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A REVIEW ON POISONOUS, PESTICIDAL AND MEDICINAL ATTRIBUTES OF *CLEISTANTHUS COLLINUS* (ROXB.) BENTH. EX HOOK.F.

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ABSTRACT

Cleistanthus collinus or Garari *(*Phyllanthaceae) is a small poisonous tree encountered in dry forests of southern and central parts of India. The plant is the cause of deliberate and accidental poisoning in rural parts of India. The fresh leaves or decoction is the common manner of consumption for suicidal purposes. Patients exhibits eye symptoms, vomiting, abdominal pain, hypokalemia, elevated levels of AST, CPK-MB, LDH, abnormal ECG, metabolic acidosis, renal injury and hypoxia. Treatment is supportive and symptomatic. Cardiac glycoside property of inhibition of ATPase activity is major mechanism of toxicity. Respiratory and cardiac arrest are the usual mode of death. Diphyllin, Cleistanthin A, Cleistanthin B are prominent phytoconstituents that belong to category of arylnalpthalide lignans and glycosides. The active ingredients of plant exhibit cytotoxic, anti-inflammatory, pesticidal and antioxidant properties. The plant extract inhibits adult emergence in mosquito vector species, is useful against many broad range of bacteria ranging from *E. coli* to *Staphylococcus*, and also suppresses viral core protein p24 expression in HIV-1. Many cancer cell lines of carcinoma, lymphoma and sarcoma are suppressed by Cleistanthins. The plant shows considerable anticandidal activity, is useful as acaricide, insecticide, piscicide, rodenticide, and is also beneficial in killing aphides. The plant helps to control agricultural pests and is traditionally used as goat feed, in hair growth, and to make wooden articles and equipment thus serving many needs.

KEYWORDS: *Cleistanthus collinus*, poison, glycosides, hypokalemia, cytotoxic, pesticide.

INTRODUCTION

Cleistanthus collinus (Roxb.) Benth. ex Hook.f. commonly referred to as Garari (Hindi) from the family Phyllanthaceae is a plant species described by Roxburgh within its contemporary name after Bentham and Hooker. The plant is native to India and Sri Lanka, is categorized as vulnerable according to IUCN, and is specially encountered in dry forests of southern and central parts of India.[1] The outer crust of the capsule along with the leaves and roots are reportedly known for its high toxicity. The plant is mainly ingested as poison leading to a large number of deaths in many parts of rural south India.[2] The freshly prepared leaf extract is also used as abortifacient, cattle and fish poison.[3] The major phytochemicals responsible for poisoning belong to the category of glycosides, tannins, saponins, lignans and lactones of which the prominent active constituents are cleistanthins, oduvin and diphyllin. In severe cases of poisoning in humans, the toxicity causes hypokalemia, hypotension, cardiac arrhythmias, mixed metabolic and respiratory acidosis, and renal failure.[4] The major mechanism through which the poison acts seems to be inhibition of vacuolar ATPase proton pump.

Recently, the plant has been researched for its therapeutic properties, as it has been known to have antifungal, antibacterial, antiseptic and diuretic properties. The active ingredients being cytotoxic in nature are experimented on several cancer cell lines, and they also seem to have potency against HIV. The plant extract possesses larvicidal activity which is exploited in controlling populations of mosquitoes involved in diseases like malaria and dengue. In addition to this, the plant is also used in traditional systems of medicines like ayurveda, siddha and homeopathy. In the agriculture sector the plant has proven to be of great importance by its inherent insecticidal nature and therefore it is used to control agriculture pests like red flour beetles and black moths. The farmers additionally utilize the plant concentrate to control ticks in animals[5], and utilize the wood to manufacture window panes, doors and cots.[6] Looking at the immense potential and usage of the plant *C. collinus*, the paper summarizes its capability as a multipurpose tree.

Taxonomy
Classification (**APG III**)
Kingdom- Plantae; Phylum- Tracheophyta; Division- Angiosperms; Class- Eudicots; Sub-class Rosids; Order- Malpighiales; Family- Phyllanthaceae (Euphorbiaceae); Genus- *Cleistanthus* Species- *collinus*

Synonyms
Amanoa collina (Roxb.) Baill., *Andrachne cadishaco* Benth., *Andrachne orbiculata* Roth., *Bridelia collina* (Roxb.) Hook. & Arn., *Clutia collina* Roxb., *Emblica palasis* Buch.-Ham., *Lebidieropsis collina* (Roxb.) Müll. Arg., *Lebidieropsis orbiculata* (Roth) Müll. Arg., *Lebidieropsis orbiculata* var. *collina* (Roxb.) Müll. Arg., *Lebidieropsis orbiculata* var. lambertii Müll. Arg.[7]

Vernacular names
Hindi- Garari, Garrar; Kannada- Badedarige, Bodadaraga, Kadagargar; Malayalam- Nilappala, Odaku, Odugu; Marathi- Garari; Oriya- Korodo, Karada; Sanskrit- Indrayava, Kaudigam, Kutaja, Nandi; Tamil- Nilaippalai, Oduvanthazhai, Odaichi, Odan, Odishi; Telugu- Kadise, Korshe, Korsi, Vadise, Kandishe; West bengal: Karlajuri; Andhra Pradesh : Kadishe, Vadisaaku; Other- Garari, Karra, Oddan thazhai, Odukkan thazha;.[7]

Botanical Description
Small deciduous trees, 2-15 m tall; branchlets pilose when young, soon glabrous. Young branches red, older spreading, rigid, smooth. Leaves simple, alternate, distichous; stipules lateral, linear; Lamina 3-11 cm long, 1.5-8 cm broad, ovate-elliptic to oblanceolate, sub-orbicular, obovate, acute or rounded; apex round, retuse, obtuse, subacute or apiculate; margin entire; texture chartaceous to firmly coriaceous, glabrous, glaucous underneath, regularly glossy; lateral nerves pinnate, thin, prominent, horizontal, 4-8 pairs, intercostae reticulate; petiole 3-10 mm long, puberulose to glabrous, slender; Inflorescences silky villous, up to 6 cm long, borne on unique leafless, or main leafy, short horizontal branches. Flowers unisexual, greenish-yellow, in clusters, male 3-5 flowered, female up to 5 flowers, in the axil of upper leaves; bracts minute, broadly based, subulate, 1.5–3 mm long. Male flower: pedicels 1-2 mm long; sepal lobes 5, occasionally 6, triangular-oblong or lanceolate-elongated, 3-4 mm long, 1-2 mm broad, adpressed fulvous, pilose outside; petals 5, fleshy, pale-yellowish-white, incurved, linear; staminal column ca.1.5 mm long; fibers 1.2 mm long; anthers oblong, 1-1.5 mm long; pistillode ovoid, 0.5 mm long. Female flower: pedicels 0.5-1.5 mm long, grey pillose; calyx-tube ca.1 mm long; sepals triangular-lanceolate, 4-5 mm long, 1-3 mm broad; petals subulate, 2 x 1 mm; disc plate shortly cupular-annular; ovary superior, subglobose, 2-2.5 x 2-3 mm, glabrous; styles thick, 3-4 mm long, almost free or basal column ca.1.5 mm long; Stigma fleshy, lobed, bifid above. Fruits (capsule) subglobose or widely elongated, truncate at apex, shallowly 3-lobed or 3-angled, 18-22 x 17-22 mm, dark brown or black when dry, shining, glabrous, with prominent reticulate venation; seeds 3, globose, black, pedicels 0.5-1.5mm long. Flowering and fruiting from November - April. Chromosomes number: 2n = 22.[7, 8, 9]

