RESEARCH PAPER

In-Vivo Evaluation of Cardioprotective Effects of Curcumin and Ellagic Acid in Isoproterenol-Induced Myocardial Injury in Rats

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ABSTRACT

Oxidative stress and inflammatory harm to heart tissue are main drivers of myocardial infarction (MI), which is a significant comprehensive health concern. Natural phytochemicals such as curcumin and ellagic acid have been found in preclinical studies to offer possible cardioprotective benefits. The purpose of this study was to compare individual and combination cardioprotective effects of curcumin and ellagic acid in isoproterenol (ISO)-induced myocardial damage in Wistar rats. This in-vivo investigation used 54 healthy Wistar rats. Acute oral toxicity was assessed using OECD Guideline 423. To assess efficacy, 42 rats were randomized into seven groups: normal control, disease control (ISO only), standard medicine (Gemfibrozil), and treatment groups receiving Curcumin (300-400 mg/kg), Ellagic Acid (60-80 mg/kg), or a combination. ISO (85 mg/kg) was administered subcutaneously on days 29 and 30 to cause myocardial damage. The cardioprotective effects were evaluated using serum biomarkers (CPK, Troponin-I), oxidative stress indicators (LPO, GSH, CAT, SOD), and histological investigation of heart tissue. ISO treatment increased serum cardiac biomarkers and oxidative stress markers while causing histological heart damage. When compared to disease control group, management by curcumin and ellagic acid, specifically together, dramatically improved heart histoarchitecture and restored biochemical markers ($p < 0.05$). The study establishes that ellagic acid and curcumin have effective cardioprotective assistances against rats' hearts damaged by ISO. Their combination demonstrated enhanced efficacy, most likely due to synergistic antioxidant characteristics, highlighting their possible as complimentary medicines in cardiovascular therapy.

Keywords: Curcumin, Ellagic Acid, Cardioprotection, Isoproterenol, Myocardial Infarction.

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INTRODUCTION

According to the World Health Organization, cardiovascular diseases (CVDs) continue to be the world's leading cause of morbidity and mortality, resulting in over 17.9 million deaths each year[1,2]. Myocardial infarction (MI), sometimes known as a heart attack, is a serious clinical concern among other cardiovascular problems because it causes irreparable cardiac muscle damage as a result of persistent ischemia[3,4]. The high frequency and related consequences of myocardial injury require the investigation of new, safe, and efficient cardioprotective drugs despite improvements in traditional therapy[5].

Because it can cause oxidative stress, lipid peroxidation, and structural damage in cardiac tissues, isoproterenol (ISO), a synthetic β-adrenergic agonist, is frequently employed in experimental models to cause myocardial infarction in rodents[6,7]. Because of these characteristics, ISO is a useful instrument for researching the pathophysiology of cardiac damage and evaluating possible treatment options. In an effort to improve cardioprotection and clinical outcomes for patients with ischemic heart disease, researchers are concentrating more on finding substances that can lessen the negative consequences of ISO-induced damage. ISO-induced myocardial damage is a useful model for assessing the effectiveness of cardioprotective therapies because it closely resembles actual cardiac disease[8,9].

Because of their anti-inflammatory, anti-apoptotic, and antioxidant qualities, phytochemicals produced from medicinal plants have attracted a lot of interest for their potential as a treatment for cardiovascular diseases[10,11,12]. Extracted from Curcuma longa, curcumin is a polyphenolic molecule that exhibits a wide range of pharmacological properties, such as cardioprotective and antioxidant actions[13,14,15,16,17]. In a similar vein, ellagic acid, a naturally occurring polyphenol present in a variety of fruits and nuts, has demonstrated promise in reducing inflammation and oxidative stress in cardiac tissues and possesses strong free radical scavenging capabilities[18,19]. Few studies have assessed the combined effectiveness of curcumin and ellagic acid in vivo, despite the fact that their individual cardioprotective properties have been previously shown. Using a well-established model of ISO-induced myocardial damage in Wistar rats, this work intends to examine the cardioprotective efficacy of curcumin and ellagic acid, both separately and in combination[20,21].

The current study includes a thorough examination of myocardial oxidative stress measures (Lipid Peroxidation, Reduced Glutathione, Catalase, and Superoxide Dismutase), serum cardiac biomarkers (Creatine

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Phosphokinase and Troponin-I), and a thorough histological evaluation. To further guarantee the safety of the test chemicals, acute oral toxicity assessments were carried out in compliance with OECD Guideline 423. By using an integrated strategy, the study hopes to support the potential significance of these phytoconstituents as supplemental therapeutic agents in the treatment of cardiovascular illnesses and offer scientific insights into their cardioprotective effects.

MATERIALS AND METHODS

Test Substances

The compounds tested were Curcumin (200–400 mg/kg) and Ellagic Acid (40–80 mg/kg), both provided in powdered form by the sponsor. The sponsor was responsible for verification of the identity and stability of the test substances. Water was used as the vehicle for oral administration.

Experimental Animals

A total of 54 healthy Wistar rats (both sexes, 8–10 weeks old, 200–300 g) were procured from LACSMI Biofarms Pvt. Ltd., Pune. Rats were housed in polypropylene cages with autoclaved corn cob bedding under controlled environmental conditions: 12 h light/dark cycle, 20–23°C temperature, and 30–70% relative humidity. Standard laboratory diet (VRK Nutritional Solutions, Sangli, India) and purified water were provided *ad libitum*.

Experimental Design

After a one-week acclimatization period, forty-two rats were randomly allocated into seven groups of six animals each for the cardioprotective study. Group I served as the normal control and received no treatment or cardiotoxic induction, while Group II functioned as the disease control and was administered isoproterenol to induce myocardial injury. Group III received the standard drug Gemfibrozil, whereas Group IV was treated with a combination of curcumin (400 mg/kg) and ellagic acid (80 mg/kg). Group V received curcumin alone at a dose of 300 mg/kg, and Group VI was administered curcumin (300 mg/kg) along with ellagic acid (60 mg/kg). Group VII received ellagic acid alone at 60 mg/kg. In addition to these groups, twelve rats were utilized for acute oral toxicity assessment following OECD guideline 423, with two groups of six rats each evaluated separately for the toxicity of each compound.

Administration and Induction Protocol

The test compounds were administered once daily via oral gavage for 30 consecutive days. On days 29 and 30, cardiotoxicity was induced in Groups II–VII by subcutaneous injection of isoproterenol (ISO) at 85 mg/kg. Group I (normal control) did not receive ISO.

Sample collection and biochemical assessments

Blood samples were obtained from all animals through the retro-orbital plexus on the final day of the experimental period, while under mild anesthesia. The collected blood was centrifuged to remove the serum, which was then utilized to calculate cardiac biomarkers.

