



Review Article

www.ijrap.net

(ISSN Online:2229-3566, ISSN Print:2277-4343)



IN VIVO STUDY OF PHARMACOLOGICAL ACTIVITIES OF *CALOTROPIS PROCERA*: AN OVERVIEW

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Received on: 30/03/22 Accepted on: 25/04/22

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DOI: 10.7897/2277-4343.130356

ABSTRACT

Calotropis is one of the widely distributed plants along the world's geographical area. Many studies have been conducted to prove the pharmacological activities of *Calotropis procera* (Arka). It is classified as Upavisha in Ayurveda by Bhavprakasha and Rasatarangini. In its therapeutic application mentioned in various classical texts, *Calotropis procera* is treating almost 58 diseases having its 547 formulations which are indicated. It contains many phytochemical constituents like Cardenolides, Benzoyllineolone, Calactin, Calotropagenin etc. These phytoconstituents have proven anti-inflammatory, anticonvulsant, hepatoprotective, antitumor, and cytotoxic properties. In this review, a thorough literature search has been done through various textbooks, research magazines and internet search engines like PubMed, Google Scholar etc. Different *In Vivo* studies carried out on *Calotropis procera* to assess its pharmacological activities are considered. Meticulous analysis of these studies has been done with special reference to plant parts and their extraction method, animal models, assessment criterion and study outcomes. This provides additional information to carry out further research on *Calotropis procera*. The present review may be useful to researchers as a ready reckoner for their studies.

Keywords: *Calotropis procera*, Upavisha, pharmacological activity, Phytochemicals.

INTRODUCTION

Medicinal plants have incredible importance because of the presence of many components, and they become the source for the synthesis of active pharmaceutical ingredients (API). The plant *Calotropis procera* is widely distributed along the world's geographical area.

By Bhavaprakasa and Rasatarangini, *Calotropis procera* is classified as Upavisha in Ayurveda. In various classical texts, plant *Calotropis procera* (Arka) therapeutic application mentioned, *Calotropis procera* has 547 formulations, which are indicated in treating almost 58 diseases¹. This plant is a rich source of phytoconstituents. It has many proven pharmacological activities such as anticancer, cytotoxic, antitumor, antiproliferative, anthelmintic, hepatoprotective, anti-inflammatory, antidiarrheal, antibacterial and antinociceptive and many more²⁻⁷.

This plant contains many phytochemical constituents like Cardenolides, Benzoyllineolone, Calactin, Calotropagenin etc. These phytochemicals constituents are effective against cancer and various diseases⁸.

The plant *Calotropis procera* and its phytoconstituents have been extensively studied for various biological and anticancer activities. Various preclinical studies have been reported on *Calotropis procera* to prove its activities by using different animal models and cell lines such as HCT-15, HCT-8, HCT-29, Hep-2, KB (Oral), SNB-78, MCF- 7 etc.

Searching for new therapeutic agents is a big challenge for scientists of the present modern era, and plants are the most

significant source of these agents. Screening plants for their pharmacological properties with the hope of finding a safe and effective agent is essential. Many synthetic compounds are available, but their use is restricted due to their hazardous effects on the environment and adverse effects on the human body. To find the agents from plant sources which are safe, effective, and environmentally friendly, *C. procera* is one of them.

The initial phase of the drug development process consists of the preclinical phase, which helps assess the safety and efficacy of a new drug by using *In Vitro* and *In Vivo* models. *In Vivo* studies act as a middle ground between *In Vitro* experiments and human trials. Researchers often use *In Vitro* methods for fundamental investigations like disease processes at the cellular level. *In Vivo* studies expand on data from *In Vitro* studies by monitoring biological responses in a living organism.

The objective of the present study is to provide systematic information about the pharmacological actions of various extracts of *Calotropis procera* and its parts. Thus, this is an attempt to collect all possible references of *In Vivo* pharmacological activities of *Calotropis procera*.

