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# Vasodilatation, cardioprotective effect against Isoproterenol induced myocardial infarction and *in vitro* antioxidant effect of *Calotropis gigantea*

**A Krishnaveni[1*], Sandhiya S[2], Vaishnavi G[2], Manjula B[2], Kavin T[3]**
[1] Assistant professor, Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai, Tamil Nadu, India
[2] Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai, Tamil Nadu, India
[3] Veterinary assistant surgeon, Madurai Medical college, Madurai, Tamil Nadu, India

## Abstract
**Background:** *Calotropis gigantea* belongs to Apocynaceae, a large erect lactiferous shrub. Native to continental Asia and South East Asia and has been introduced in the pacific islands, Australia, Central and northern South America, South Africa, India, Srilanka, China, Thailand, Philippines, Malaysia, Indonesia and Cambodia. People of Bihar and Orissa used leaves to treat chest diseases. People of Shevaroy hills, Tamil Nadu used shoots and seeds were used by people of Birbhum, West Bengal for chest pain.
Tribal communities used this plant to treat bronchial asthma, cholera, convulsions, pneumonia, toothache, ringworm and small pox infections, fever, rheumatism, leprosy, constipation, wounds. The phytochemical survey reported the presence of cardenolides, triterpenoids, anthocyanins and hydrocarbons. The plant exhibited antimicrobial, antioxidant, antiasthmatic, anticonvulsant, hepatoprotective, hypoglycemic, procoagulant, abortifacient, cytotoxic, insecticidal, ovicidal and antisolar activity. A study was undertaken to investigate vasodilatation, myocardial infarction and *in vitro* antioxidant effect of *Calotropis gigantea.*
**Materials and methods:** Leaves were collected from Mangalakudi village, Madurai district, Tamil Nadu, in the month of February 2025. It was identified and authenticated by Dr. Stephen. The collected leaves were washed with water; shade dried, powdered and aqueous extract was prepared. The extract was concentrated and stored in container for further use.
**Results and conclusion:** The present study reported the significant vasodilatation activity of *Calotropis gigantea* and It also evidenced that it has the potential to act against isoproterenol induced myocardial infarction in comparison with standard and control for all three activities.

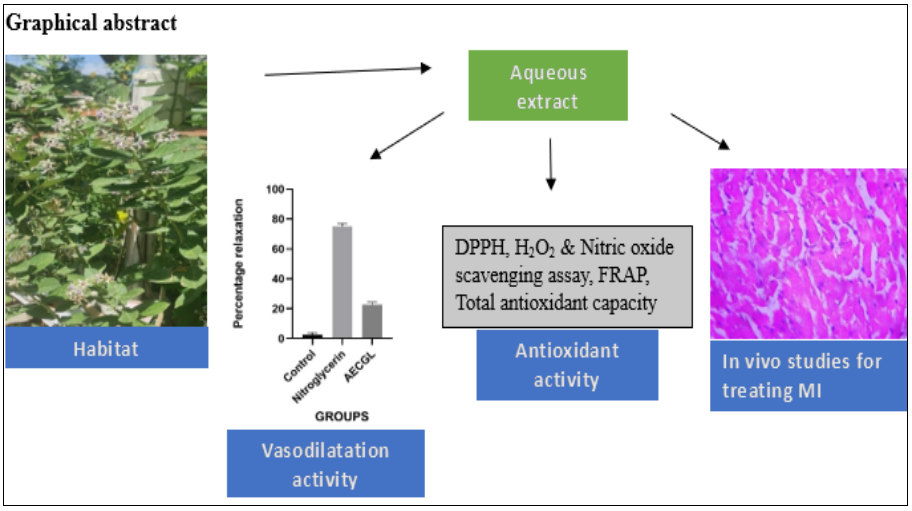
**Keywords:** Anti-oxidant, *Calotropis gigantea*, Isoproterenol induced, myocardial infarction, vasodilatation