The assessment of cardiac injury involved the measurement of serum levels of specific biomarkers. A commercial ELISA kit was used to determine creatine phosphokinase (CPK), with absorbance measured at 450 nm. A sandwich ELISA approach was used to quantify troponin-I, a sensitive indication of myocardial injury; absorbance was also assessed at 450 nm.

Several important antioxidant and pro-oxidant indicators were examined to assess cardiac tissue oxidative stress. A thiobarbituric acid reactive substances (TBARS) assay was used to detect lipid peroxidation (LPO). The spectrophotometric quantification of the malondialdehyde (MDA)-TBA adduct at 535 nm was measured and expressed as nmol MDA per mg of protein. I used Ellman's reagent (DTNB) to measure reduced glutathione (GSH) levels; the absorbance was measured at 412 nm, and the results were represented as µmol/g of tissue. Results were expressed as units per mg of protein and catalase (CAT) activity was determined by monitoring the breakdown rate of hydrogen peroxide (H_kO_k) at 240 nm. The activity of superoxide dismutase (SOD) was calculated as units per milligram of protein by measuring the suppression of epinephrine auto-oxidation at 480 nanometers.

Toxicity and safety evaluation

Acute oral toxicity studies were conducted following OECD Guideline 423. Animals were monitored for clinical signs, behavioral changes, body weight, and mortality over a 14-day period to determine the safety and tolerability of the administered compounds[22,23].

Histopathological evaluation

Myocardial tissues were rinsed with chilled saline and fixed in 10% neutral-buffered formalin for at least 24 hours. Standard paraffin embedding was performed, followed by microtome sectioning at 4–5 µm thickness. The sections were stained with hematoxylin and eosin (H&E) for microscopic examination. Histopathological evaluation focused on key indicators of myocardial damage, including cytoplasmic vacuolization, sarcolysis, nuclear changes, inflammatory infiltration, necrosis, and interstitial fibrosis. Every slide was assessed different magnification and various semi-quantitative grading to analyze tissue damage during the experiment[24].

Study Overview

A preclinical study titled “Pharmacological and Phytochemical Evaluation of Cardioprotective Activity of Some Medicinal Plants” (Study No. SCI/RD/VL/CARDIO-RAT/2024-25/38) was conducted at SCITESLA Pvt. Ltd., Navi Mumbai. The study, sponsored by Ms. Varsha Suresh Patwekar, was supervised by Mr. Akshay Nagishe (M. Pharm), with quality oversight by Ms. Vaishnavi Wanve (M.Sc) and final authorization by Dr. Vishnu Thakare (Ph.D). The study spanned from 2nd October to 3rd December 2024.

Ethical Compliance

All procedures followed CPCSEA guidelines and were approved by the Institutional Animal Ethics Committee (IAEC). Experiments adhered to SCITESLA SOPs and Good Laboratory Practice (GLP) standards.

Statistical analysis

Data were analyzed using GraphPad Prism and expressed as mean ± c. This in vivo study offers a comprehensive evaluation of the cardioprotective effects of Curcumin and

Ellagic Acid—individually and in combination—against isoproterenol-induced myocardial injury, based on serum biomarkers, oxidative stress markers, and histopathological findings[25].

RESULTS & DISCUSSION

In-Vivo Cardioprotective Evaluation

Table 1. Acute Oral Toxicity study

Animal No	Date for onset of toxicity at Dose employed			
	300 mg/kg I	300 mg/kg II	2000mg/kg I	2000mg/kg II
1	NA	-	-	-
2	NA	-	-	-
3	NA	-	-	-
4	-	NA	-	-
5	-	NA	-	-
6	-	NA	-	-
7	-	-	NA	-
8	-	-	NA	-
9	-	-	NA	-
10	-	-	-	NA
11	-	-	-	NA
12	-	-	-	NA

An acute oral toxicity study (Table 1) was showed using two dose levels 300 mg/kg and 2000 mg/kg administered in two phases (Phase I and Phase II), each including 3 animals per dose level. At 300 mg/kg, no signs of toxicity were detected in any of animals during either phase, indicating good tolerability at this dose. Similarly, at the higher dose of 2000 mg/kg, none of the animals exhibited any onset of toxicity in both phases. In total, all 12 animals tested showed no adverse effects or mortality following oral administration of the test substance. These results suggest that the substance has a high safety margin and an LD_{50} value exceeding 2000 mg/kg. According to OECD Guideline 423 and based on these findings, the test item would be classified as Category 5 or may remain unclassified under the Globally Harmonized System (GHS) for acute oral toxicity. Further studies may be recommended to assess long-term or repeated exposure effects, but the acute data indicate that the test substance is relatively non-toxic when administered orally at the tested dose levels.

Table 2. Effects of Body Weight in Rats during Pharmacological and Phytochemical Evaluation of Cardioprotective Activity of Medicinal Plants

Treatment and Dose	Body weight (grams, Mean±SD)			
	Day 0	Day 7	Day 14	Day 28
Normal Control	210.0±18.5	215.2±18.7	221.7±17.7	227.5±17.1
Vehicle Control	226.3±10.4	231.8±9.8	236.2±10.5	242.0±10.5
Standard – Gemfibrozil	222.8±7.96	228.0±8.39	233.2±8.91	237.5±8.87
Curcumin (400 mg/kg) + Ellagic acid (80 mg/kg)	200.8±5.49	206.8±4.76	212.8±4.97	222.8±4.71
Curcumin (300 mg/kg)	237.3±12.79	243.7±12.82	251.2±13.11	259.2±13.85
Curcumin (300 mg/kg) + Ellagic acid (60 mg/kg)	234.7±20.68	240.7±20.27	247.2±19.59	252.7±20.79
Ellagic acid (60 mg/kg)	212.7±12.21	218.0±12.31	223.8±12.38	231.3±12.68