Fig: 1 *Cleistanthus collinus* Plant, (Courtesy: Yercaud Elango, CC BY-SA 4.0).[10]

Distribution
Commonly found in dry deciduous forests, scrubs, on rocky soil along the streams up to an altitude of 1400 m. Andhra Pradesh: Districts Anantapur, Chittoor, Kadapa, East Godavari, Guntur, Krishna, Kurnool, Nellore, Prakasam, Srikakulam, Vishakapatnam, Vizianagaram

and West Godavari. Telangana: Nalgonda, Khammam and Mehboobnagar districts. Maharashtra: Sawantwadi, Nagpur, Ettapalli, Arkapalli, Mesa, Pambhurna, Morchandi, Bittergaon, Aurangabad, and Nanded. Karnataka: Ballari, Kolar, Chamarajanagar, Hassan and Dakshina Kannada. Kerala: Wayanad, Malappuram, Palakkad and Thrissur districts. Odisha: Districts Angul, Balasore, Bargarh, Bolangir, Boudh, Cuttack, Deogarh, Dhenkanal, Gajapati, Ganjam, Kalhandi, Kandhamal, Kendrapara, Keonjhar, Khurda, Koraput, Malkangiri, Mayurbhanj, Puri, Rayagada, Sambalpur and Sundergarh. Tamil Nadu: Coimbatore, Dindigul, Dharmapuri, Kanchipuram (Changalpattu-CGP), Karur, Krishnagiri, Madurai, Namakkal, Pudukkottai, Sivaganga, Salem, Tiruchirapalli, Tiruvannamalai, Villipuram and Vellore. In addition to these places, the plant also occurs in states of Madhya Pradesh, West Bengal and dry hills of India from Himachal Pradesh to Bihar. The plant is widely distributed in Malaysia, Sri Lanka, Africa and most of the South-East Asian countries.

Phytochemistry

Preliminary Phytochemical Studies

The preliminary phytochemical studies on different leaf extracts using hot and cold extraction methods have shown affirmative results for alkaloids, glycosides, steroids, phlobatannins, saponins, terpenoids and tannins.

Phytoconstituents

The major category of phytoconstituents in this plant belong to arylnalpthalide lignans which are present in free forms or as glycosides. The sugar moiety of these glycosides are *O*-methylxylose (major) and *O*-methyl derivatives of glucose (minor). Diphyllin is mostly present in leaves and heartwood, while Collinusin and Cleistonone are constituents of aerial parts of the plant (leaves). Many more glycosides of diphyllin having sugar moiety on C-4 have been isolated. The monoglycosides in the above category isolated from leaves and heartwood of the plant include Cleistanthin A having 3,4-di-*O*-methyl-β-D-xylopyranose, sugar moiety; Cleistanthin B with β-D-glucose moiety; 4-*O*-(3-*O*-methyl-β-D-glucopyranosyl)-diphyllin having 3-*O*-methyl-β-D-glucose moiety. Cleistanthin D, also a monoglycoside has 2,3,5-tri-*O*-methyl-D-xylofuranose sugar. The diglycoside Cleistanthin C obtained from the heartwood has 2,3-di-*O*-methyl-β-D-xylopyranosyl [1→4]-β-D-glucopyranose sugar moiety. The 4-*O*-Diphyllin diglycosides are also present in this plant. The plant also shows presence of Cleistanthin E, a triglycoside of 4-*O*-Diphyllin with 2,3,5-tri-*O*-methyl-D-xylofuranosyl-2,3-di-O-methyl- D-xylofuranosyl [1→4]-2,3-di-*O*-methyl-β-D-gluco-pyranose sugar moiety. Taiwanin C, 3,4-dihydrotaiwanin C, Taiwanin E, and 4-*O*-3,4-di-*O*- methyl-β-D-xylopyranosyl taiwanin E are also other arylnaphthalide lignans obtained from heartwood of this plant besides Diphyllin. The Furofuranoid lignans isolated by various workers are (+)-Sesamin (heartwood), Paulownin, Wodeshiol, and 4-hydroxysesamin. Dihydrocubebin isolated from heartwood is the only dibenzylbutane lignan reported, besides ellagic acid.[11]

The other important phytoconstituents present in aqueous, dry and fresh leaf extracts of *C. collinus* are 3-O- methyl-D-glucose (32-41%), Benzenetriol (22-25%), 1,6- Anhydro-β-D- glucopyranose (15-16%), n-Hexadecanoic acid (10-11%), Heptacosane (4-9%), 2-Hydroxy-7methoxy-4, 5-diphenyl-5H-indeno[1,2-d] pyrimidine (6-7%), 1,2-Benzenedicarboxylic acid diisooctyl ester (3-4%), and Eicosane (3-4%).[12]

Recent studies have also shown the presence of other compounds that possess considerable biological activities, which are Undecanoic acid; Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester; Pentanoic acid; α-methyl mannofuranoside; 5-*O*-Methyl-D-gluconic acid dimethylamide; Octadecanoic acid, 9,10-epoxy-18-(trimethylsiloxy)-, methyl ester, cis-; 3,6-methano-8H- 1,5,7-trioxacyclopenta [IJ] cycloprop[A]azulene- 4,8(3H); **(Table-1).** Similarly the other detected compounds with unknown activities include 2,3-anhydro-D-galactosan, pentanoic acid, 2-(aminooxy)-; 4,4-dimethyl-3- (3-methylbut-2-enylidene) octane- 2,7-dione; pseduosarsasapogenin-5,20-dien methyl ether; sulfurous acid, 2-propyl tridecyl ester; 9- octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis-; and dihydroartemisinin, 6-deshydro-5-deshydroxy-3-desox.[13]