## Introduction
*Calotropis gigantea* belongs to Apocynaceae, a large erect lactiferous shrub. Native to continental Asia and South East Asia and has been introduced in the pacific islands, Australia, Central and Northern South America, South Africa, India, Srilanka, China, Thailand, Philippines, Malaysia, Indonesia and Cambodia [1, 2]. *Calotropis* genus contains two different species *gigantea* (Purple flowered plant) and *procera* (White flowered plant) [3]. People of Bihar and Orissa used leaves to treat chest diseases [4]. People of Shevaroy hills, Tamil Nadu used shoots and seeds were used by people of Birbhum, West Bengal for chest pain [5]. Santals tribe of Jharkhand and West Bengal used this plant to treat bronchial asthma, cholera, convulsions, pneumonia, toothache, ringworm and small pox infections. People of Kolayat tehsil, Rajasthan used the plant to treat fever. People of Chitheri Hills, Dharmapuri and people of Raipur, Chhatisgarh plains used the plant preparations in rheumatism, People of North Kanara, Karnataka used to treat leprosy. People of Kamrup, Assam and tribals of Eastern Rajasthan used to treat constipation and to get rid of wounds respectively [6]. The phytochemical survey reported the presence of cardenolides from the latex and leaves, triterpenoids, anthocyanins from flowers and hydrocarbons [7]. It exhibited antimicrobial [8] antioxidant [9], antiasthmatic [10], anticonvulsant [11], Hepatoprotective [12], Hypoglycemic [13], Procoagulant [14], abortifacient [15], cytotoxic [16], insecticidal [17], ovicidal [18, 19] and anti solar activity [20].

An investigation was studied to identify the vasodilatation, cardio protection against isoproterenol induced myocardial infarction and *in vitro* antioxidant effect of *Calotropis gigantea.*

## Materials and methods
### Preparation of aqueous extract of *Calotropis gigantea* leaves [AECG$_L$]
The fresh matured leaves of *Calotropis gigantea* were collected, thoroughly washed with water and shade-dried. The dried leaves were pulverized into a fine powder. About 10 g of the powdered material was boiled in 200 ml of water for 2 hours. The extract was filtered and evaporated to dryness. As per the requirement, respective batches were prepared.

### Evaluation of vasodilatation activity of [AECG$_L$] using isolated rat aorta
**Materials**
- **Animals:** Adult male Wistar rats (250–300 g)
- **Drugs & Chemicals:** Phenylephrine (PE), Acetylcholine (ACh), Krebs–Henseleit (KH) solution, AECG$_L$
- **Instruments:** Organ bath setup with isometric transducer, PowerLab data acquisition system, aerator (95% $O_2$ + 5% $CO_2$), tissue force transducer, thermostat.




## Method

- **Animal Preparation**

1. Euthanize rat humanely according to CCSEA guidelines.
2. Dissect thoracic aorta carefully and place in cold, oxygenated KH solution.

- **Tissue Preparation**

1. Remove connective tissue and fat.
2. Cut into 3–4 mm rings; avoid damaging endothelium unless testing endothelium-independent relaxation.

- **Mounting in Organ Bath**

1. Mount ring between two stainless steel hooks: one fixed, one connected to a transducer.
2. Maintain in 20–25 mL KH solution at 37°C, bubbled with 95% $O_2$ + 5% $CO_2$.
3. Apply 1 g resting tension and equilibrate for 60 min; wash every 15 min.

- **Pre-contraction**

1. Contract the tissue with PE(Phenylephrine) 1 µM until steady plateau contraction is reached.

- **Treatment**

1. Add $AECG_L$cumulatively (e.g., 10, 30, 100 µg/mL) at 5–7 min intervals.
2. Record tension changes after each dose.

- **Standard**

After PE contraction, add ACh (1 µM) to confirm endothelial integrity.

- **Data Recording**

1. Calculate % relaxation using:

$$\% \text{ aortic vasodilation} = \frac{\Delta g\,(g)}{\text{Initial tone at submaximal contraction (g)}} \times 100$$

2. Statistical analysis was performed by one-way ANOVA, significance was determined by Bonferroni test as post hoc using Graphpad Prism [version 10.6.0(890)] [21].

***In vivo* studies of $AECG_L$ against isoproterenol induced myocardial infarction in rat's model**

**Materials**

- **Animals:** Adult male wistar rats of four groups each containing six in it.
- **Drugs & Chemicals:** Isoproterenol, Nitroglycerin, $AECG_L$

**Methods**

- **Animal preparation**

Ethical committee clearance no. 13/2025 for 24 rats. Adult male Wistar rats (200–250 g) were obtained. They were kept in a temperature-controlled environment (22 ± 4 ◦ C) with a 12 h dark/light cycle. The animals had access to normal laboratory feed and water ad libitum.

- **Induction of Myocardial Infarction**

Daily subcutaneous injection of Isoprenaline (8.5 mg/kg) was given to rats on days 8 and 9 to induce experimental Myocardial infarction [22].

- **Symptoms to identify Myocardial Infarction induced in rats**

Anxiety/ Depression
Interest in environment
Mobility
Social interaction [23]

- **Experimental groups**

**Normal:** The control group received saline for 9 days via intra peritoneal route.

**Disease control (Isoprenaline injected):** The Disease control group received saline for 9 days via intra peritoneal route. On the 8th and 9th days in Isoprenaline group, 0.3 ml (85 mg/kg body weight) was subcutaneously injected with an interval of 24 h.

