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# A Review on, Pharmacological Evaluation of Cardioprotective Activity of Methanolic Leaf Extract of Calotropis Gigantean Linn in Isoprenaline Induced Myocardial Infarction in Swiss Albino Rats.

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**ABSTRACT**

Cardiovascular diseases (CVDs) are the most prevalent cause of death and disability worldwide. CVD, a group of disorders of the heart and the vasculature, includes high blood pressure, coronary heart disease, myocardial infarction, congestive heart failure, stroke and congenital heart defects[3]. The world health organization (WHO) estimates that 17 million people die of cardiovascular disease annually. WHO predicts that deaths due to circulatory system diseases are projected to double by 2015.

It is the acute condition of myocardial necrosis that occurs as a result of imbalance between coronary blood supply and myocardial demand. The patient may experience significant disability or die.

**Keywords:** CVD, WHO

## Introduction

Herbal medicine, also called botanical medicine or phytomedicine, refers to the use of seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Use of herbs and traditional systems of medicine is becoming more main stream as improvements in analysis and quality control along with advances in clinical research show their value in the treatment and prevention of diseases.

From ancient time, plants have used as the major source of medicine and food for human being, and they have continued to provide mankind with new, novel therapeutic medicine and remedies. Since the last five decades, there has been a remarkably research in the study and use of herbal plants.

This current global interest in the study and use of medicinal plants has led to the characterization and identification of novel lead molecules, and isolation of active chemical compounds of therapeutic importance. The current world scenario of utilization of plant-derived natural remedies hascreated a dire need for accurate and up to date information on the characteristic properties and therapeutic uses, efficacy, safety and quality of medicinal plant products[1].

Due to toxicity and side effects of allopathic medicines, has led to rapid increase in the number of herbal drug manufacturers for reduced these problems. Herbal drugs/products have reached extensive adequacy as beneficial agents like anti arthritic, sedative, antidepressant, anti anxiety, antispasmodic, analgesic, anti-inflammatory antimicrobial, anti diabetic, anti fertility, anti ageing, etc. Herbal drugs have been recognized for approximately 5000 years. These drugs have survived real world testing and thousands of years of human testing[2].

Lifestyles of populations across the world have changed dramatically in the 20th century.

These changes (collectively termed as epidemiological transition) have been brought about by a number of developments in science and technology that now affect every facet of human existence. Most human societies have moved from agrarian diets and active lives to fast foods and sedentary habits. Combined with increasing tobacco use, these changes have fuelled the epidemic of obesity, diabetes, hypertension, dyslipidaemia and cardiovascular diseases (CVD).

**Cardiovascular diseases (CVDs).** Cardiovascular diseases (CVDs) are the most prevalent cause of death and disability worldwide. CVD, a group of disorders of the heart and the vasculature, includes high blood pressure, coronary heart disease, myocardial infarction, congestive heart failure, stroke and congenital heart defects[3].

The world health organization (WHO) estimates that 17 million people die of cardiovascular disease annually. WHO predicts that deaths due to circulatory system diseases are projected to double by 2015[4].

The most important clinical complication is an acute occlusion due to blood clot formation during rupture of the lesion, resulting in myocardial infarction. The

**Myocardial infarction**, commonly known as heart attack. It occurs when myocardial ischemia surpasses the critical threshold level for an extended time resulting in irreversible myocardial cell damage. Although clinical care is improved, public awareness is raised and health innovations are widely used, myocardial infarction still remains the leading cause of death worldwide[5].

According to the World Health Organization it will be the major cause of death in the world by the year 2020. In India, the number of patients being hospitalized for myocardial infarction, commonly known as heart attack, is increasing over the past 35 years and male patients have shown a more striking increase[6].

It is the acute condition of myocardial necrosis that occurs as a result of imbalance between coronary blood supply and myocardial demand. The patient may experience significant disability or die. Experimental and clinical studies have shown that there is increased generation of reactive oxygen species such as superoxide anion ($H_2O_2$ ) and hydroxyl radicals (-OH) in heart failure, which involved in the formation of lipid peroxides, damage of cell membrane, and destruction of antioxidative defense system. Therapeutic intervention via suppression of free radical generation and/or enhancement of endogenous antioxidant enzymes may limit the infarct size and attenuate myocardial dysfunction[7]

**Diagnosis of CARDIAC toxicity**

- To diagnose heart disease can include:
- Electrocardiogram (ECG). An ECG is a quick and painless test that records the electrical signals in heart. It can spot abnormal heart rhythms.
- Echocardiogram. This noninvasive exam uses sound waves to produce detailed images of your heart's structure. It shows how your heart beats and pumps blood.
- tress test. This type of test involves raising your heart rate with exercise or medicine while performing heart tests and imaging to check how your heart responds.