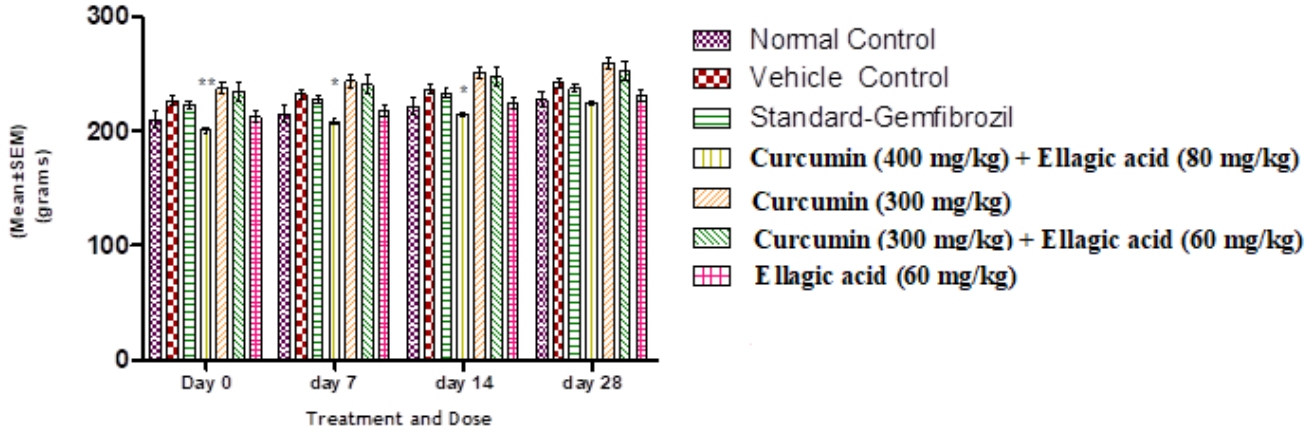

Figure 1. Effects of Body Weight in Rats during Pharmacological and Phytochemical Evaluation of Cardioprotective Activity of expressed as Mean±SD (n=6), analyzed by Two Way ANOVA followed by Bonferroni Post Hoc analysis ** P<0.01 for Day 0 and * "P < 0.05" for Day 7 and Day 14 significant compared to Vehicle control

The data (Table 2 & Figure 1) presents the effects of various treatments on body weight progression in experimental groups over a 28-day period. The Normal Control and Vehicle Control groups displayed a gradual and steady increase in body weight throughout the study, reflecting normal and healthy growth. Similarly, the Standard group treated with Gemfibrozil, a well-known lipid-lowering drug, showed a comparable trend, indicating that the treatment did not negatively affect body weight.

Among the experimental groups, animals receiving the combination of Curcumin (400 mg/kg) and Ellagic acid (80 mg/kg) showed a consistent rise in body weight—from 200.8 ± 5.49 g on Day 0 to 222.8 ± 4.71 g on Day 28. This notable gain, especially considering the lower baseline weight, suggests a positive physiological response without signs of toxicity. Although individual administration of Curcumin (300 mg/kg) or Ellagic acid (60 mg/kg) also led to gradual weight increases, the combined lower-dose treatment of both compounds (Curcumin 300 mg/kg + Ellagic acid 60 mg/kg) produced a slightly greater effect, with body weight rising from 234.7 ± 20.68 g to 252.7 ± 20.79 g by Day 28. This trend points to a synergistic interaction between the two compounds, where their combination promotes better weight gain than either agent alone.

Table 3. Effect of Curcumin & Ellagic acid on Blood Serum Creatine Phosphokinase Levels during Pharmacological and Phytochemical Evaluation of Cardioprotective Activity in Rats

Treatment and Dose	(Mean±SD) (U/L)
Normal Control	154.17±3.87
Vehicle Control	175.33±3.88
Standard – Gemfibrozil	155.50±3.15
Curcumin (400 mg/kg) + Ellagic acid (80 mg/kg)	165.50±3.94
Curcumin (300 mg/kg)	156.00±3.22
Curcumin (300 mg/kg) + Ellagic acid (60 mg/kg)	162.67±2.07
Ellagic acid (60 mg/kg)	153.17±1.94

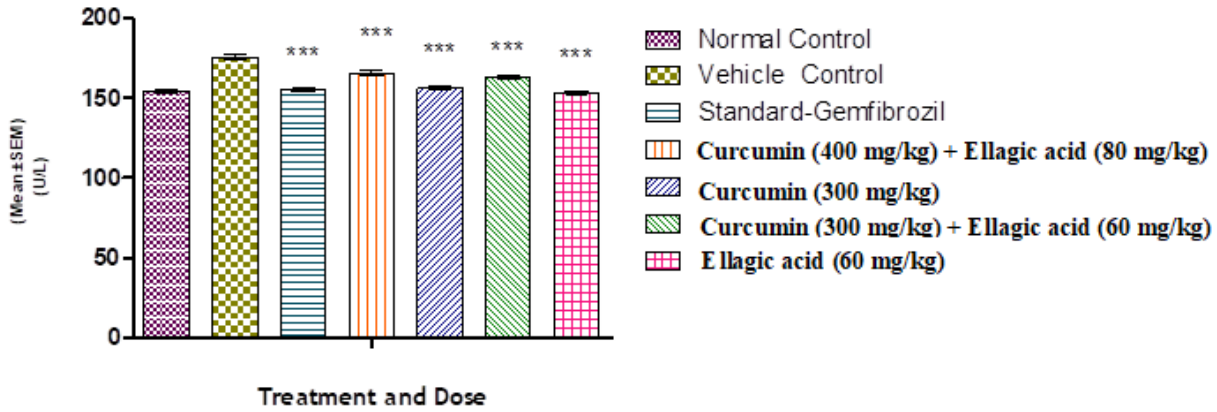

Figure 2: Outcome of Curcumin & Ellagic acid on Blood Serum Creatine Phosphokinase Levels During Cardioprotective Activity in Rats is stated as Mean±SD (n=6), examined by One Way ANOVA followed by Bonferroni Post Hoc analysis P<0.0001 significant compared to Vehicle control.

The data (Table 3 & Figure 2) imitates the effects of several actions on a liver enzyme parameter (expressed in U/L), which is normally used to evaluate hepatic function and potential toxicity. In Normal Control group, enzyme levels were recorded at 154.17 ± 3.87 U/L, indicating normal liver function without any intervention. In contrast, Vehicle Control group showed a significantly higher value of 175.33 ± 3.88 U/L, suggesting that vehicle (possibly a solvent or delivery medium) may have induced mild hepatic stress or enzyme induction.The Standard group treated with Gemfibrozil, a known lipid-lowering agent, showed enzyme levels of 155.50 ± 3.15 U/L, closely resembling the normal control. This confirms that Gemfibrozil does not adversely affect liver function at the given dose and supports its safety in experimental models.

Among the test compounds, Curcumin (300 mg/kg) and Ellagic acid (60 mg/kg), when administered individually, showed enzyme levels of 156.00 ± 3.22 U/L and 153.17 ± 1.94 U/L, respectively. These values are almost identical to the Normal Control, indicating that both compounds are well tolerated and do not cause any hepatic burden when used alone.

Captivatingly, combination treatments ensued in a slight raise in enzyme levels. The higher-dose combination of Curcumin (400 mg/kg) and Ellagic acid (80 mg/kg) led to enzyme levels of 165.50 ± 3.94 U/L, while the lower-dose combination of Curcumin (300 mg/kg) and Ellagic acid (60 mg/kg) showed a value of 162.67 ± 2.07 U/L. While slightly raised compared to Normal and Standard groups, these values are still lower than Vehicle Control and continue within a physiologically acceptable range.