Table-1: Major chemical constituents of *C. collinus*, their biological roles and few uses.[11, 12, 13]

Phytoconstituents	Biological Role / Use	Phytoconstituents	Biological Role / Use
Diphyllin ($C_{21}H_{16}O_7$)	V-ATPase blocker, antiviral, antitumor, cytotoxic, anticandidal,	1,1,3-triethoxypropane ($C_9H_{20}O_3$)	Toxic, irritant
4-O-(3-O-methyl-β-D-glucopyranosyl)- diphyllin	antiviral, antitumor, cytotoxic,	2-Methoxy-4-vinylphenol ($C_9H_{10}O_2$)	Aromatic, flavoring, cytotoxic, anti-inflammatory, germination inhibitor, insect repellent,
Collinusin ($C_{21}H_{18}O_6$)	Toxic,	1,2,3-Benzenetriol ($C_6H_6O_3$)	Oxidative, anti-psoriatic,

Cleistanone ($C_{30}H_{48}O_2$)	Cytotoxic,	1, 6- Anhydro-β-D-glucopyranose (Levoglucosan) ($C_6H_{10}O_5$)	Chemical tracer
Cleistanthin A ($C_{28}H_{28}O_{11}$)	Toxic, Apoptotic, antiviral, anticancer, oxidative, diuretic	Cedrene ($C_{15}H_{24}$)	Antiseptic, Antifungal, anti-inflammatory, diuretic
Cleistanthin B ($C_{27}H_{26}O_{12}$)	Toxic, apoptotic, antiviral, anticancer, oxidative, diuretic	2-(3-Isopropyl-4-methyl-pent-3-en-1-ynyl)-2-methylpropyl ester ($C_{14}H_{20}O$)	-----
Cleistanthin C ($C_{34}H_{38}O_{16}$)	Toxic, hypotensive	2-methyl-Heptadecane ($C_{18}H_{38}$)	Pheromone of tiger moth, petroleum industry use
Cleistanthin D ($C_{29}H_{30}O_{11}$)	Toxic	n-Hexadecanoic acid ($C_{16}H_{32}O_2$)	Anti-inflammatory
Cleistanthin E ($C_{42}H_{52}O_{20}$)	Toxic	3-O-methyl-d-glucose ($C_7H_{14}O_6$)	Metabolic marker
Cleistanthoside A ($C_{34}H_{38}O_{16}$)	Toxic, genotoxic, anticancer	Oleic acid ($C_{18}H_{34}O_2$)	Anticholesteremic, anti-inflammatory
Cleistanthoside B	Toxic	Octadecanoic acid ($C_{18}H_{36}O_2$)	Surfactant, soaps, cosmetics, detergents, lubricant, softening agent
3,4-dihydrotaiwanin C	Cytotoxic	1, 2-Benzenedicarboxylic acid ($C_8H_6O_4$)	Endocrine disruptor, Ingredient of polyesters, resins, chemical industry
Taiwanin E ($C_{20}H_{12}O_7$)	Apoptotic, anticancer	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl ester) ($C_{16}H_{22}O_4$)	Cytotoxic, endocrine disruptor,
4-O-3,4-di-O methyl-b-D-xylopyranosyl taiwanin E	Cytotoxic	1,2- Benzenedicarboxylic acid, diisooctyl ester ($C_{24}H_{38}O_4$)	Cytotoxic, fungitoxic
(+)-sesamin ($C_{20}H_{18}O_6$)	Fat reducing, antioxidant, anti-inflammatory	Eicosane ($C_{20}H_{42}$)	Anti-inflammatory, analgesic, antipyretic, petrochemical industry, paraffin waxes
Paulownin ($C_{20}H_{18}O_7$)	Slightly toxic, anticancer, anti-inflammatory, analgesic, hypoglycemic	Heptacosane ($C_{27}H_{56}$)	Antimicrobial
Wodeshiol ($C_{20}H_{18}O_8$)	Cytotoxic, anticancer	Leucodelphinidin ($C_{15}H_{14}O_8$)	Hypoglycemic
Dihydrocubebin ($C_{20}H_{22}O_6$)	Inhibits histamine release	Lupeol ($C_{30}H_{50}O$)	Anti-inflammatory, antioxidant, antidiabetic, anticancer
Ellagic acid ($C_{14}H_6O_8$)	Antibacterial, anticancer, antiviral, antioxidant, anti- inflammatory	β-sitosterol ($C_{29}H_{50}O$)	Anticholesteremic, antioxidant, anticancer
A- methyl mannofuranoside ($C_7H_{14}O_6$)	Antibacterial, antifungal	Undecanoic acid ($C_{11}H_{22}O_2$)	Cytotoxic, anticancer, antimicrobial
5-O-Methyl-D-gluconic acid dimethylamide ($C_9H_{19}O_6N$)	Antimicrobial, antioxidant	Pentanoic acid ($C_5H_{11}O_3N$)	Antioxidant, lubricant, adhesive agent
Cyclopropanatettradecanoid acid, 2-octyl-, methyl ester ($C_{26}H_{50}O_2$)	Antimicrobial	Octadecanoic acid, 9,10-Epoxy-18- (trimethylsiloxy)-, methyl ester, cis ($C_{22}H_{44}O_4S$)	Insecticidal, anticandidal, antifungal

3,6-Methano- 8H-1,5,7-trioxacyclopent a[IJ]cycloprop[A]azulene- 4,8(3H) ($C_{15}H_{18}O_6$)	Cytotoxic	4,4-Dimethyl- 3-(3-methylbut-2-enylidene)octa ne-2,7-dione ($C_{15}H_{24}O_2$)	-----