**Diagnosis-**

- Cardiac computerized tomography (CT) scan. In a cardiac CT scan, you lie on a table inside a doughnut-shaped machine. An X-ray tube inside the machine rotates around your body and collects images of your heart and chest.
- Cardiac magnetic resonance imaging (MRI). A cardiac MRI uses a magnetic field and computer-generated radio waves to create detailed images of your heart.

**Calotropis gigantean**

*Calotropis* is a genus of flowering plants in the family Apocynaceae. They are commonly known as milkweeds because of the latex they produce. *Calotropis* species are considered common weeds in some parts of the world. The flowers are fragrant and are often used in making floral tassels in some mainland Southeast Asian cultures. Fibers of these plants are called madar or mader. *Calotropis* species are usually found in abandoned farmland.

**Scientific classification**

Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperms
Clade: Eudicots
Clade: Asterids
Order: Gentianales
Family: Apocynaceae
Subfamily: Asclepiadoideae
Tribe: Asclepiadeae
Genus: Calotropis


***Calotropis gigantea* (L.)**

**Species**

- *Calotropis acia*
- *Calotropis gigantea* (L.)
- *Calotropis procera* (Aiton)

**Morphological characteristics of leaf:**

*Calotropis* is a large, bushy shrub with decussate, obovate, coriaceous, auriculate, leaves with acute, subsessile apices extraaxillary, umbellate, panicale inflorescene with purple corolla and erect lobes. The morphological studies revealed the leaves to be subsessile, 6-15 cm by 4.5-8 cm, broadly ovate, ovate-oblong, elliptical, or obovate, acute, pubescent when young and glabrous on both sides on maturity

**Geographical distribution, Origin, and habitat:**

*Calotropis gigantea, the crown flower, is a species of Calotropis native to Cambodia, Vietnam, Bangladesh, Indonesia, Malaysia, the Philippines, Thailand, Sri Lanka, India, China, Pakistan, Nepal, and tropical Africa. It is a large shrub growing to 4 m (13 ft) tall.*

*Habit: A large shrub, much branched, gregarious, young branches covered with white, cottony hairs, contains milky latex.*

- Stem: Erect, branched, cylindrical, solid, contains milky latex.
- Leaves: 100–200 mm (4–8 in) long, decussate, obovate or elliptic-oblong, shortly acute, subsessile, cordate or often amplexical at the base.
- Inflorescence: Umbellate cymes.
- Flowers:

Large, white, not scented, peduncles arising between the petioles. Flower-buds ovoid, angled, Calyx lobes 5, divided to the base, white, ovate; corolla broadly rotate, valvate, lobes 5, deltoid ovate, reflexed, coronate-appendages broad, obtusely 2-auricled below the rounded apex which is lower than the staminal-column. Stamens 5, anthers short with membranous appendages, inflexed over

the depressed apex of the pentagonal stigma. Pollinium one in each cell, pendulous caudicles slender. Carpels 2 distinct, styles 2, united to the single pentagular stigma, ovary 2-celled, ovules many.

- Fruit: A pair of follicles with many, hairy seeds.
- Flowering and Fruiting Time: November-April

**Phytochemical Constituents:**

Calotropis procera contained many biological active chemical groups including, **cardenolides, steroids, tannins, glycosides, phenols, terpenoids, sugars, flavonoids, alkaloids and saponins**.

*Significance*

- Common as a weed in will bete lands.
- The root, bark and milk used in medicine for the treatment of dysentery cutaneous affections.
- The leaves are applied on paralysed parts, painful joints.
- The milk is useful in leprosy and ringworm.

**Chemical Constituents**

- Carbohydrate (57.2% w/w).
- Protein (22% w/w)
- Fat (0.50% w/w)
- Calcium (287 mg)
- Phosphorus (311 mg)
- Iron (6.77 mg)
- Vitamins like Thiamine (0.4 mg), Riboflavin (0.2 mg) and Niacin (1.5 mg) per 100 grams of dry matter
- Calotropis gigantea also contained many biological active chemical groups including, cardenolides, steroids, tannins, glycosides, phenols, terpenoids, sugars, flavonoids, alkaloids and saponins.