Review of Literature

Calotropis procera (Arka)

Latin Name: *Calotropis procera* (Ait.) R.Br

Family: Asclepiadaceae

Vernacular Names:

- Sanskrit: Raktarka, Arkaparna, Kshiraparna, Arkanama, Vikirana, Shuklaphala,
- Marathi: Rui
- Hindi: Aak, Madar

- English: Madar

Types– Shwetarka- *Calotropis gigantea* (Linn.) R. Br Ait.
Raktarka- *Calotropis procera* (Ait.) R. Br

Classification: Modern: Irritant, Organic, Vegetative poison.
Ayurved: Akritrim, Sthavara, Vanaspatija visha, Upavisha

Botanical Description: *Calotropis procera* is an erect shrub of about 1-2 m. Leaves are subsessile, broad, ovate, usually 6-15 cm long and 4.5-8 cm broad. Flowers are in umbellate cymes, white, purple-spotted or pink. Root bark taproots with prominent tops and rounded heads. Complex roots are greyish white. The bark is yellowish-grey outside and yellowish-white inside. The root is corky, soft and rough with longitudinal fissures.

Parts used: Roots, Leaves, Root Bark, Flowers, Latex (Kshira).

Active Principles: β -amyryn, Calotropin, Calotoxins, Calactin, Uscharin, Trypsin in Kshira

Fatal Dose: Not determined

Fatal period: About 12 hours.

In *Calotropis procera*, different parts of the plant have been reported to possess various phytochemicals containing cardiotoxic agents such as calotropin, calotropagenin, calotoxin, calotropagenin and voruscharine, steroids, di and triterpenes such as stigmasterol, β -sitosterol, flavonoids, polyphenol compounds, and various newer reported hydrocarbons and proteins shown in Table 2.

The pharmacological activities of major primary phytoconstituents are tabulated in Table 3.

A thorough literature search has been done through various textbooks, research magazines and internet search engines like PubMed, Google Scholar etc., to find out different *In Vivo* studies carried out on *Calotropis procera* to assess its pharmacological activities. These studies are tabulated according to plant parts and their extraction method, animal models, assessment criterion and study outcomes in Table 4.^{9,10,11}

DISCUSSION

Currently, established drugs are primarily synthetic and derived from chemical origin. These synthetic drugs have shown some side effects like drug induce toxicity. So, it is today's need to search for alternative safe medicines. Medicines derived from plant origin are supposed to be safer and more effective. Hence various attempts have been made to discover the new safe alternative drug.

The plant *Calotropis procera* is a rich source of phytoconstituents with proven pharmacological activities. Various preclinical studies have been reported on *Calotropis procera* to prove different pharmacological activities. Other plant parts of *Calotropis procera* contain many phytoconstituents, as shown in Table 2. These phytoconstituents have many pharmacological activities such as antitumor, cytotoxic, antiulcer, antioxidant, anti-inflammatory, cardioprotective, and antipyretic, as shown in Table 3.

Table 1: Properties and action of Arka¹²

Rasadipanchak	Arka
Rasa	Katu, Tikta
Guna	Tikshna, Sara
Virya	Ushna
Vipaka	Katu
Doshagnata	Kapha, Vata
Karma	Vatahara, Vranahara, Vishaghna, Dipana, Krimighna, Shwashara
Therapeutic Uses	Shotha, Shwasa, Sleshmodara roga, Kandua, Krimirog, Vrana, Gulma, Pliharogah, Arsah
Dose	250-750mg of the drug in powder form

Table 2: Phytoconstituents of Various Parts of *Calotropis procera*¹³

Parts used	Phytoconstituents
Latex	α - amyryn, - β amyryn, - β sitosterol and calotoxin (0.15%), Calactin (0.15%), Calactin composed of calotropagenin, Calotropin, Calactinic acid, Voruscharin (0.45%), and hexose, Uzarigenin, Syriogenin, Proceroiside, Uscharin,
Leaves	β - amyryn, cardenolides, calotropagenin, calotropin.
Root Bark	Benzoyllineolone, Benzoylisolineolone, β - amyryn, Three oleanane type triterpenes namely calotropoleanyl ester (olean - 13 (18)- ene- 3 β - yl acetate), Proceroleanenol A and (olean- 13(18)- ene-9 α - ol) and Proceroleanenol B (olean-5, 13(18)- diene-3 α -ol
Flower	β - amyryn, cyanidin 3-rhamnoglucoside Esters of β - calotropeols, Evanidin 3-rhamnoglucoside, volatile fatty acids and long-chain fatty acids, esters of waxy acids, evanidin- 3- rhamnoglucosides and alcohols.
Stem bark	d- and - β calotropeols - β amyryn, giganteol, a colourless wax, tetracyclic terpene (small quantity), sterols