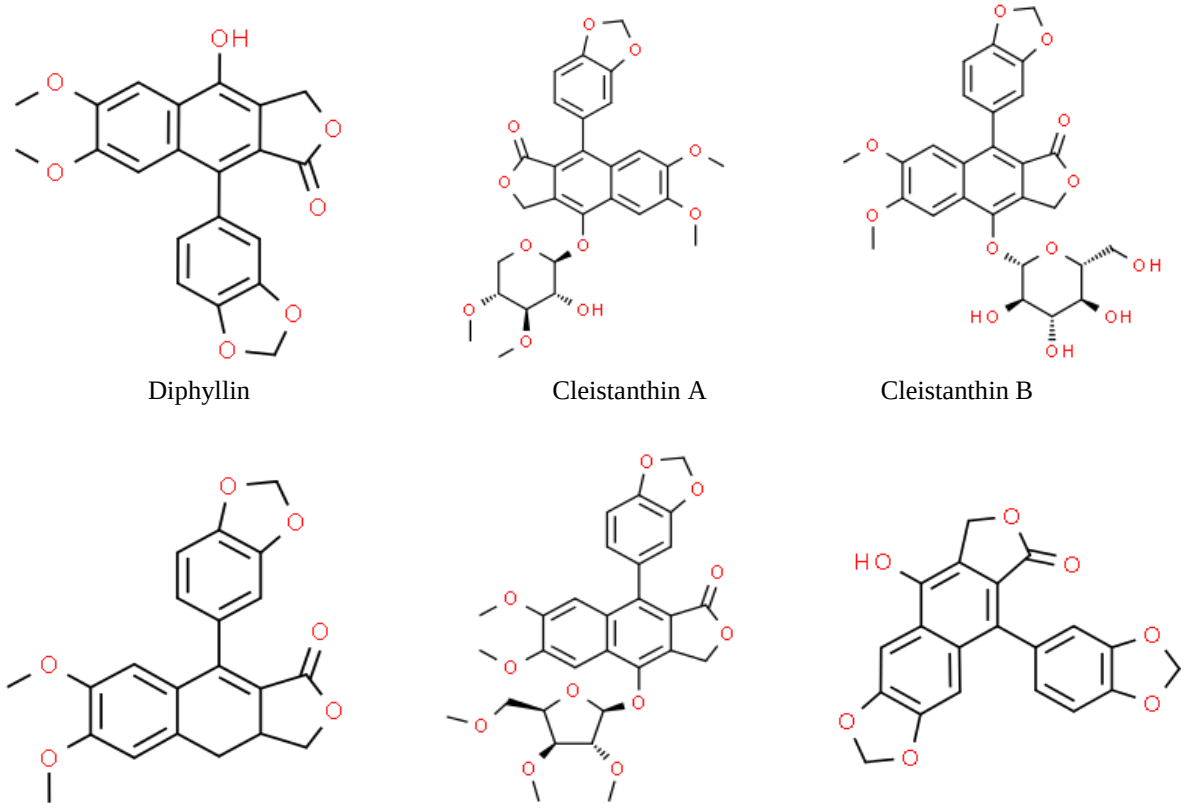

Fig 2. Chemical structures of major phytoconstituents. Courtesy: ChemSpider[14, 15, 16, 17, 18, 19]

Poisoning in Humans

The occurrence of poisoning cases in India is high and it is assessed that more than 50,000 individuals die due of the toxic exposure of various kinds. There are few plants like *Cerbera odollum*, *Cleistanthus collinus*, *Strychnos nux-vomica*, *Abrus precatorius*, *Colchicum autumnale* and *Datura metel* that are extremely poisonous and utilized for suicidal and homicidal purposes. In India 19% of suicidal deaths were due to consumption of plant poison as reported by Bose.*et al.* The harming due to this plant is mostly restricted toward the southern Indian provinces of Tamil Nadu and Pondicherry. Between 1926 and 1985, more than 1000 instances of *C. collinus* poisoning were reported from different parts of Tamil Nadu.[20] Mysteriously, regardless of the plant being distributed in different districts of India, no reports about harming are accessible from other parts of India, however there was notice of cases from pre-independence Bengal.[20] The plant poisoning seems to be an issue of the country populace, mostly used by women for deliberate self-harm. A review on acute poisoning in village residents showed that more than 87% of women took plant poisons of which 44.5% of whom devoured *C. collinus*.[21]

Mode of consumption

All the parts of the plant (leaves, twigs, fruits, bark, roots) are equally poisonous to humans as well as animals but leaves are commonly consumed as poison. Leaves are consumed as freshly crushed pulp, filtered juice of freshly crushed leaves or as boiled decoction. The boiled extract of leaves is more poisonous than freshly ground leaves according to the studies. Higher death rates were found in patients who had ingested a bigger number of leaves (>60) and boiled leaf extract. The glycosides and arylnaphthalene lignan lactones are toxic ingredients responsible for poisoning. The lignan lactones including Cleistanthin A, Cleistanthin B, Diphyllin and Collinusin are collectively called as Oduvin.

Common symptoms of poisoning

The common external symptoms of poisoning include vomiting, epigastric pain, breathlessness, dyspnoea, tachypnoea, visual disturbances, giddiness, headache, drowsiness, fever, tachycardia and hypotension. Many survivors are without symptoms or mild symptomatic with abdominal pain and giddiness.

Clinical Parameters

The following symptoms and clinical parameters may or may not be present or be severe depending on the toxicity profile which in turn depends on susceptibility of the patient, part of plant consumed, way of consumption, growth stage of plant, amount of toxin consumed and manner of consumption. In the cases studied, the respiratory rate was high (>30/min), fine bilateral crepitations were heard in few, sometimes bilateral peripheral infiltrates were seen in chest X-ray, lower PaO_2/FiO_2 (P/F ratio 135-155), indicated non cardiogenic pulmonary oedema caused by acute respiratory distress syndrome (ARDS).

The heart parameters (Fig. 3 & 4) include high pulse rate (128/min) which is seen in most cases, hypotension (SBP <90 mmHg), tachycardia (after 40-60 hours), low central venous pressure (CVP), ST depression, prolonged QTc, inverted T-waves, leading to distributive shock. Arterial blood gasses (ABG) may show slightly lower partial pressure of oxygen (PaO_2), lower partial pressure of Carbon dioxide ($PaCO_2$), and patients with respiratory failure may show widened alveolar-arterial O_2 gradient/ difference ($AaDO_2$ gap) and hypoxia. This might suggest a cardiotoxic effect which causes myocardial injury leading to intraventricular conduction defects.[22,23]

The blood biochemistry may reveal hypokalemia (K <3.1 mEq/L), hyponatremia (Na <130 mEq/L), and WBC with predominant neutrophils, slightly lower levels of blood pH (7.28-7.35), HCO_3 (10.6-21.7 mEq/L), and elevated levels of chloride (104-114 mEq/L), SAP, ALT, AST, CPK, CPK-MB, LDH, total bilirubin (hyperbilirubinemia) and blood urea. A decreasing trend in the values of calcium (6.2-8.0 mg/dL) and magnesium (0.6-1.5 mg/dL) is also seen which results in QTc prolongation on ECG.[23, 24]

