus leaving the heart tissues with no known defense mechanism against ROS. Therefore, hearts from CYP-treated rats are more vulnerable to ROS. Interestingly, GOH treated rats showed improved activities of antioxidants than CYP intoxicated group. This clearly proves the antioxidant capacity of GOH due to its free radical encountering activity. Moreover, many studies in our laboratory reporting the antioxidant potential of various compounds against cardiotoxicity as well as hepatotoxicity which includes Ferulic acid [57], Ascorbic acid [57], Squalene [56] and Carvacrol [22].

SOD and CAT are the primary antioxidants involved in the inactivation of environmental carcinogens and in the direct elimination of toxic free radicals and electrophiles, resulting in the amelioration of oxidative damage [22, 56-59]. In accordance with the present findings, the significant decrease in the levels of SOD and CAT activity was due to exhaustion of the enzymes as a result of oxidative stress and increase in LPO caused by CYP. GPx is a well-known first line of defense against oxidative stress and several studies have reported the decreased activities of GPx in various toxicity conditions. GST is a group of multifunctional proteins that perform functions ranging from catalyzing the detoxification of electrophilic compounds to protection against peroxidative damage. GR plays a major role in regenerating GSH from GSSG, thus maintaining the balance between the redox couple. This enzyme requires NADPH for its activity, which is provided by the action of G6PD. Besides, the diminished activity of GPx, GST and GR might be owing to the decreased availability of the substrate, GSH and increase in the levels of peroxides during cardiotoxicity on CYP administered rats. Nevertheless, GOH treatment bolster the antioxidant defense mechanism as depicted by the increased tissue levels of SOD, CAT, GPx, GST and GR antioxidants. Non-enzymic antioxidants such as GSH, Vitamin-E, Vitamin-C and Vitamin-A are

closely interlinked to each other and play an excellent role in protecting the cell from oxidative damage. Glutathione is a ubiquitous tripeptide which functions as a free radical scavenger in the oxidative stress conditions [58]. Depletion of GSH impairs the ability of the cells to protect against the free radicals and results in enhanced LPO. Vitamin E is a lipid soluble antioxidant which is present in cellular membranes where it plays an important role in the suppression of free radical induced LPO [59]. Vitamin C is a free radical scavenger and functions as an antioxidant in recycling the Vitamin E radical back to Vitamin E. GSH in tissues keeps up the cellular levels of vitamin C and vitamin E in active form. These data clearly exemplify the reduced activities of the vitamin C and E, as pronounced GSH depletion is observed in the CYP administered group. GOH supplemented animals significantly increased the levels of all the above antioxidants which may be due to ability GOH to interact with hydroxyl and superoxide radicals thereby subsequently having the property to scavenge them. The alterations in lipoprotein transport and metabolism play an essential role in the context of changes in plasma lipids. Data from this study revealed that CYP significantly increased cholesterol and triglycerides in serum. Hypercholesterolemia, hypertriglyceridemia induced by CYP, which are well-known risk factors in cardiovascular diseases, has been reported previously. Interestingly, GOH supplementation completely reversed the CYP-induced increase in cholesterol and triglycerides to the control values. Biochemical data were further confirmed by histopathological studies of the cardiac tissues. As CYP induction (10 days of its administration) caused marked myocardial degeneration in the form of myofibrillar loss, inflammatory cell infiltration, cytoplasmic vacuole formation, interstitial edema and hemorrhage. These histopathological changes have been previously reported in CYP-induced cardiotoxicity [15, 34, 55-57, 60]. GOH treated animals revealed the normal architecture of the heart and showed better cardioprotection as observed by the absence of adverse pathological changes in the heart of CYP induced cardiac damage. Thus, GOH may reduce oxidative stress through the direct antioxidant effect, leading to the prevention of CYP-induced cardiotoxicity.

## CONCLUSION

The present study from our research findings clearly elaborates the significant protective effect of GOH on CYP induced cardiotoxic properties by enhanced the levels of antioxidants and reduced levels of marker enzymes. It is hypothesized that GOH protects the tissues by scavenging the toxic metabolite, which is proved by the normalization of the biochemical parameters. Hence, GOH may be often considered as an effective chemoprevention model for CYP-induced cardiotoxicity.

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## CONFLICT OF INTERESTS

Declared None

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