**Nitroglycerin treated:** The standard group received nitroglycerine for 9 days via subcutaneous route. Isoprenaline (8.5 mg/kg body weight) was administered on the 8th and 9th days with an interval of 24 h.

**$AECG_L$:** The test group receives $AECG_L$ given by gavage at doses of body weight for 9 days. Subcutaneous injection of Isoprenaline (8.5 mg/kg body weight) was administered on the 8th and 9th days with an interval of 24 h.

**Table 1:** Experimental groups of Isoproterenol induced myocardial infarction

| Group | Treatment | Dose | Duration | |
|---|---|---|---|---|
| | | | Day 1 to 7 | Day 8 & 9 |
| Group I Normal (n=6) | Saline | Normal saline | Normal saline | Normal saline |
| Group II Disease control (n=6) | Isoprenaline Injected | 8.5mg/kg | Normal saline | Isoprenaline |
| Group III Standard (n=6) | Nitroglycerin treated | 25mg/kg | Nitroglycerin | Nitroglycerin + Isoprenaline |
| Group IV Test (n=6) | $AECG_L$ | 200mg/kg | $AECG_L$ | $AECG_L$ + Isoprenaline |

- **Blood collection**

The rats were anesthetized with ketamine hydrochloride/xylazine and were sacrificed on day 10. Blood was collected by cardiac puncture and heart tissue was removed immediately, cleaned with ice saline, and dried on filter paper. The heart tissue was immediately frozen in liquid nitrogen and stored at 80 ℃ for measurement and further analysis of the indicators [22].

- **Evaluation**

The following biochemical parameters were evaluated in the serum of experimental groups

1. Creatine-kinase (CK-MB)
2. Serum Glutamic-Oxaloacetic Transaminase (SGOT)
3. Serum Glutamic Pyruvic Transaminase (SGPT)

Statistical analysis was performed by one-way ANOVA, significance was determined by Bonferroni test as post hoc using Graphpad Prism [version 10.6.0(890)].
Histopathological examination is performed for heart tissue of experimental groups.

***In vitro* Antioxidant assay**

**Determination of DPPH free radical scavenging assay**

The effect of $AECG_L$ on DPPH radical was estimated using the method of Liyana Pathiranan *et al*. (2005) [24]. About 0.1 ml of DPPH-methanol solution (0.135 mM) was mixed with 1.0 ml of different concentrations of sample. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Vitamin C was used as standard drug [24]. The results are obtained in Tab 5&Fig 4. The percentage of free radical scavenging was calculated according to the following equation

$$\%\text{Percentage inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} X\ 100$$

**Determination of Hydrogen peroxide scavenging assay**

Hydrogen peroxide scavenging assay was determined according to the method of Rana M G *et al* (1996) [25]. Various concentrations are prepared. 1ml of $AECG_L$ were added to 2 ml of hydrogen peroxide solution (10 mM) in phosphate buffer (50 mM, pH 7.4). The $AECG_L$ was replaced by methanol for control. Reaction mixture was incubated at room temperature for 30 min. The unreacted hydrogen peroxide was determined by measuring the absorbance of the reaction mixture at 230 nm with respect to the blank (methanol) using UV/visible spectrophotometer [25]. The percentage inhibition was calculated according to the following equation. The results are obtained in Tab 6&Fig 5 and $IC_{50}$ values were estimated graphically using a non-linear regression.

**Determination of nitric oxide scavenging assay**

Nitric oxide was generated from sodium nitroprusside and its scavenging effect was determined as per Green *et al*., 1982 [26]; Morocci *et al*., 1994 [27]. Different concentration of $AECG_L$ in phosphate buffer was incubated with sodium nitroprusside for 5 hours at 25℃. Control experiments were performed with equal amount of buffer instead of extract solution. After 5 hours of incubation, 0.5ml of supernatant liquid was removed and 0.5ml of Griess reagent was added. The absorbance of the chromophore formed during diazotization with sulphanilamide and its subsequent coupling was read at 546nm. Vitamin C was used as standard and the nitric oxide scavenging was expressed in terms of ascorbic acid equivalents [26, 27] and its results are presented in Tab 7&Fig 6

**Determination of ferric reducing antioxidant power (FRAP) activity**

Ferric reducing antioxidant power activity was determined according to the method of Maruthamuthu Vijayalakshmi (2016) [28]. Different concentrations of $AECG_L$ and its various fractions was added to 2.5ml of 0.2M sodium phosphate buffer (PH 6.6) and 2.5ml of 1% potassium ferricyanide solution. The reaction mixture was vortexed well and then incubated at 50℃ for 20min using vortex shaker. At the end of the incubation, 2.5ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000rpm for 10 min. The supernatant (2.5ml) was mixed with 2.5ml of deionized water and 0.5ml of 0.1% ferric chloride. The colored solution was read at 700nm against the blank with reference to standard using UV-Spectrophotometer. Vitamin C was used as standard [28]. The results are presented in Tab 8& Fig 7.