The increased levels are also seen in urine gap, urinary excretion of potassium and changes in urine osmolality in addition to oliguria which is due to toxin induced inappropriate vasodilation leading to distal tubular renal acidosis and shock. In addition to this, global tubular dysfunction and proximal tubular injury may also occur.[24, 25] Some cases show diarrhea, polyurea, and also fractional excretion of bicarbonate on bicarbonate supplementation. In critical cases (Fig. 4) neuromuscular weakness, cardiac arrhythmias, acute kidney injury (AKI), acute respiratory failure requiring mechanical ventilation (AcRFMv), respiratory and renal failure are witnessed.[24, 25]

Mortality rate with *C. collinus* is around 40% and occurrence of deaths were mostly seen on third day in most cases or within 7 days of poison ingestion. Sudden cardiac arrest was the usual mode of death seen followed by respiratory failure, cardiac arrest, cardiac arrhythmia, exclusive renal failure, ARDS, multiple organ failure and refractory hypotension.[25]

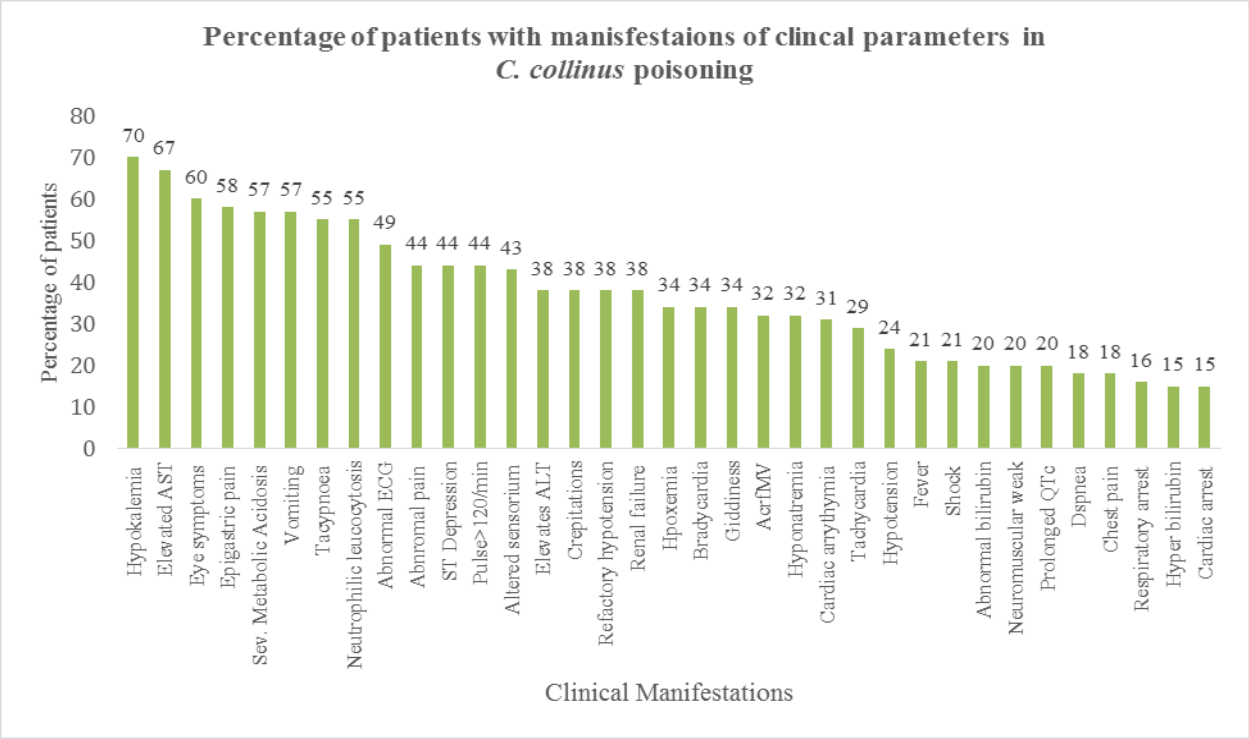

Fig 3. Percentage of patients showing clinical manifestations due to *C. collinus* poisoning.

Treatment

Continuous monitoring of breathing, circulation, ECG with rhythm strip, cardiac parameters and close monitoring and estimation of serum potassium, sodium and creatinine is necessary. The focus should be correction of metabolic acidosis which needs to be done aggressively with fluid resuscitation with soda bicarbonate IV followed by Shohl's solution and potassium citrate, Correction of hypokalemia with intravenous KCl and potassium citrate for normalization of ECG. At later stages oral supplementation of sodium bicarbonate and potassium could be given. The poisoning management can be done using N-acetylcysteine between 50-100 mg/Kg for 24 hours, also melatonin and thiol containing compounds to help restore glutathione depletion. Usage of adequate fluids for compensation of urinary loses and blood pressure is necessary.[24, 25] Depending on the type of poison, activated charcoal can be advised in single or multi dose with potassium and magnesium supplementation. Some studies also show usage of therapeutic strategies to bypass the inhibition of vacuolar ATPase in renal brush border membrane (BBM). Management of ARDS can be done by PEEP and prone ventilation. Arrhythmias which occur at high levels of toxin in blood are offset by these treatments. In rare case a patient showed neuromuscular weakness, dysphagia and myasthenic like syndrome with ptosis which was treated by neostigmine. In one treatment, multidose activated charcoal (MDAC) was used with 30 patients where initially gastric lavage was given followed by activated charcoal with 1 gm/Kg dose and continued with MDAC for 48 hours with doses at an interval of every 6 hours. The results showed decrease in hypokalemia, hypomagnesemia, arrhythmias, neuromuscular weakness, ARDS, and most importantly there was reduction in mortality of poisoned patients.[26]