**Phytochemical Investigations:**

- Extraction of whole plant material
- Preliminary Phytochemical screening

**Pharmacological Investigations:**

**A. *In-Vitro***

- Inhibition of calcium oxalate crystallization in human urine
- In-Vitro antioxidant activity:

1. DPPH Free radical scavenging activity
2. Phosphomolybdenum reduction assay
3. Nitric oxide radical-scavenging assay
4. Reducing power assay

**B. *In-Vivo***

- Evaluation of Cardioprotective activity by Isoproterenol induced myocardial infarction
- Evaluation of **Antioxidant potential** of Cardioprotective activity by Isoproterenol induced myocardial infarction in Swiss albino rats.
- **Histopathology of heart.**

**MATERIALS AND METHODS[8-12]**

**MATERIALS**

**Drugs –**

- Ketamine hydrochloride injection (Aneket from Neon Laboratories Limited)
- Isoproterenol (Samarth Life Science Pvt Ltd)
- Verapamil (VPL from Samarth Life Science Pvt Ltd.)

**Chemicals-**

**Table 1: List of chemicals**

| S.No. | List of Chemicals |
|---|---|
| 1 | Hydrogen peroxide ($H_2O_2$) |
| 2 | 5,5-Dithiobis 2-nitrobenzoic acid (DTNB) |
| 3 | Ether |
| 4 | Acetic acid |
| 5 | Thio barbituric acid |
| 6 | n-butanol pyridine |
| 7 | EDTA |
| 8 | Sucrose |
| 9 | Adrenaline |
| 10 | Trichloro acetic acid |

| | |
|---|---|
| 11 | Formalin |
| 13 | Potassium phosphate buffer |
| 14 | Sodium dodecyl sulphate |
| 15 | Ethanol |
| 16 | Sodium carbonate |
| 17 | Sodium hydroxide |
| 20 | Phosphate buffer |
| 22 | Formic acid |
| 23 | Hydrochloric acid |
| 24 | Sodium Nitroprusside |
| 25 | Chloroform |
| 26 | Sulphuric acid |

Reagents will be prepared according to the need and some will be purchased from commercial sources.

**Diagnostic kits:** Diagnostic kits used for the estimation of marker enzymes CK-MB, LDH, SGOT and SGPT will be procured from **Span Diagnostic Ltd. India** and **AGAPPE Diagnostic**. **Instruments:**

- Micro centrifuge ("Microfuge" M/S Remi instruments Pvt. Ltd., Maharashtra, India).
- Semi Auto Analyser (MS 500 e, M/S Maysum technology Pvt. Ltd).
- ECG machine [Cardiart108DG (BPL)]
- Dhona balance (M/S Dhona instruments Pvt. Ltd., Kolkata, India)
- Colorimeter (Systronics, Photoelectric Colormeter-112)
- Tissue homogenizer (M/S Remi instruments Pvt. Ltd.,Maharashtra, India)
- Autoclave

## METHODOLOGY

**Extraction:**

The authenticated plants parts will be shade dried and powdered coarsely. Extraction will be done according to standard procedures using analytical grade solvents. Coarse powder of *Caesalpinia pulcherrima*, *Bauhinia varigata* and *Caesalpinia crista* will be soxhlet extracted successively with petroleum ether and absolute alcohol (at 60º C). The aqueous extract will be prepared coarse powder by maceration process. The *Caesalpinia pulcherrima*extracts will be concentrated under reduced pressure to yield ethanolic (8.2%) and the aqueous extracts (12.4 %). The extracts of *Bauhinia variegata* Linn obtained will be concentrated under reduced pressure to yield ethanolic (10.5%) and the aqueous extracts (17.8 %). Same procedure will be also used for extraction of coarse powder of seed of Caesalpinia *Crista.* The extracts obtained will be concentrated under reduced pressure to yield ethanolic (13.5%) and the aqueous extracts (18.6 %).

**QUALITATIVE CHEMICAL TEST:**

**Preliminary phytochemical investigation of extract:**

Qualitative chemical tests will be conducted for ethanolic and aqueous extracts of *Caesalpinia pulcherrima* L., *Bauhinia variegata* Linn and *Caesalpinia Crista*to identify the various phytoconstituents. The various tests conducted are given below and observations are recorded and tabulated.

**1. Tests for Carbohydrates:**

**Molisch's test (General test):**

To 2-3 ml aqueous extract, few drops of α-naphthol solution in alcohol will be added, shaken and concentrated $H_2SO_4$ will be added from the sides of the test tube. It will be observed for violet ring at the junction of two liquids.