Table 3: Main Phytoconstituents of *Calotropis procera* and their Pharmacological activities^{14, 15, 7}

Phytoconstituents	Pharmacological activities
α amyryn	Anti-inflammatory, Hepatoprotective, Antioxidant, Analgesic, Cytotoxic, Antitumor, Antiulcer, Gastroprotective.
β amyryn	Anti-inflammatory, Hepatoprotective, Gastroprotective, Antioxidant, Analgesic, Antiulcer,
β sitosterol	Anti-inflammatory, Antioxidant, Anticancer, Antitumor, Anorexic, Antibacterial, Antifertility, Antifeedant, Antihyperlipoproteinaemic, Antileukemic, Antilymphomic, Antimutagenic, Antiviral, Antipyretic, Hepatoprotective, Hypoglycaemic, Hypolipidemic, Ulcerogenic.
Calotropin	Antitumor, Cardioactive, Proteolytic
Calactin and Calotoxin	Cardioactive
Stigmasterol	Antioxidant, Antinociceptive, Antiviral, Cancer-preventive, Hypocholesterolaemia, Sedative.

Table 4: In Vivo Pharmacological Activities in *Calotropis procera* with special reference to its different parts

Activities	Parts/Extract used	Animal Model	Assessment	Outcome
Cytotoxic Chemopreventive ⁴	Dried Latex (aqueous extract)	X-15 Transgenic mouse model	Hepatocellular carcinoma	Cytotoxic Chemo preventive effect
Antitumor ³	Stem extracts- (Ethyl acetate, acetone and methanol extract)	Adult Swiss albino mice	Sarcoma 180 tumours	Antitumor activity
Antidiarrheal ¹⁶	Leaves (Methanol extract)	Albino mice	-	Methanol extract shows significant Antidiarrheal activity.
Anti hyperglycaemic effect ¹⁷	Leaves (Hydroalcoholic extract)	1.Male Wistar rats 2. Swiss mice	1.Acute toxicity 2.Oral glucose tolerance test	The hydroalcoholic extract has an anti-hyperglycaemic effect in streptozotocin-induced diabetic rats.
Antioxidant and anti-hyperglycaemic ¹⁸	Dried latex (aqueous suspension)	Wistar rats	1. Blood glucose estimation 2. Estimation of serum insulin levels 3. Detection of urinary glucose and protein 4. Estimation of GSH and TBARS in renal tissue 5. Histological analysis of kidney	Antioxidant and anti-hyperglycaemic property and protection against the complications associated with diabetes.
Antidiabetic ¹⁹	Latex (Phyto-modulatory proteins)	Wistar rats	1. Effect of LP administration in fed animals 2. Measurement of hepatic TNF- α 3. Measurement of insulin levels 4. Intraperitoneal glucose (ipGTT), insulin (ipITT) and pyruvate (ipPTT) tolerance testing 5. Intraperitoneal glucagon tolerance test 6. Western blot analysis 7. Real-time PCR	Therapeutic potential in DM2.
Anti-hyperbilirubinemic and wound healing ²⁰	Leaves (Aqueous extract)	Wistar rats	-	AECP showed significant bilirubin Lowering and wound healing properties in Wistar rats.
Anti-inflammatory Analgesic ²¹	Leaves (Ethanol extract)	1.Wistar rats 2.Swiss mice	1. Anti-inflammatory study Carrageenan induced paw oedema in rats 2. Formalin paw lick test in rats. 2. Analgesic study 3. Acetic acid writhing response in mice 4. Tail flick test.	Ethanol extract shows potent anti-inflammatory and analgesic properties.
Anti-inflammatory ²²	Latex (Methanol Extracts)	Wistar rats	1. Rat paw oedema test 2. Histological changes associated with carrageenin-induced rat paw oedema	Extracts of DL exert their anti-inflammatory effects mainly by inhibiting histamine and BK and partly by inhibiting PGE2.
Anti-inflammatory Proteolytic ²³	Latex (proteins derived from the latex)	Male Swiss mice	1. Platelet count 2. Proteolytic activity 3. Plasma clotting activity 4. Activated partial thromboplastin time 5. Prothrombin time 6. Fibrinogen-agarose plate assay 7. Fibrinogen polymerisation assay 8. Fibrinogenolytic activity 9. Electrophoresis 10. Human plasma clot hydrolysing activity	Anti-inflammatory And proteolytic and therapeutic potential in conditions associated with coagulation abnormalities.
Anti-inflammatory ²⁴	Latex (Protein Sub-fraction)	Wistar albino rats	1. Joint inflammation 2. Stair climbing ability 3. Motility 4. Dorsal flexion pain 5. Estimation of tissue glutathione level 6. Estimation of tissue thiobarbituric acid reactive substances level 7. Histological analysis	Therapeutic potential in the treatment of arthritis.
Anticonvulsant ²⁵	Roots (Chloroform and aqueous extract of root)	Female Albino Wistar rats	1. Maximal electroshock seizure (MES) test 2. Pentylene-tetrazole-induced seizure test 3. Lithium-pilocarpine-induced seizures 4. Electrically kindled seizures	Chloroform extract and aqueous extract may be beneficial in the absence (pfit mal) and tonic clonic (grand mal) seizures.
Anticonvulsant ²⁶	leaves (aqueous extract)	Wistar rats	1. Acute study 2. Chronic study 3. Estimation of GABA in the brain	Inhibited PTZ induced seizures in rats.