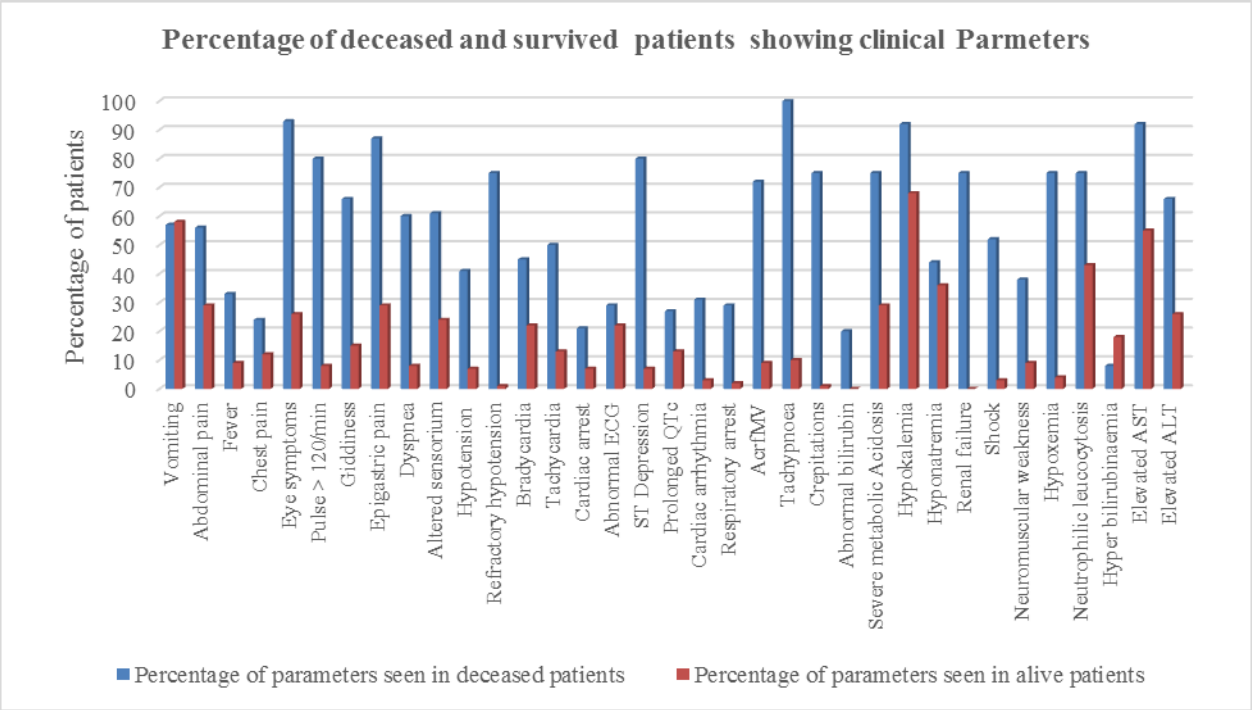

Fig 4. Percentage of dead and alive patients showing clinical parameters due to *C. collinus* poisoning.

Mechanism of toxicity

Inhibition of ATPase activity- The symptoms like hypokalemia, diuresis and kaliuresis in patients can be attributed to cardiac glycoside property of inhibition of ATPase activity specially Na^{+}-K^{+} ATPase, magnesium ATPase, and vacuolar ATPases.

Neuromuscular blockade- According to the studies done by Nandkumar *et.al*,1996 on mice showed the probable reason for symptoms such as respiratory failure, muscle weakness and cramps. There was reduction in response of compound nerve action potential, compound muscle action potential (97%), isometric tension (38%), amplitude of miniature end plate potential and shortening of fall out time leading to irreversible effects due to changes at the muscle nerve junction.

Inhibition of LDH isoenzymes- The LDH activity was considerably reduced in various tissues with maximum reduction in kidneys of test animals (rabbits) when they were treated with aqueous leaf extract intravenously during experimental study.[27]

Depletion of Glutathione- The manifestation of toxicity is also due to reduction in enzymes containing thiol group as reported by Sarthachandra *et al*.,1997. This finding could be useful for selection of thiol compounds as probable antidotes to fight toxicity.[28]

Neutrophilic granulocytosis- Tests animals like mongrel cats, rhesus monkeys, Swiss mice and albino rats showed neutrophilic granulocytosis. It was

dependent on species, dose and route of administration of cleistanthins as reported by Rao *et al*.

Respiratory paralysis- In one study on rat kidney the inhibition of proton pumps in renal BBM was noted but the Na^+-K^+ ATPase activity was not affected. In another study the inhibition of vacuolar H^+ ATPase activity in BBM was responsible for development of dRTA and subsequent renal failure leading to respiratory arrest.[29] The study done on rodents, showed 100% mortality when they were treated with aqueous extract of *C. collinus* intraperitoneally, with noted manifestations of low blood potassium, basic urine and respiratory acidosis, which was due to hindered ATPase movement in rodent kidney, causing type I dRTA and respiratory arrest.[29]

Hypotension- A dose dependent hypotensive effect was seen in Wistar rats with sudden respiratory depression without affecting electrocardiogram at a dose of cleistanthins A & B of 64 µg.[30] In the electrocardiogram, cleistanthins A and B notably altered the electric pastime of the heart, the modifications had been brief and of no similar consequence [30]. In another study done by Kumar *et.al* in guinea pigs the crude extract of plant leaves blocked phenylephrine induced contraction of aorta. Both these effects were attributed to blocking alpha- adrenergic receptors by cleistanthins.

Alpha-adrenergic receptor blockade- Cleistanthins A & B have shown to block both alpha 1- and 2-adrenoreceptors which was seen by inhibited platelets aggregation with a degree comparable to that of yohimbine, and the compounds also blocked phenylephrine from relaxing the rat jejunum.[31] As platelets have a population of alpha 2- adrenoreceptors, a role similar to thrombin receptors and ADP is played by platelets in its aggregation, and therefore defect in platelet function can be attributed to defect in alpha 2-adrenoreceptors, clinically manifesting as hemostatic abnormalities, and change in affinity and number of these receptors in platelet membrane. The effect of many alpha 2-adrenoreceptors antagonists on platelet aggregation have been studied under the threshold concentration of collagen with epinephrin as an agonist.[32,33]

Pharmacological studies

Antibacterial activity: The studies have shown that methanol extract of plant leaves is most effective on many strains of bacteria like *Escherichia coli, Klebsiella pneumoniae, Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus*, with zone of inhibition (ZOI) ranging from 18-30 mm. The anti- bacterial activity of 1 mg of concentration of plant extract, minimum inhibitory concentration (MIC) in mg/mL and minimum bactericidal concentration (MBC) for hot methanol extract (HME) of plant material is shown in **table 2**.[34] In other studies the hot water extract (HWE) was most effective for *Salmonella paratyphi* (11.8 mm ZOI), and for *Vibrio cholerae* (11.5mm ZOI) the methanol extract was most effective. The mild temperature acetone extract (AE) was effective against Methicillin Resistant *Staphylococcus aureus* (MRSA) and *Enterococcus* species with 13.0 mm and 10.0 mm ZOI respectively.