**Determination of total antioxidant capacity content**

Total antioxidant capacity was determined according to the method of Prieto P (1999) [29]. $AECG_L$ in different concentrations were prepared individually containing 3ml of distilled water and 1ml of Molybdate reagent solution. These tubes were kept incubated at 95℃ for 90min. After incubation, these tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695nm. Mean values from three independent samples were calculated for each $AECG_L$ Vitamin C was used as standard [29]. The results were displayed in Tab 9&Fig 8

**Results and discussion**

**Evaluation of vasodilatation activity of [$AECG_L$] using isolated rat aorta**

Various concentration (1 to 100 µg/ml) of $AECG_L$ were prepared and vasodilatation was observed by using isolated rat aorta.

**Table 2:** Evaluation of vasodilatation of [$AECG_L$] using isolated rat aorta

| S.No. | Concentration (µg/ml) | Percentage Relaxation (Mean ± SEM) |
|---|---|---|
| 1. | 1 | 5.4 ± 0.6 |
| 2. | 3 | 12.8 ± 0.8 |
| 3. | 10 | 26.3 ± 1.2 |
| 4. | 30 | 51.7 ± 1.8 |
| 5. | 100 | 78.9 ± 2.3 |

**Table 3:** Evaluation of vasodilatation of [$AECG_L$], ACh using isolated rat aorta

| Group | Dose (µg/mL) | Initial Tension (g) | Tension After treatment with $AECG_L$ (g) | Percentage Relaxation | Remarks |
|---|---|---|---|---|---|
| Control (Vehicle) | — | 1.00 ± 0.07 | 0.98 ± 0.06 | 2.5 ±1.5$^{††††}$ | No significant relaxation |
| $AECG_L$ | 30 | 1.01 ± 0.08 | 0.78 ± 0.05 | 22.8 ± 3.0$^{****††††}$ | Moderate vasodilatory effect |
| Acetyl choline (ACh) | 1 µM | 1.00 ± 0.04 | 0.25 ± 0.03 | 75.0 ± 2.5$^{****}$ | Maximal endothelium-dependent relaxation |

$^{****}$ - P <0.0001 Extremely significant with control †$^{†††}$- P <0.0001 Extremely significant with standard

Statistical analysis was performed by one-way ANOVA, significance was determined by Bonferroni test as post hoc using Graphpad Prism [version 10.6.0(890)]. In isolated rat aorta, $AECG_L$ produced a concentration-dependent relaxation, with a maximal effect of 78.9 ± 2.3% at 100 µg/ml displayed in Tab 2. At 30 µg/ml, $AECG_L$ induced a significant reduction in phenylephrine (1 µM) induced aortic contraction (22.8 ± 3.0% relaxation) compared with acetylcholine (1 µM) produced a maximal endothelium-dependent relaxation of 75.0 ± 2.5% displayed in Tab 3.

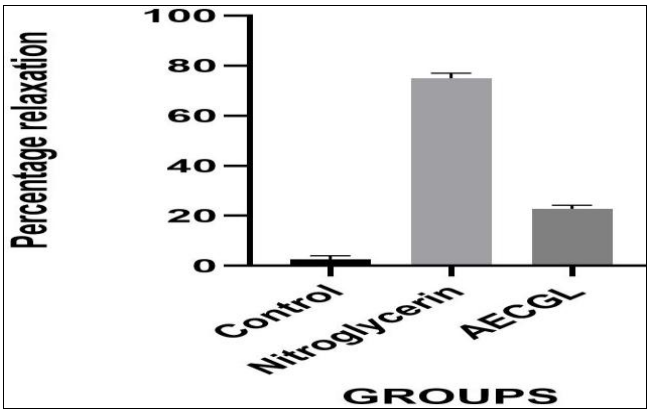



**Fig 1:** Graphical representation of % relaxation of [$AECG_L$], ACh using isolated rat aorta

### *In vivo* studies of $AECG_L$ against isoproterenol induced myocardial infarction in rat's model

Biochemical parameters such as SGPT, SGOT and CK-MB were evaluated in rat's serum in order to know the protective effect of $AECG_L$ using kits.