Hepatoprotective ²⁷	Flowers (Hydro-ethanol extract 70%)	Wistar rats	1. Hepatoprotective activity 2. Biochemical analysis	Biochemical markers to the near-normal levels of the treatment group.
Hepatoprotective ²⁸	Dried extracts	Wistar rats	1. Acute toxicity studies 2. Hepatoprotective study	Hepatoprotective activity
Immunomodulatory ²⁹	Root bark (Ethanol extract)	Swiss albino mice	1. Phytochemical tests 2. Acute toxicity studies 3. Haemagglutinating antibody (HA) titre 4. Delayed-type hypersensitivity (DTH) reactions 5. Peritoneal Macrophage count 6. Vascular Permeability in Mice 7. Peripheral blood count 8. Cyclophosphamide-induced immunosuppression	Immunomodulatory activity
Antipyretic effect ³⁰	Latex	Albino rats	-	Antipyretic activity
Antinociceptive ³¹	Latex (protein fraction)	Male Swiss mice	Pharmacological tests a) Acetic acid-induced writhing b) Formalin test c) Hot plate test	Antinociceptive activity
Cardioprotective ³²	Latex (alcoholic extract)	Albino rats	1. Anti-myocardial-infarction activity 2. Estimation of serum enzyme levels in rats 3. Estimation of lipid peroxide and glutathione content in heart homogenates 4. Histopathological studies	Cardioprotective activity
Gastroprotective ³³	Dried latex and its methanol extract (MeDL)	Wistar rats	1. Effect of DL and MeDL on the gastric mucosa of normal rats 2. Estimation of antioxidant level and protein in gastric tissue 3. Estimation of thiobarbituric acid reactive substances (TBARS) in gastric tissue 4. Histological analysis of stomach	Gastroprotective activity
Lipolytic effect ³⁴	Leaf (extract)	Albino Wistar rats	Histopathology	Lipolytic activity