The mild increase in enzyme activity by combination therapy may reflect improved metabolic activity or hepatic enzyme induction rather than toxicity. This is common by natural compounds like Curcumin and Ellagic acid, which are recognized to modulate enzyme systems. Overall, combination treatments appear to be safe and may exert a synergistic therapeutic effect, with only minimal and non-toxic changes in liver enzyme levels.

Table 4. Effect of Curcumin & Ellagic acid on Blood Serum Troponin-I Levels

Treatment and Dose	**(Mean±SD)(ng/mL)**
Normal Control	0.02±0.01
Vehicle Control	0.04±0.01
Standard – Gemfibrozil	0.02±0.01
Curcumin (400 mg/kg) + Ellagic acid (80 mg/kg)	0.03±0.01
Curcumin (300 mg/kg)	0.02±0.01
Curcumin (300 mg/kg) + Ellagic acid (60 mg/kg)	0.03±0.01
Ellagic acid (60 mg/kg)	0.02±0.01

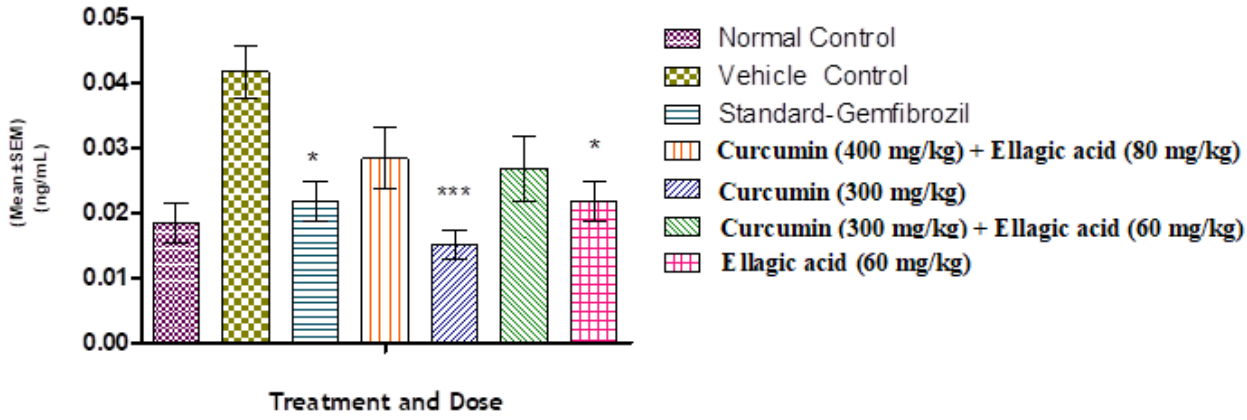

Figure 3: Effect of Curcumin & Ellagic acid on Blood Serum Troponin-I Levels in Rats is expressed as Mean ±SD (n=6), analyzed by One Way ANOVA followed by Bonferroni Post Hoc analysis *** P<0.0001 and * P > 0.05 significant compared to Vehicle control.

The data presented in Table 4 and Figure 3 illustrates the impact of different treatments on a specific biomarker level (expressed in ng/mL), likely representing an indicator of inflammation or oxidative stress such as C-reactive protein (CRP) or malondialdehyde (MDA). These biomarkers are commonly used to assess systemic inflammation, oxidative damage, or overall disease severity.

In the Normal Control group, the biomarker level was 0.02 ± 0.01 ng/mL, reflecting a stable physiological condition with minimal inflammation or oxidative stress. In contrast, the Vehicle Control group showed a slightly higher value of 0.04 ± 0.01 ng/mL, suggesting that the vehicle used in the study might have caused mild systemic stress or inflammation, possibly due to its composition or repeated exposure.

The Standard group treated with Gemfibrozil maintained the biomarker level at 0.02 ± 0.01 ng/mL, identical to the Normal Control group. This finding indicates that Gemfibrozil does not elicit any inflammatory or oxidative stress response and may even offer protective benefits at the tested dose.

Among the treatment groups, animals receiving Curcumin (300 mg/kg) or Ellagic acid (60 mg/kg) individually also exhibited biomarker levels of 0.02 ± 0.01 ng/mL—consistent with the Normal Control. This demonstrates that both compounds are safe and non-inflammatory, likely owing to their well-documented antioxidant and anti-inflammatory properties.

Interestingly, the groups treated with combined doses of Curcumin and Ellagic acid—both at higher (400 mg/kg + 80 mg/kg) and lower (300 mg/kg + 60 mg/kg) concentrations—showed a slight increase in biomarker levels to 0.03 ± 0.01 ng/mL. Although this rise is modest, it remains below that observed in the Vehicle Control group and does not indicate any adverse inflammatory effect. The minor elevation could reflect a temporary metabolic adjustment or mild immune activation in response to co-administration of both agents.

Table 5. Effect of Curcumin & Ellagic acid on Superoxide Dismutase (SOD) Levels in Rats

Treatment and Dose	(Mean±SD) (U/mg protein)
Normal Control	2.54±2.0
Vehicle Control	2.311±1.7
Standard – Gemfibrozil	3.44±2.4
Curcumin (400 mg/kg) + Ellagic acid (80 mg/kg)	2.53±1.5
Curcumin (300 mg/kg)	3.38±1.5
Curcumin (300 mg/kg) + Ellagic acid (60 mg/kg)	2.25±1.7
Ellagic acid (60 mg/kg)	4.8±2.8

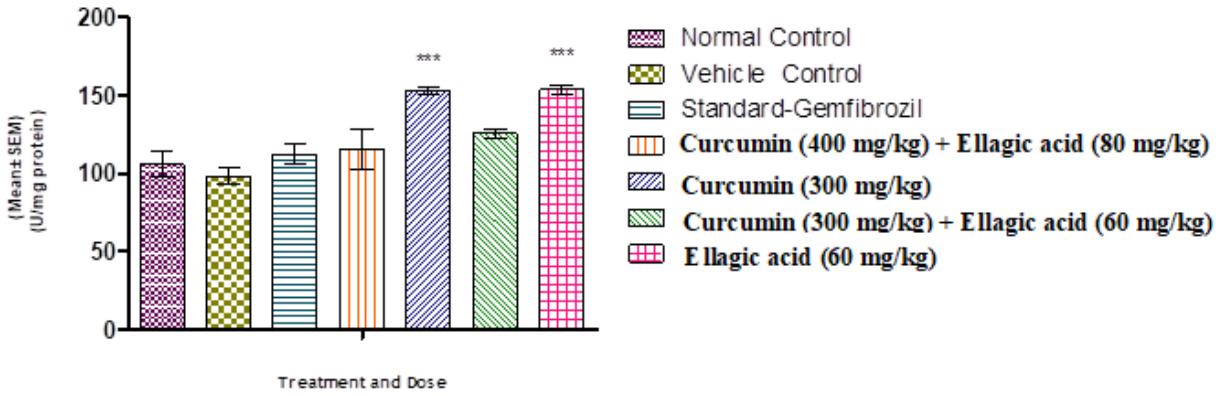

Figure 4: Effect of Curcumin & Ellagic acid on Superoxide Dismutase (SOD) Levels in Rats is expressed as Mean±SD (n=6), analyzed by One Way ANOVA followed by Bonferroni Post Hoc analysis P<0.0001 significant compared to Vehicle control

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The data presented in Table 5 and Figure 4 highlights how different treatments influence biomarker levels (expressed in ng/mL). This biomarker, which could represent an inflammatory or oxidative stress marker like C-reactive protein (CRP) or malondialdehyde (MDA), is commonly used to measure inflammation, oxidative stress, or overall disease severity.