**Table 4:** Biochemical parameters evaluation of [$AECG_L$]in isoproterenol induced MI in rat's models

| Parameters | Normal | Disease control (Isoprenaline injected) | Nitroglycerin treated | $AECG_L$ |
|---|---|---|---|---|
| SGPT | 91±2.80$^{††}$ | 76.5±2.59$^{†}$ | 43±2.30$^{**}$ | 56±3.46$^{*}$ |
| SGOT | 252±3.50$^{††}$ | 314.5 ±3.17$^{**}$ | 328.5 ±3.75$^{**}$ | 350 ±4.61$^{**}$ |
| CK MB | 12±2.62$^{††}$ | 22.5±2.02$^{†}$ | 53±3.46$^{**}$ | 40.5±2.02$^{*}$ |

$^{*}$ - P<0.05 significant with normal; $^{**}$- P<0.01 highly significant with normal
$^{m†}$- P<0.05 significant with standard; $^{††}$- P<0.01 highly significant with standard

Statistical analysis was performed by one-way ANOVA, significance was determined by Bonferroni test as post hoc using Graphpad Prism [version 10.6.0(890)].

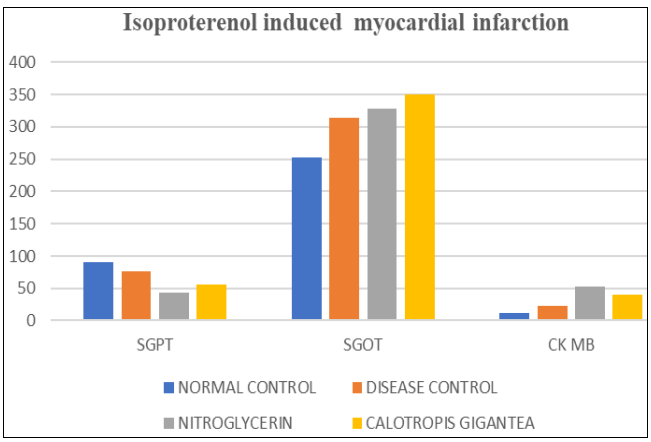



**Fig 2:** Graphical representation of changes inbiochemical parameters in isoproterenol induced MI in rat's models

The biochemical evaluation of $AECG_L$ in isoproterenol-induced myocardial infarction (MI) models reveals notable modulation of cardiac marker (CK-MB). SGPT levels were significantly reduced in the $AECG_L$ group (56±3.46) compared to the disease control, SGOT levels, however, remained elevated across all groups, with $AECG_L$ showing the highest value (350±4.61), suggesting persistent myocardial stress. CK-MB levels were markedly increased in the standard and $AECG_L$ groups, with $AECG_L$ (40.5±2.02) showing a moderate rise compared to the standard (53±3.46), implying partial cardioprotection. The statistical significance observed across these parameters underscores the therapeutic potential of $AECG_L$ in mitigating biochemical disturbances associated with myocardial infarction.

### Histopathological studies of heart

**Normal:** Heart section shows myocardium with normal architecture.

**Disease control:** Multiple section studied show focal areas in the showing fibers with extensive coagulative necrosis with complete loss of nuclei and cross striations. There shows dense neutrophilic infiltration in the centre zone with macrophages on the periphery. Interstitial edema is evident. Surrounding myocardium shows viable myocytes with mild reactive changes.

**Nitroglycerin:** Heart section shows myocardium with normal architecture. In stroma focal area shows scattered inflammatory infiltrates.

**AECG$_L$:** Heart section shows myocardium with mild altered architecture. Stroma shows focal areas of inflammatory infiltrates with mild edema. Surrounding stroma shows normal myocytes. Stroma shows no significant pathology in normal control, nitroglycerin.
Blood vessels showing no significant pathology in normal control and AECG$_L$.