In table 4, a meticulous analysis of *In Vivo* studies to assess pharmacological activities of *Calotropis procera* with special reference to plant parts, their extraction method, animal models, assessment criterion and study outcomes has been done. Upvisha, *Calotropis procera*, is a beneficial plant as per Ayurveda and modern view. Many studies are available on the internet about *Calotropis procera* in which 15900 preclinical pharmacological studies have been registered in the last 20 years, including 3470 *In Vivo* studies. 21 *In Vivo* studies with full research papers have been selected for the current review. Also, ten important pharmacological activities out of 27 proven activities have been selected. Animal studies are a middle ground between *In Vitro* experiments and human trials. Most animal studies use laboratory-bred mice, rats, hamsters, guinea pigs etc., rodent species which are almost genetically identical. *In Vitro* methods are deployed for foundational investigations like disease processes at the cellular level. At the same time, *In Vivo* studies expand on data generated from *In Vitro* studies by monitoring biological responses in a living organism.

After assessing these studies, it is observed that anti-inflammatory, anticonvulsant, hepatoprotective, anti-hyperglycaemic, and anticancer activities are frequently evaluated. Four studies on anti-inflammatory activity, two studies on anti-hyperglycemic activity, two studies on hepatoprotective, and two studies on anticonvulsant activity are found. Commonly used plant parts are latex and leaves of *Calotropis procera*, but latex is used most frequently. Various extracts have been tested like aqueous extract, ethyl acetate extract, methanol extract, hydroalcoholic extract, protein extract, chloroform extract etc. Most commonly prepared extracts are protein and aqueous extract. These studies' widely used animal models are Wistar rats and Swiss albino mice.

Assessment criteria deliver knowledge about the qualities, characteristics and aspects of an assessment task that will be used to measure their achievement outcomes. Assessment criteria make it clear for researchers what factors will be taken under consideration while making conclusions about their study.

Two anti-hyperglycaemic activity studies have been carried out using two extracts, viz. hydroalcoholic extract of leaves and aqueous extract of dried latex in Wistar rats. After comparing the assessment parameter blood glucose level, the p-value of hydroalcoholic extract of leaves is < 0.05, and an aqueous extract of dried latex is < 0.001. Therefore, aqueous extract of dried latex has more significant anti-hyperglycaemic activity than hydroalcoholic extract of leaves.

Three anti-inflammatory activity studies have been carried out using three extracts viz. ethanol extract of leaves, methanol extract of latex and protein extract of latex in Wistar rats. In two studies, the carrageenan-induced paw oedema model was used, and in the third study, joint inflammation was assessed. By comparing p-value it is found that p-value of leaves ethanol extract is < 0.05, latex extract < 0.001 and protein extract of latex < 0.05. Out of these three studies, it is concluded anti-inflammatory activity of latex extract of *Calotropis procera* has given the most significant results.

In two anticonvulsant studies, three different extracts, viz. chloroform and aqueous extract of roots and aqueous extract of leaves, were assessed. These studies have the same model (PTZ induce convulsion model), similar study population (Wistar rats) and similar assessment criteria, i.e., delay in the time of convulsions. After comparing the p values of these studies, chloroform extract of the root is highly significant.

Two hepatoprotective activity studies were compared. In both studies, the Paracetamol induces hepatotoxicity model was used in which extracts of leaves and flowers were assessed in Wistar rats. Parameters compared were SGOT, SGPT, Alkaline phosphate, and Total bilirubin level. Both studies showed a similar p-value ($p < 0.01$), so both extracts showed significant hepatoprotective activity.

The present study provides systematic information and meticulous analysis of *In Vivo* pharmacological actions of various extracts of *Calotropis procera* and its parts. Thus, this is an attempt to collect all possible references of *In Vivo* pharmacological activities of *Calotropis procera*.

CONCLUSION

From the above review, the extract of different parts of *Calotropis procera* (Upavisha Arka) has shown other pharmacological activities in various *In Vivo* studies. Hence, *Calotropis procera* has significant potential in treating different diseases. The present review may help provide additional information to further preclinical and clinical research on its use in treating various conditions.

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Cite this article as:

Ashishkumar A. Kale et al. *In vivo* study of pharmacological activities of *Calotropis procera*: An overview. Int. J. Res. Ayurveda Pharm. 2022;13(3):44-49 <http://dx.doi.org/10.7897/2277-4343.130356>

Source of support: Nil, Conflict of interest: None Declared

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