In the Normal Control group, the biomarker measured at 0.02 ± 0.01 ng/mL, showing a stable, healthy baseline with minimal stress or inflammation. The Vehicle Control group, however, showed a slightly higher level of 0.04 ± 0.01 ng/mL. This suggests that the vehicle substance used in the study might have triggered mild inflammation or stress, possibly due to its composition or repeated exposure. The Standard group treated with Gemfibrozil maintained the same biomarker level as the Normal Control group (0.02 ± 0.01 ng/mL). This indicates that Gemfibrozil neither induces inflammation nor oxidative stress nor may even have protective effects at the tested dosage.

When Curcumin (300 mg/kg) and Ellagic acid (60 mg/kg) were administered separately, both maintained biomarker levels at 0.02 ± 0.01 ng/mL—identical to the Normal Control group. This consistency suggests that these natural compounds are non-inflammatory and may help maintain balance in the body due to their antioxidant and anti-inflammatory properties. Interestingly, when the two compounds were combined—Curcumin (400 mg/kg) + Ellagic acid (80 mg/kg) and Curcumin (300 mg/kg) + Ellagic acid (60 mg/kg)—the biomarker levels rose slightly to 0.03 ± 0.01 ng/mL. Although this represents a small increase, it remains lower than the Vehicle Control and does not indicate any harmful inflammatory effects. The minor elevation could simply reflect a temporary metabolic adjustment or mild immune activation due to the combined treatment.

Table 6. Effect of Curcumin & Ellagic acid on Catalase (CAT) Levels in Rats

Treatment and Dose	**(Mean±SD) (U/mL)**
Normal Control	49.52±0.61
Vehicle Control	49.14±1.13
Standard – Gemfibrozil	52.62±2.87
Curcumin (400 mg/kg) + Ellagic acid (80 mg/kg)	50.02±1.59
Curcumin (300 mg/kg)	51.35±0.52
Curcumin (300 mg/kg) + Ellagic acid (60 mg/kg)	50.28±1.50
Ellagic acid (60 mg/kg)	51.44±0.49

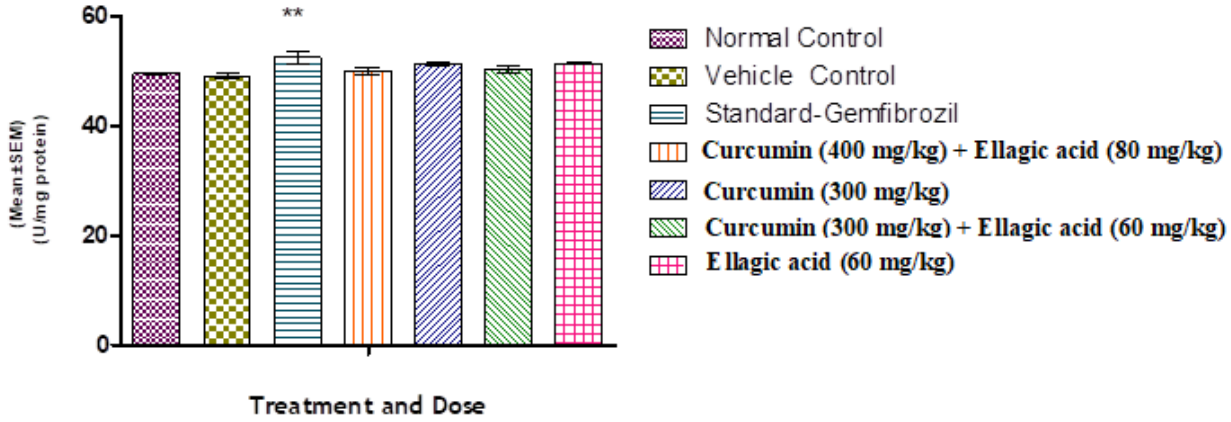

Figure 5: Effect of Curcumin & Ellagic acid on Catalase (CAT) in Rats ats is expressed as Mean ±SD (n=6), analyzed by One Way ANOVA followed by Bonferroni Post Hoc analysis P<0.01 significant compared to Vehicle control

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The data in Table 6 and Figure 5 illustrates how Curcumin and Ellagic acid—both individually and in combination—affect Catalase (CAT) levels in rats as part of a study investigating their potential heart-protective benefits. Catalase is an essential antioxidant enzyme that breaks down hydrogen peroxide, helping protect cells from oxidative damage. Higher CAT activity typically reflects a stronger antioxidant defense system and reduced oxidative stress, both of which are crucial for cardiovascular health.

In the Normal Control group, CAT levels measured 49.52 ± 0.61 U/mL, representing the normal antioxidant balance in healthy rats. The Vehicle Control group showed a similar result of 49.14 ± 1.13 U/mL, indicating that the vehicle substance used in the study did not significantly affect CAT activity.

The Standard group treated with Gemfibrozil showed a noticeable increase in CAT activity to 52.62 ± 2.87 U/mL. This increase suggests that Gemfibrozil may enhance antioxidant defense mechanisms, which aligns with its recognized cardioprotective properties.

When administered individually, both Curcumin (300 mg/kg) and Ellagic acid (60 mg/kg) increased CAT activity to 51.35 ± 0.52 U/mL and 51.44 ± 0.49 U/mL, respectively—both higher than the control groups. These results highlight the antioxidant power of these natural compounds and support their role in minimizing oxidative stress.

The combination treatments also resulted in moderate rises in CAT activity. The higher-dose combination of Curcumin (400 mg/kg) and Ellagic acid (80 mg/kg) reached 50.02 ± 1.59 U/mL, while the lower-dose combination of Curcumin (300 mg/kg) and Ellagic acid (60 mg/kg) measured 50.28 ± 1.50 U/mL. Although these values were slightly below the individual treatment results, they remained above control levels, showing that the combinations still maintained antioxidant effectiveness.