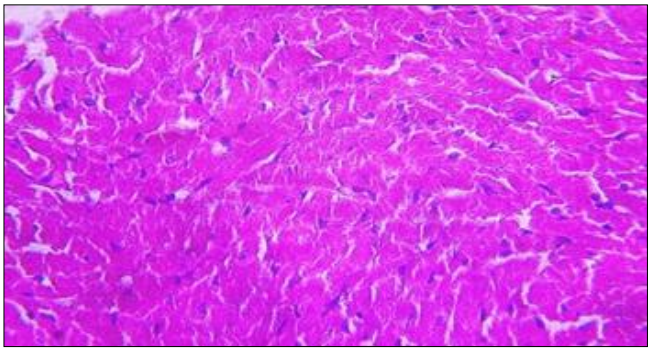


**Fig 3.1:** Normal control

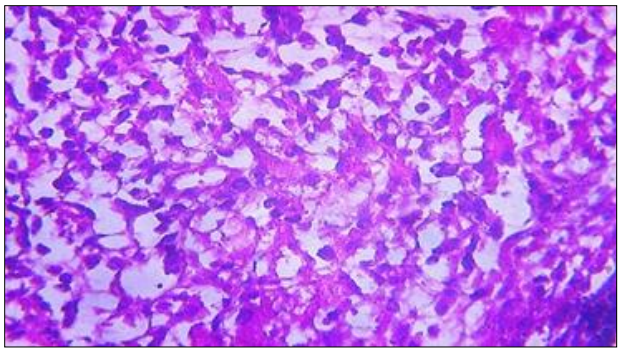


**Fig 3.2:** Disease control

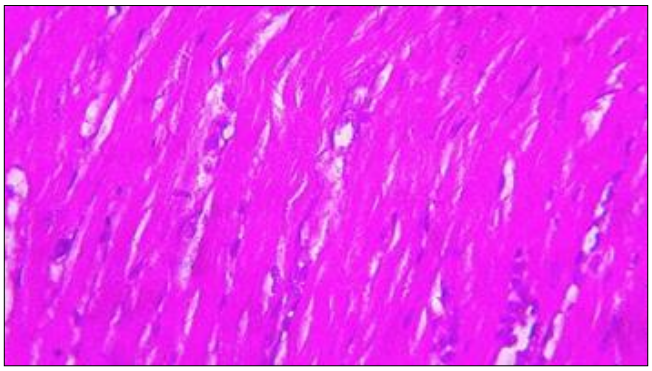


**Fig 3.3:** Nitroglycerin

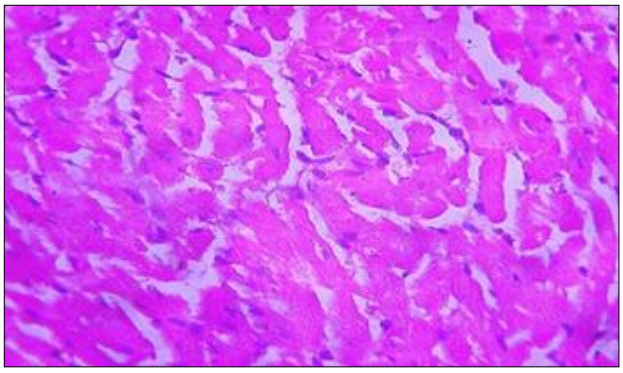


**Fig 3.4:** AECG$_L$

**Fig 3:** Histology of heart sections (under 40x); 3.1 shows normal myocytes; 3.2 shows showed loss of architecture with neutrophilic infiltrates; 3.3 shows normal myocardium with mild inflammation; 3.4 shows show mild edema infiltrates

***In vitro* antioxidant activity**
*In vitro* antioxidant activity of AECG$_L$ were determined by five methods and the results are depicted below

**DPPH free radical scavenging assay of AECG$_L$**
Various concentrations (0 to10 µg/ml) of AECG$_L$ were treated with DPPH and the absorbance was obsereved and displayed in Tab 5&Fig 4.

**Table 5:** Determination of DPPH free radical scavenging assay of AECG$_L$

| S.No. | Concentration (µg/ml) | Percentage inhibition | |
|---|---|---|---|
| | | AECG$_L$ | Ascorbic acid |
| 1. | 0 | 0 | 0 |
| 2. | 2 | 34.32 | 29.92 |
| 3. | 4 | 34.49 | 34.15 |
| 4. | 6 | 46.67 | 43.83 |
| 5. | 8 | 48.77 | 50 |
| 6. | 10 | 49.61 | 55.28 |
| | $IC_{50}$ | 8.31 | 7.92 |

The inhibitory concentration $IC_{50}$ value of AECG$_L$ was found to be 8.31µg/ml in comparison with ascorbic acid as standard 7.92 µg/ml