**For Reducing Sugars:**

**a) Fehling's test:**

**b) Benedict's test:**

**Test for Monosaccharides:**

**a) Barfoed's test:** Equal volumes of Barfoed's reagent and test solution will be added, heated for 1-2 min, in boiling water bath and cooled, and observed for red precipitate.

**Test for Hexose Sugars:**

**Tests for Non-Reducing Sugars:**

**a)** Test solution does not give response to Fehling's and Benedict's test.
**b)** Tannic acid test for starch: With 20% tannic acid, test solution will be observed for precipitate.

**2. Tests for Proteins:**

**a)** **Biuret test** (General test): To 3 ml T.S. added 4% NaOH and few drops of 1% $CuSO_4$ solution and observed for violet or pink colour.
**b)** **Millon's test** (for proteins): Mixed 3 ml T.S. with 5 ml Million's reagent, white precipitate obtained. Precipitate when warmed turns brick red or precipitate dissolves giving red colour.
**c)** **Xanthoprotein test** (For protein containing tyrosine or tryptophan): Mixed 3ml T.S. with 1 ml concentrated $H_2SO_4$, observed for white precipitate.
**d)** **Test for protein containing sulphur:** Mixed 5 ml T.S. with 2 ml 40% NaOH and 2 drops 10% lead acetate solution. Solution will be boiled, turns black or brownish due to PbS formation.
**e)** **Precipitation test:** The test solution will be observed for white colloidal precipitate with following reagents:
**i)** Absolute alcohol
**ii)** 5% mercuric chloride solution
**iii)** 5% cupric sulphate solution
**iv)** 5% lead acetate
**v)** 5% ammonium sulphate.

**3. Tests for Steroids:**

**a)** Salkowski Reaction:
**b)** Liebermann-Burchard Reaction
**c)** Libermann's reaction:

**4. Tests for Amino Acids:**

**a)** **Ninhydrin test** (General test): 3 ml T.S. and 3 drops 5% Ninhydrin solution will be heated in boiling water bath for 10 min and observed for purple or bluish colour.
**b)** **Test for Tyrosine:** Heated 3 ml T.S. and 3 drops Million's reagent. Solution will be observed for dark red colour.
**c)** **Test for tryptophan:** To 3 ml T.S. added few drops glycoxalic acid and concentrated $H_2SO_4$ observed for reddish violet ring at junction of the two layers.

**5. Tests for Flavonoids:**

**6. Tests for Alkaloids:**

**a)** **Dragendroff's test:** To 2-3 ml T.S, added few drops Dragendroff's reagent and observed for orange brown precipitate.
**b)** **Mayer's test:** To 2-3 ml T.S, added few drops Mayer's reagent and observed for precipitate.
**c)** **Hager's test:** To 2-3 ml T.S, added Hagers reagent and observed for yellow precipitate.
**d)** **Wagner's test:** To 2-3 ml T.S, added few drops of Wagner's reagent and observed for reddish brown precipitate.

**7. Tests for Tannins and Phenolic Compounds:**

**8. Tests for Vitamins:**

**9. Tests for Glycosides:**

**Tests for Cardiac Glycosides:**

**a)** Baljet's test:
**b)** Legal's test Test for deoxysugars (Kellar Killani test):
**c)** Libermann's test (For bufadenolids):
**d)** Tests for Saponin Glycosides:-

**Tests for Coumarin Glycosides:**

Test solution when made alkaline, observed for blue or green fluorescence.

**Acute toxicity studies:**

Acute toxicity studies for aqueous and ethanolic extracts of *Calotropis gigantea* will be conducted as per OECD guidelines 423 using albino Wistar rats. Each animal will be administered the aqueous solution of the extract by oral route. The animals will be observed for any changes continuously for the first 2h and up to 24 h for mortality.

**Extracts:**

Methanolic extracts of *Calotropis gigantea* at doses of X mg/kg b.w. will be used to evaluate diabetic neuropathy and cardioprotective activities. Stock solution of the extracts will be prepared in the range of X mg/ml in water according to the need of study.

**CARDIOPROTECTIVE ACTIVITY-**

**Isoproterenol Induced Myocardial Infarction[13-14]**

Albino Wistar rats either sex will be divided into various groups (n = 6). All plant extracts will be treated for 30 days. At the end of the treatment period, animals of all groups will be administered with isoproterenol at a dose of 85

mg/kg body wt., subcutaneously, twice, at an interval of 24 h.