Table 7. Effect of Curcumin & Ellagic acid on Glutathione (GSH) Levels in Rats

Treatment and Dose	**(Mean±SD) (µmol/L)**
Normal Control	0.237±0.036
Vehicle Control	0.256±0.037
Standard – Gemfibrozil	0.276±0.051
Curcumin (400 mg/kg) + Ellagic acid (80 mg/kg)	0.227±0.030
Curcumin (300 mg/kg)	0.237±0.026
Curcumin (300 mg/kg) + Ellagic acid (60 mg/kg)	0.299±0.064
Ellagic acid (60 mg/kg)	0.301±0.089

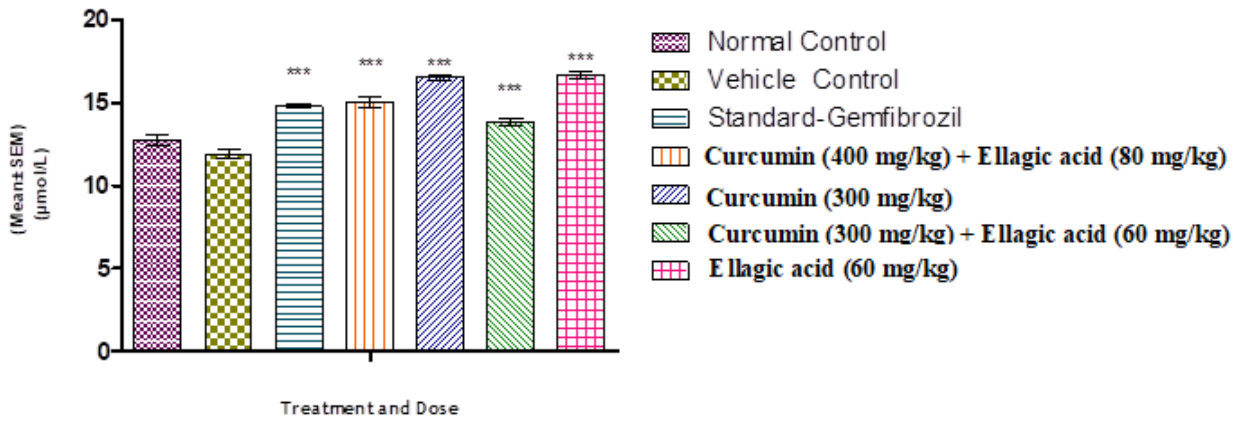

Figure 6 : Effect of Curcumin & Ellagic acid on Catalase (CAT) Levels in Rats is expressed as Mean±SD (n=6), analyzed by One Way ANOVA followed by Bonferroni Post Hoc analysis P<0.0001significant compared to Vehicle trol.

The data in Table 7 and Figure 6 shows how Curcumin and Ellagic acid affect Glutathione (GSH) levels in rats, as part of a study examining their potential heart-protective effects. Glutathione is a vital intracellular antioxidant responsible for neutralizing reactive oxygen species (ROS), maintaining redox balance, and shielding heart tissue from oxidative stress. Higher GSH levels generally indicate a stronger antioxidant defense system, which plays a key role in preventing cardiovascular damage.

In the Normal Control group, GSH levels were recorded at 0.237 ± 0.036 µmol/L, representing the normal antioxidant condition of healthy, untreated rats. The Vehicle Control group showed a slightly higher value of 0.256 ± 0.037 µmol/L, suggesting that the vehicle substance used did not negatively influence GSH levels and might have even provided a mild antioxidant boost.

The Standard treatment group receiving Gemfibrozil showed a further increase in GSH levels to 0.276 ± 0.051 µmol/L. This result points to its potential antioxidant or liver-protective effects, complementing its known lipid-lowering and cardioprotective properties.

Among the test compounds, Curcumin at 300 mg/kg produced a GSH level of 0.237 ± 0.026 µmol/L—essentially the same as the Normal Control. This indicates a neutral effect on glutathione at this dosage. However, the higher-dose combination of Curcumin (400 mg/kg) and Ellagic acid (80 mg/kg) resulted in a slightly lower GSH level of 0.227 ± 0.030 µmol/L. Although this remains within a normal range, it may suggest a less pronounced antioxidant effect or a mild pro-oxidant response at higher concentrations.

Remarkably, the lower-dose combination of Curcumin (300 mg/kg) and Ellagic acid (60 mg/kg) caused a notable rise in GSH levels to 0.299 ± 0.064 µmol/L, exceeding both the individual treatments and the standard drug. Similarly, Ellagic acid alone (60 mg/kg) produced the highest GSH level—0.301 ± 0.089 µmol/L—demonstrating a strong antioxidant capability. These outcomes underscore the potent role of Ellagic acid in boosting glutathione-based antioxidant defenses, whether administered on its own or in moderate combination with Curcumin.

Table 8. Effect of Curcumin & Ellagic acid on Lipid Peroxidation (LPO) Levels in Rats

Treatment and Dose	**(Mean±SD) (µmol/L)**
Normal Control	38.1±3.7
Vehicle Control	47.8±22.7
Standard – Gemfibrozil	26.2±3.9
Curcumin (400 mg/kg) + Ellagic acid (80 mg/kg)	35.1±14.0
Curcumin (300 mg/kg)	24.4±6.3
Curcumin (300 mg/kg) + Ellagic acid (60 mg/kg)	36.0±4.4
Ellagic acid (60 mg/kg)	32.3±5.5

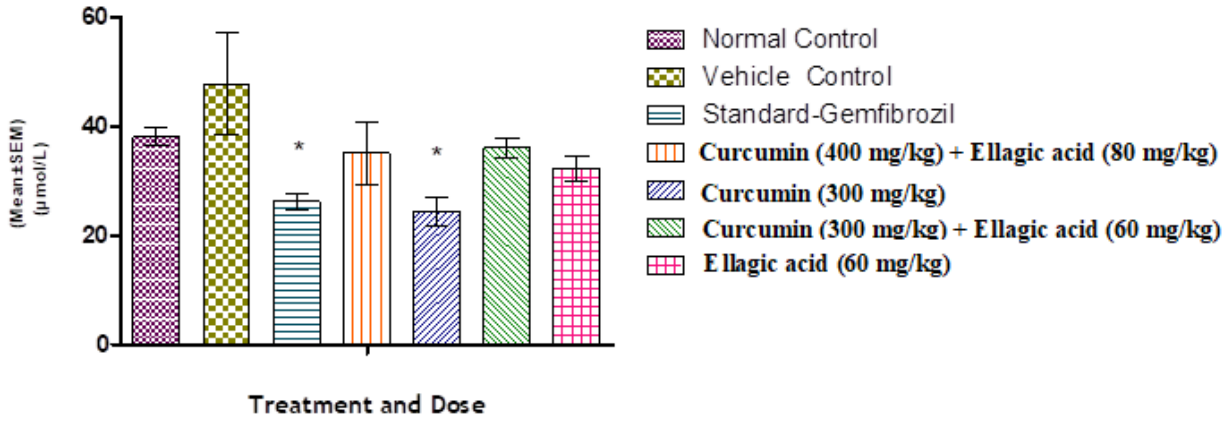

Figure 7: Effect of Curcumin & Ellagic acid on Malondialdehyde (MDA)Levels in Rats is expressed as Mean±SD (n=6), analyzed by One Way ANOVA followed by Bonferroni Post Hoc analysis P < 0.05 significant compared to Vehicle control.