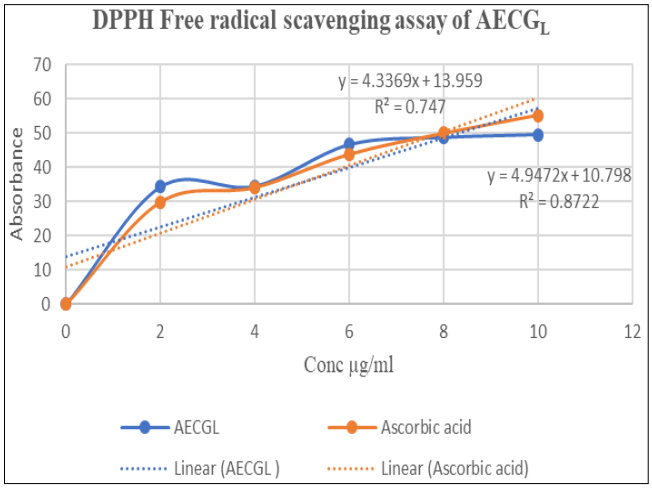



**Fig 4:** Graphical data of DPPH free radical scavenging assay of AECG$_L$

**Hydrogen peroxide scavenging assay of AECG$_L$**
Various concentrations (0 to10 µg/ml) of AECG$_L$ were treated with hydrogen peroxide and the absorbance was obsereved and displayed in Tab 6&Fig 5

**Table 6:** Determination of Hydrogen peroxide scavenging assay of $AECG_L$

| S.No. | Concentration (µg/ml) | Percentage inhibition | |
|---|---|---|---|
| | | $AECG_L$ | Ascorbic acid |
| 1. | 0 | 0 | 0 |
| 2. | 2 | 24.17 | 29.92 |
| 3. | 4 | 30.51 | 32.03 |
| 4. | 6 | 40.84 | 47.23 |
| 5. | 8 | 54.76 | 50.08 |
| 6. | 10 | 58.69 | 63.26 |
| | $IC_{50}$ | 7.68 | 7.3 |

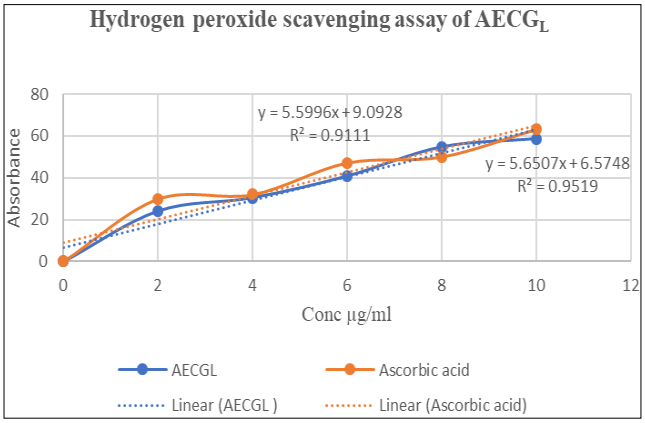



**Fig 5:** Graphical data of Hydrogen peroxide scavenging assay of $AECG_L$

The inhibitory concentration $IC_{50}$ value of $AECG_L$ was found to be 7.68 µg/ml in comparison with ascorbic acid as standard 7.3 µg/ml

**Nitric oxide scavenging assay of $AECG_L$**

Various concentrations (0 to 25 µg/ml) of $AECG_L$ were treated with griess reagent and the absorbance was obsereved and displayed in Tab 7&Fig 6

**Table 7:** Determination of nitric oxide scavenging assay of $AECG_L$

| S.No. | Concentration (µg/ml) | Percentage inhibition | |
|---|---|---|---|
| | | $AECG_L$ | Ascorbic acid |
| 1. | 0 | 0 | 0 |
| 2. | 2 | 10.89 | 27.25 |
| 3. | 4 | 23.83 | 32.03 |
| 4. | 6 | 33.11 | 38.4 |
| 5. | 8 | 36.25 | 44.24 |
| 6. | 10 | 38.18 | 50.08 |
| | $IC_{50}$ | 11.65 | 9.09 |

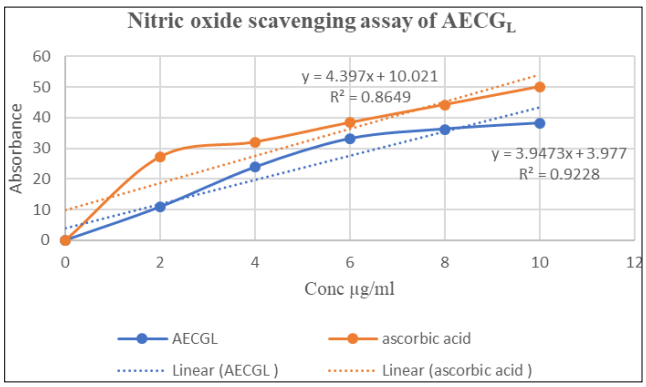



**Fig 6:** Graphical data of Nitric oxide scavenging assay of $AECG_L$

The inhibitory concentration $IC_{50}$ value of $AECG_L$ was found to be 11.65µg/ml in comparison with ascorbic acid as standard 9.09 µg/ml