The green synthesized silver nanoparticles (SNPs) using *C. collinus* extract also exhibited significant anti-bacterial activity with ZOIs in *Bacillus subtilis* (16.0 mm), *Shigella sonnei* (13.5 mm), *Staphylococcus aureus* (21.0 mm) and *S. dysentriae* (21.5 mm).[35]

Table-2: Antibacterial activity of *C. collinus*. (Modified from Elangomathavan *et al*, 2013).[34]

Pathogens	Zone of Inhibition in (mm)		Minimum Inhibitory Concentration (mg/mL)	Minimum Bactericidal Concentration (mg/mL)
	Streptomycin (20µg)	HME (1mg)		
Escherichia coli	35.0	26.0	0.40	0.45
Klebsiella pneumoniae	35.0	18.0	0.42	0.50
Listeria monocytogenes,	37.0	18.0	0.32	0.40
Pseudomonas aeruginosa	33.0	22.0	0.36	0.45
Salmonella typhi	11.0	30.0	0.27	0.35
Staphylococcus aureus,	32.0	22.0	0.42	0.50
Salmonella paratyphi	21.6	(HWE 100mg/ml)11.8	-	-
Vibrio cholerae	22.0	(HWE	-	-

		100mg/ml)10.8		
MRSA	25.0	13.0	-	-
Enterococcus sps	20.0	10.0		

Larvicidal activity

The vector mosquito suppression becomes very important to control the spread of diseases like dengue, malaria, chikungunya, filarial fever and many more. In a study done on adult emergence inhibition activity by *C. collinus* aqueous leaf extract on mosquito species, caused death at larval-pupal molt stage and pupal- adult eclosion stage. The adult emergence, larval and pupal mortality percentage at 1000 mg/L extract per mosquito species and control were- *Anopheles stephensi* (4.8, 78.8, 16.4 and 89.4, 9.2, 1.4), *Aedes aegypti* (10.4, 73.4, 16.2 and 90.6, 8.2, 1.2), and *Culex quinquefasciatus* (31.0, 56.4, 12.6 and 93.8, 4.4, 1.8). It was also seen that there was increase in larval, pupal and total developmental periods (days) in comparison to control with *A. stephensi* (10, 3, 13 and 8, 2, 10), *A. aegypti* (9, 3, 12 and 8, 2, 10), *C. quinquefasciatus* (10, 3, 13 and 8, 2, 10). The effect on these species of mosquitoes was dose dependent, also showing metamorphic abnormalities like inability to moult to the next stage, inability of adults to completely shed its exuvia, and to fly above normal level.[36]

The larvicidal activity on *A. aegypti* using green synthesized SNPs from *C. collinus* extract was shown to be very effective with 100% mortality rate after 24 hours of treatment at even 1% of NP concentration. As the concentration of NPs increased from 1-5% the time in hours for mortality of larvae also decreased. The death of larvae is generally due to easy permeating properties of SNPs inside the cell, and binding specifically with DNA or sulfur containing proteins and other molecules causing structural changes in DNA, enzymes and organelles (Rai *et al*, 2009, Benelli, 2016).

Anti-cancer activity

The active constituents Clesitanthin A & B are potent against many cancer cell lines causing inhibition of DNA synthesis, DNA damage, chromatid exchange, chromosomal aberrations, hampering of cell growth and proliferation, interfering with transcription and replication leading finally to apoptosis.

The first report of the plant extract (alcoholic) activity was shown against carcinoma of nasopharynx human epidermoid which was done by Bhakuni *et. al* who also indicated that tannin fractions of extract contain the anti-cancer agents, and just 28 hours of incubation led to the cytotoxicity level of 10 µg/L. Dioctyl phthalate derivatives which possess anti-cancer activity were isolated in acetone extract during one GC-MS Study. In comparison to 5 anticancer drugs, Cleistanthin A was found to be more effective against cervical carcinoma cell line (SiHa), and oral carcinoma cell line KB. Breaks in DNA strand and resultant apoptosis was seen in Chinese hamster ovary cells and p53 deficient cell line on longer exposure and high concentrations of Cleistanthin A.[37] Cleistanthin B was also effective against cervical carcinoma cell line (SiHa), whereas T-cell leukemia (molt-4) was least sensitive to it. The cytotoxicity effects were also confirmed on mouse normal cell lines. Rajkumar S *et. al* observed reduction in transcription and replication in K-562 tumor cell lines due to Cleistanthin A & B. Cytotoxicity against MT-2 cell lines were seen due to Cleistanone and its acetyl derivative (Ramesh *et.al,* 2003). The tumor size drastically decreased and the life span increased in mice affected with Dalton's ascites lymphoma and sarcoma when treated with Cleistanthin A. Significant anti-proliferative effects of aqueous and ethyl acetate extracts of *C. collinus* were observed in normal cell line.[38] The ethyl acetate extract containing diphyllin showed 23-59% anti-proliferative activity against 3T3- L1 preadipocytes cell proliferation with 180 mg/mL concentration necessary to inhibit cell growth at 50%.

Antifungal activity

In humans and veterinary animals, the major fungal infection is candidiasis caused by *Candida* species. Diphyllin extracted from leaf extract of the plant showed considerable anticandidal activity against *Candida albicans*, *C. glabrata* and *C. tropicalis* at 200 µg concentration was 11.0 mm, 3.5 mm and 9.5 mm diameter of ZOI respectively. Similarly the minimal fungicidal concentration (MFC) for *C. albicans*, *C. glabrata* and *C. tropicalis* was 85 µg/mL, 145 µg/mL and 110 µg/mL respectively. Diphyllin was twice more active than miconazole against *C. glabrata*. In another study the ZOI for *C. non albicans* was 9 mm for acetone extract.[39]

Anti-HIV activity

The most promising naturally derived anti-HIV compounds are flavonoids, terpenoids, alkaloids, proteins and some polysaccharides. The effect of crude aqueous extract of *C. collinus* on HIV-1 replication in-vitro was tested by viral core protein p24 expression. It was seen that the extract inhibited the p24 antigen expression, also showing increased inhibition with increasing concentration of extract, with more than 50% and 72% inhibition at 20 and 40 mg/mL of extract concentration respectively.[40] Thus the crude aqueous extract prepared from the fresh leaves of *Cleistanthus collinus* can serve as a potent fluid providing potential lead compounds for the discovery of drugs that possess anti-HIV activity.