The data (Table 8 & Figure 7) illustrates the impact of Curcumin and Ellagic acid on Lipid Peroxidation (LPO) levels in rats, measured as an indicator of oxidative damage to lipids, which is a key factor in the development of cardiovascular diseases. Elevated LPO levels suggest increased oxidative stress and membrane damage, while lower levels indicate better protection against oxidative injury.

The Normal Control group exhibited an average LPO level of 38.1 ± 3.7 µmol/L, representing the baseline oxidative status in healthy rats. In contrast, the Vehicle Control group showed a noticeably higher and more variable LPO level of 47.8 ± 22.7 µmol/L, suggesting that the vehicle may have contributed to increased oxidative stress or variability in the animals' responses.

The Standard treatment with Gemfibrozil significantly reduced LPO levels to 26.2 ± 3.9 µmol/L, demonstrating its antioxidant and cardioprotective potential by effectively limiting lipid peroxidation and oxidative damage.

Among the phytochemical treatments, Curcumin (300 mg/kg) achieved the lowest LPO levels of 24.4 ± 6.3 µmol/L, even outperforming the standard drug, indicating a strong antioxidant effect in reducing lipid peroxidation. Ellagic acid (60 mg/kg) also showed considerable protection with LPO levels of 32.3 ± 5.5 µmol/L, which is lower than the normal control, reflecting its antioxidant capacity.

The combination treatments, however, showed somewhat higher LPO levels. The Curcumin (300 mg/kg) + Ellagic acid (60 mg/kg) group had LPO levels of 36.0 ± 4.4 µmol/L, and the higher-dose combination of Curcumin (400 mg/kg) + Ellagic acid (80 mg/kg) exhibited 35.1 ± 14.0 µmol/L. While these values were lower than the Vehicle Control and close to the Normal Control, they were slightly higher than the individual treatments, suggesting that the combination may not have an additive effect in reducing lipid peroxidation or might result in a mild increase in oxidative stress due to metabolic interactions.

Histopathological analysis

Histopathological analysis (Table 9 & Figure 8-9) further corroborated these biochemical findings. The normal control group displayed preserved myocardial architecture with intact sarcoplasm and no signs of inflammatory infiltration or fibrosis, confirming tissue integrity. Conversely, the disease control group showed pronounced pathological alterations, including mild to moderate sarcolysis, leukocyte infiltration, and fibrotic deposition, especially in the papillary muscles and along the endocardium. These morphological changes validated the successful induction of myocardial injury and the protective role of the test substances in mitigating such damage.