**Table 2:**

| Groups | Treatment |
|---|---|
| Group I | Normal Control |
| Group II | Isoproterenol (IP) (85 mg/kg, s.c.) |
| Group III | IP + Plant Extract |
| Group IV | IP + Plant Extract |
| Group V | IP + Plant Extract |
| Group VI | IP + Standdrugard |

**Electrocardiography**

At the end of the experimental period, A lead II electrocardiogram (ECG) will be monitored by using Cardiart108DG (BPL) with sensitivity 20 mm mV at a paper speed 25 mm/s. and changes in ECG pattern will be considered.

**Estimation of Plasma lipid profile in rats**

Plasma total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL) will be analyzed using commercially available kits. Very low density lipoproteins (VLDL) and low density lipoprotein (LDL) will be measured.

**Estimation of serum enzyme levels in rats**

After experimental period blood will be withdrawn from the retro orbital sinus, the serum will be separated by centrifugation and will be used for the estimation of marker enzymes CK-MB, LDH, SGOT and SGPT using AGAPPE diagnostic kits.

Animals will be sacrificed and heart will be isolated and subjected to histopathological studies.

**RESULTS:**

Reported Pharmacological Activity

**Hepatoprotective Activity**

- Argal *et al* (2010) investigated Evaluation of Hepatoprotective Activity of Calotropis gigantean[15].
- Lodhi G *et al* (2009) investigated Hepatoprotective effect of Calotropis gigantea extract against carbon tetrachloride induced liver injury in rats[16].

**Antioxidant Activity:**

- Singh N et *al* (2010) investigated *In vitro* antioxidant activity of *Calotropis gigantea* hydroalcohlic leaves extract[17].
- Amit JS *et al* (2010) investigated Phytochemistry and evaluation of antioxidant activity of whole plant of Calotropis gigantea Linn[18].
- Elakkiya P *et al* (2009) investigated A study on phytochemical screening and in vitro antioxidant activity of Calotropis gigantea L[19].

**Cardiprotective activity:**

- Bhat SK *et al* (2013) investigated Therapeutic potential of cardiac glycosides of Calotropis gigantean for breast cancer[20].

**Antimicrobial Activity:**

- Kumar G, *et al* (2010) investigated Antimicrobial activity of latex of *Calotropis gigantea* against pathogenic microorganisms an *in vitro* study[21].
- Karthik L *et al* (2010) investigated Antibacterial activity of aqueous extract of *Calotropis gigantea* leaves–an *in vitro* study[22].
- Garg LC *et al* (1963) investigated Anthelmintic activity of Calotropain and Bromelain[23].
- Habib MR *et al* (2009) investigated Antimicrobial and cytotoxic activity of di-(2-ethylhexyl) phthalate and anhydrosophoradiol-3-acetate isolated

from *Calotropis gigantea* (Linn.) flower[24].

- Alam MA, *et al* (2008) investigated Antimicrobial activity of akanda (*Calotropis gigantea* L.) on some pathogenic bacteria[25].

**Insecticide activity:**

- Alam MA, *et al* (2009) investigated Insecticidal activity of root bark of *Calotropis gigantea* L. against *Tribolium castaneum* (Herbst)[26].

**Antidiarrheal activity:**

- Chitme HR *et al* (2004) Studies on antidiarrhoeal activity of Calotropis gigantean[27].

**Wound healing activity**

- Deshmukh PT, *et al* (2009) investigated Wound healing activity of *Calotropis gigantea* root bark in rats[28].
- Nalwaya N, *et al* (2009) investigated Wound healing activity of latex of *Calotropis gigantea*[29].

**Analgesic Activity:**

- Pathak AK *et al* (2007) investigated Analgesic activity of Calotropis gigantea flower[30].

**Antitumor and Cytotoxic Activity:**

- Wang Z, et *al* (2008) investigated A new cytotoxic pregnanone from *Calotropis gigantea*[31]

**Antifugal activity:**

- Kumar G, *et al* (2010) investigated *In vitro* anti-*Candida* activity of *Calotropis gigantea*[32].

**Toxicity studies**

- Kshirsagar, *et al* (2010) investigated Acute and subacute toxicity study of the ethanolic extract from Calotropis gigantean in rodents[33].

**Keywords**

- Cardiovascular
- Myocardial infarction
- Phytochemicals
- Isoproterenol

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