Use in agriculture

Acaricide

Studies show that 6% alcoholic and 8% aqueous extract of *C. collinus* was very effective against ticks leading to more than 97% and 98% mortality in 7 and 5 days respectively. The aqueous extract is easy to prepare (80gm leaf powder/L), environmentally safe, socially acceptable and has potential that is equal to chemicals (Amitraz) acaricides. The LC50/LD50 of 6% at 5 hours or 7% at 1 hour using aqueous extract, and 4 % at 2 hours using alcoholic extract could be used against tick *Boophilus microplus.*[41]

Insecticide

The activity potential of *C. collinus* against insects, beetles, larva, aphides and other pests is same as synthetic insecticides. The plant has potential to control pests in cereal crops like ragi, jowar, bajra, wheat, rice and others and is used in Madhya Pradesh and Chhattisgarh. Studies done by Ahirawar, 2011 revealed that fresh leaf juice, decoction and alcoholic extracts were very effective as natural insecticide in killing all the insects in rice field in addition to common paddy pest rice caseworm *Nymphula depunctalis* of Chhattisgarh region of India. Leaf decoction (50%) was found to be most effective producing effect within 12 hours. The extract did not damage the rice plants but instead promoted growth and controlled insects till harvest. The rice grains were healthy and yield was higher in crops treated with extracts compared to control and benzene hexachloride treated ones. Thus Garari promotes organic farming by being less toxic then chemical pesticides and also being potent against many pests. The alcoholic extract was not so effective, and adversely affected growth, producing brown stains on leaves and sometimes causing death of plants.[42] The methanolic extract of *C. collinus* at 5 mg/mL caused 90% mortality of red flour beetle *Tribolium castaneum* in between 8-10 hours with LD_{50} of 3 mg/mL. Similarly the repellency test using 5 mg/mL of extract showed 90% repellency in 9-10 hours. There was increase in percent mortality and repellency with increase in dose from 1mg/mL to 5 mg/mL. This beetle generally originates from infested grain or from dry stored cereal products such as flour, cornmeal.[43] Another insect, the African cotton leafworm *Sporodora littoralis* whose larvae feed on more than 85 plant species including plants of economic importance like tomato, sweet potato, capsicum, wheat and many grasses, was also affected by the plant extract. The antifeedant activity experiments carried out on 3rd instar larvae (10-12 mg) of *S. littoralis* showed % AI (antifeedant activity index) which was calculated according to the formula given by Han *et al*., 2006. The AI was 0.328 and 0.244 for concentration of 2 mg/mL and 4 mg/mL of methanolic extract in 4-6 and 6-8 hours respectively.

The most difficult pest to control the diamondback moth *Plutella xylostella* which has developed resistance to almost every insecticide worldwide is an important pest of cruciferous crops. The study done to see the larval and pupal mortality of this pest showed leaf powder hot water extract (HWE) to be more fruitful than just simple water extract (WE). Maximum larval mortality of 80% was seen in 11% HWE, and respective pupal mortality & malformation ranged from 20-35%, but was 55% for WE.[44] The plant extract was also potent against aphides like brown plant hopper (*Nilapurvata lugens*) thus helping in crop management and protection.

Rodenticide

The other effect of aqueous extract of *C. collinus* showed rodenticide potential which disclosed the values of LC50/LD50 for controlling mouse and rats as 12.5mg/Kg and 10.5mg/Kg respectively. Similarly for rabbit the control values were 13.5mg/Kg.

Traditional uses

The tribal (santhal, lodha) and non-tribal villagers in south-west Bengal use this plant as goat fodder (stall feed) since very long time. The goats also browse and eat the young leaves of the plant from the forest. Although studies have shown that Cleistanthin A and B act as anti-neoplastic agents against mice, monkeys, rats and cats, but no toxicity was reported for goat. This might be due to presence of some enzymes that detoxify the toxic content of the plant in goat's digestive tract. The villagers in the south-west Bengal region also use the plant for thatching roof of huts, and as fencing in the home land and farm land.[45]

C. collinus along with onion and lime juice in 5:3:2 proportion is applied on scalp to grow new hairs and to prevent hair loss and white hairs.[46] The plant wood forms important construction material for cattle sheds, houses and temporary settlements and is used to make cots, doors and windows by Malayali tribals in Yercaud hills of south-eastern ghats of Salem district of Tamil Nadu.[47] The plant is used against amenorrhea, diarrhea and as an antiseptic. The extract of leaves is used as fish poison. Diphyllin and methyl ether of diphyllin are the active constituents that have piscicidal activity (Kawazu *et al*).

CONCLUSION

The toxic plant *Cleistanthus collinus* which is widely distributed is consumed mostly by rural female folks in parts of southern India with suicidal intention. The plant has an interesting and considerable source of biologically active secondary phytochemical constituents, the major falling into the category of arylnaphthalide lignans and their glycosides, beside furfuranoid lignans and pimarane diterpenes.

The patients after consuming plant parts commonly present abdominal pain and vomiting as usual features. Hypokalemia and metabolic acidosis develops gradually persisting till end. The poisoning can be life threatening which is seen through manifestations like renal tubular injury, electrolyte, acid-base, cardiac and respiratory

disturbances. The treatment given should be supportive and symptomatic as specific antidote of poison is absent. The routine testing should include estimation of serum potassium, sodium and ABG analysis. The manner in which leaves are consumed (fresh or boiled extract) has predictive effect. The animal studies have helped to understand the mechanisms of actions like inhibition of ATPase, LDH isoenzymes, and depletion of glutathione. The understanding of pathophysiology in *C. collinus* poisoning will lead to prognostic and therapeutic implications.

In addition to several pharmaceutical activities and benefits in the field of medicine, the plant compounds Cleistanthin A and B and other arylnapthalide lignan glycosides that exhibit anticancer activity against many tumor cell lines seems very beneficial. The anticancer properties disclosed by several animal studies of the plant toxins will need support of human studies, which if shown to be true will be of massive advantage in the management of cancer patients. The treatment of particular types of cancer could be done by understanding the mode of action of the anticancer compounds from the plant. The potent anti-HIV and anticancer activity of the plant can show way for potential lead compounds that could be obtained from this plant or derived from its active constituents. The toxic property of the plant can become medicinal property if the extracts are used in right dosages, thus composing new efficient green medicines for treatment of infectious diseases in humans as well as veterinary animals.

In the field of agriculture also the plant can be utilized in controlling pests on a good number of crops. The plant *C. collinus* as herbal pesticide will be economical, safe for non-target organisms, environment friendly, and will decrease insecticide resistance. It seems that the fresh plant leaves are not toxic to animals, humans and insects, but it is the degradation product which might form due to sunlight heating the atmosphere and the heat produced in animal or plant degradative system that converts plant extract to concentrated decoction which then gains pesticidal property.

This indigenous plant should be carefully thought and cultivated in agroforestry system, which will lead to decrease in cost, and will also address social and ecological issues. Thus, the plant has tremendous scope and potential that can be sustainably exploited in the field of medicine, agriculture and forestry.

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