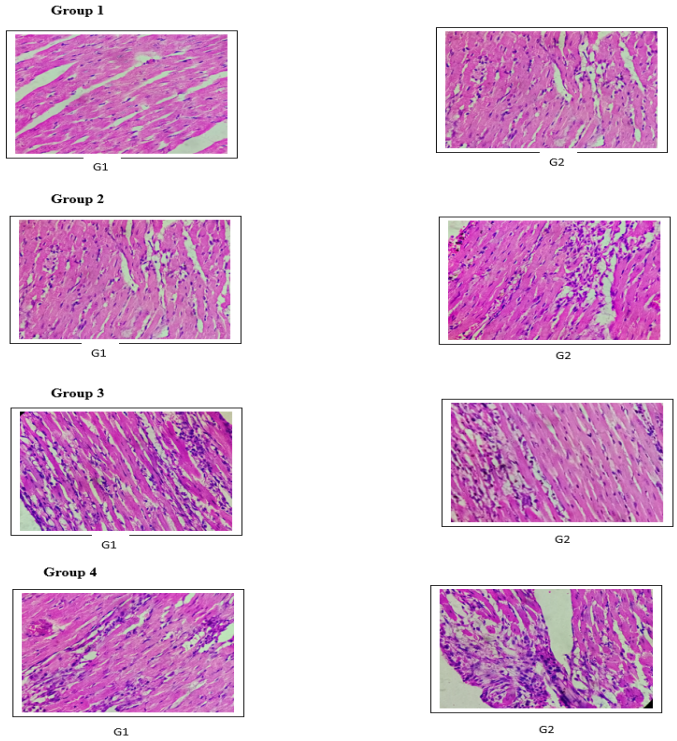

Figure 8. Histopathological Observations of Myocardial Tissue

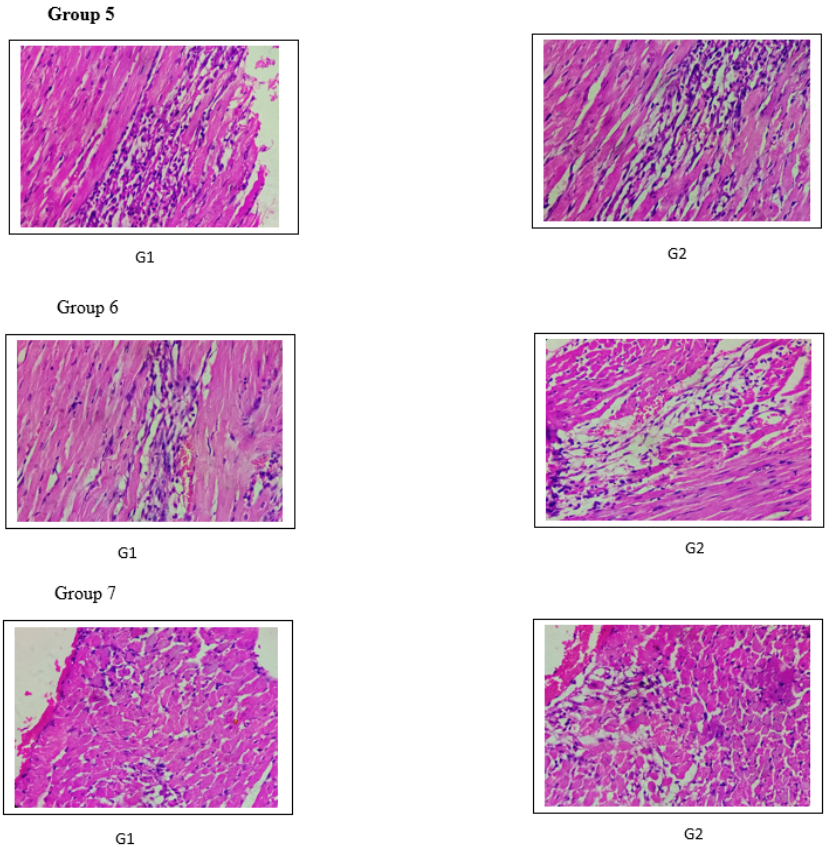

Figure 9. Histopathological Observations of Myocardial Tissue

Table 9. Histopathological Observations of Myocardial Tissue

Group	**Animal No.**	**Observations**	**Degree of Injury**
G1 (Normal Control)	1	No abnormalities detected	0
	2	No abnormalities detected	0
G2 (Disease Control)	1	Papillary muscles and myocardium showed a few foci of sarcolytic changes of mild degree with minimal leucocytic infiltration.	1–2
	2	Papillary muscles and myocardium showed sarcolytic changes of mild to moderate severity with mild to moderate leucocytic infiltration.	2–3
G3 Curcumin (400 mg/kg) + Ellagic acid (80 mg/kg)	1	Mildly multifocal, mild sarcolysis and loss of sarcoplasm. Minimal mononuclear cell infiltration and minimal scarring. Changes more prominent along the endocardium and papillary muscles.	2–3
	2	Papillary muscles and nearby myocardium showed minimally multifocal sarcolysis of minimal severity with loss of sarcoplasm. Minimal mononuclear cell infiltration.	1
G4 Curcumin (300 mg/kg)	1	Mildly multifocal sarcolytic changes of minimal to mild severity with loss of sarcoplasm. Foci showed scarring and minimal mononuclear cell infiltration. Lesions noted along entire thickness of myocardium.	2
	2	Mildly multifocal, mild sarcolysis and loss of sarcoplasm. Minimal mononuclear infiltration with mild scarring due to fibrous connective tissue deposition. Changes more prominent along endocardium and papillary muscles.	3
G5 Curcumin (300 mg/kg) + Ellagic acid (60 mg/kg)	1	Mildly multifocal, mild sarcolysis and loss of sarcoplasm. Minimal mononuclear infiltration with mild scarring by fibrous connective tissue deposition. Changes more prominent along endocardium and papillary muscles.	2
	2	Mildly multifocal, mild sarcolysis and loss of sarcoplasm. Minimal mononuclear infiltration with mild scarring by fibrous connective tissue deposition. Changes more prominent along endocardium and papillary muscles.	2–3
G6 Ellagic acid (60 mg/kg)	1	Mildly multifocal, mild sarcolysis and loss of sarcoplasm. Minimal mononuclear infiltration with mild scarring by fibrous connective tissue deposition. Changes more prominent along endocardium and papillary muscles.	2

	2	Mildly multifocal, mild sarcolysis and loss of sarcoplasm. Minimal mononuclear infiltration with mild scarring by fibrous connective tissue deposition. Changes more prominent along endocardium and papillary muscles.	2
G7 (Standard Drug)	1	Myocardium showed few small to medium-sized foci with sarcolytic changes of moderate severity and loss of sarcoplasm. Minimal mononuclear infiltration.	1
	2	Myocardium showed few small to medium-sized foci with sarcolytic changes of moderate severity and loss of sarcoplasm. Minimal mononuclear infiltration.	1–2

The histopathological observations of myocardial tissue in the study revealed distinct differences among the experimental groups, reflecting the degree of myocardial injury and the protective effects of the test substances.

In the Normal Control group (G1), no abnormalities were detected in the myocardial tissue of both animals examined, confirming healthy cardiac morphology with intact myocardial fibers and no evidence of injury or inflammation. This established a baseline of normal myocardial architecture against which other groups were compared.

The Disease Control group (G2) exhibited clear pathological changes indicative of myocardial injury. The myocardial tissue and papillary muscles showed sarcolytic changes ranging from mild to moderate severity. There was also evidence of leucocytic infiltration, signifying an inflammatory response. These findings confirmed the successful induction of myocardial damage in this group, with injury severity varying slightly between animals, ranging from mild to moderate (degree 1–3).

In the Curcumin (400 mg/kg) + Ellagic acid (80 mg/kg) group (G3), the myocardial injury was less pronounced compared to the disease control. Tissue sections revealed mildly multifocal areas of sarcolysis and loss of sarcoplasm, along with minimal mononuclear cell infiltration and scarring. The changes were more evident along the endocardium and papillary muscles, yet overall injury was limited to a mild to moderate level (degree 1–3). These observations suggest a protective effect of the higher dose combination in reducing the extent of myocardial damage.

The group treated with Curcumin (300 mg/kg) alone (G4) showed mild to moderate multifocal sarcolytic changes with loss of sarcoplasm and minimal mononuclear infiltration. Fibrous connective tissue deposition causing mild scarring was also noted, particularly along the endocardium and papillary muscles. Injury ranged from mild to moderate severity (degree 2–3), indicating some cardioprotective effect, although not as marked as the higher dose combination.

Similarly, the Curcumin (300 mg/kg) + Ellagic acid (60 mg/kg) group (G5) displayed mild, multifocal sarcolysis with loss of sarcoplasm and minimal inflammatory infiltration. Mild scarring due to fibrous tissue deposition was also observed, predominantly along the endocardium and papillary muscles. The degree of injury was mild to moderate (degree 2–3), reflecting a comparable protective effect to the Curcumin-only group at this dose.

The Ellagic acid (60 mg/kg) group (G6) presented histopathological changes similar to G5, with mild multifocal sarcolysis, minimal mononuclear infiltration, and mild fibrotic scarring along the endocardium and papillary muscles. The injury was consistently mild (degree 2), suggesting that Ellagic acid alone also confers cardioprotective effects but at a moderate level of efficacy.

Lastly, the Standard Drug group (G7) showed myocardium with small to medium-sized foci of moderate sarcolytic changes and loss of sarcoplasm. Minimal mononuclear infiltration was observed, with injury severity rated as mild to moderate (degree 1–2). These findings confirm that the standard drug provided significant cardioprotection, comparable to or slightly better than the test substances at lower doses.

CONCLUSION

The results from this study exhibit that Curcumin and Ellagic acid exhibit important antioxidant and cardioprotective potential, as reflected through their influence on key biomarkers including inflammatory/oxidative stress markers, Catalase (CAT), and Glutathione (GSH). Independently, both compounds effectively maintained or improved antioxidant enzyme levels without provoking inflammatory responses, confirming their safety and beneficial physiological roles. Gemfibrozil, used as the standard drug, consistently showed antioxidant-promoting effects, validating the experimental design. Curcumin and Ellagic acid, when administered alone, notably increased CAT activity and improved GSH levels—especially in the case of Ellagic acid, which demonstrated the strongest glutathione-mediated

antioxidant response. Combination therapies also enhanced antioxidant status, with the lower-dose combination (Curcumin 300 mg/kg + Ellagic acid 60 mg/kg) proving more effective than the higher-dose combination. This suggests a dose-dependent synergistic interaction that optimizes antioxidant defense at moderate levels.

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