**Ferric reducing antioxidant power (FRAP) activity of $AECG_L$**

Various concentrations (0 to10 µg/ml) of $AECG_L$ were treated with potassium ferricyanide and the absorbance was obsereved and displayed in Tab 8&Fig 7

**Table 8:** Determination of ferric reducing antioxidant power (FRAP) activity of $AECG_L$

| S.No. | Concentration (µg/ml) | Percentage inhibition | |
|---|---|---|---|
| | | $AECG_L$ | Ascorbic acid |
| 1. | 0 | 0 | 0 |
| 2. | 2 | 28.96 | 31.1 |
| 3. | 4 | 32.50 | 37.29 |
| 4. | 6 | 31.35 | 43.32 |
| 5. | 8 | 31.92 | 47.23 |
| 6. | 10 | 48.63 | 53.42 |
| | $IC_{50}$ | 10.88 | 8.18 |

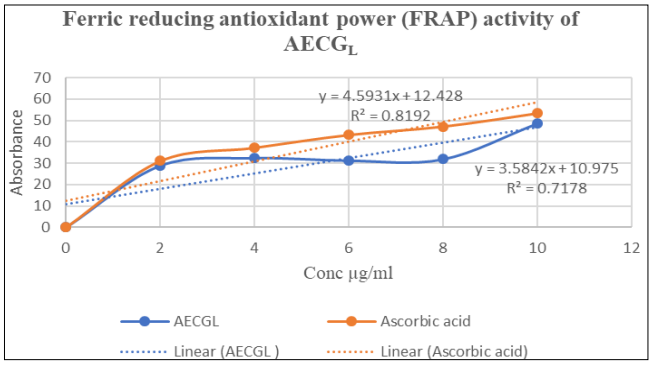



**Fig 7:** Graphical data of ferric reducing antioxidant power (FRAP) activity of $AECG_L$

The inhibitory concentration $IC_{50}$ value of $AECG_L$ was found to be 10.88 µg/ml in comparison with ascorbic acid as standard 8.18 µg/ml

**Total antioxidant capacity of $AECG_L$**

Various concentrations (0 to10 µg/ml) of $AECG_L$ were treated with molybdate reagent and the absorbance was obsereved and displayed in Tab 9&Fig 8

**Table 9:** Determination of total antioxidant capacity of $AECG_L$

| S.No. | Concentration (µg/ml) | Percentage inhibition | |
|---|---|---|---|
| | | $AECG_L$ | Ascorbic acid |
| 1. | 0 | 0 | 0 |
| 2. | 2 | 39.23 | 28.23 |
| 3. | 4 | 47.12 | 36.47 |
| 4. | 6 | 56.78 | 53.23 |
| 5. | 8 | 65.34 | 73.67 |
| 6. | 10 | 65.79 | 91.17 |
| | $IC_{50}$ | 5.72 | 5.33 |

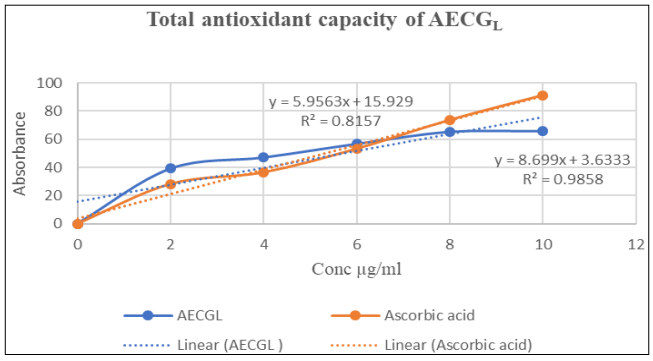



**Fig 8:** Graphical data of total antioxidant capacity of $AECG_L$

The inhibitory concentration $IC_{50}$ value of $AECG_L$ was found to be 5.72 µg/ml in comparison with ascorbic acid as standard 5.33 µg/ml

## Conclusion

The present investigation demonstrates that the leaf extracts of *Calotropis gigantea* possess significant cardioprotective potential against experimentally induced myocardial infarction. Biochemical and histopathological findings revealed attenuation of myocardial damage, reduction in serum cardiac marker enzymes, and improvement in cardiac tissue integrity. These findings support the traditional claims of *Calotropis gigantea* in cardiovascular disorders and suggest its potential as a natural source for developing cardioprotective agents. However, further studies are warranted to isolate the active constituents, explore the precise mechanisms, and establish safety through long-term preclinical and clinical evaluations.

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