Introduction
1. Malaria Epidemic

Malaria is the most threatening disease all over the world after the aids and tuberculosis because of its spread and ubiquitousness. Infectious diseases are most prevalent and fatal next only to the cardiovascular diseases. Out of the most dangerous six infectious diseases malaria rank fifth in number producing more than 12% death. Globally, every year, out of 300 million malarial patients 1.5 to 2.7 million die and majority of the death is amongst children (Kain and Keystone, 1998). The most endemic part of the world for malaria is sub-Saharan Africa. The infected pregnant woman may die or experience miscarriage or deliver premature infant due to malaria. The acute malaria with high fever (discontinuous or continuous) and shivering result in to convulsions of limbs while the chronic form of malaria leads to cerebral malaria leading to coma and ultimately to death within 24 hours (Anonymous, 1999). *Plasmodium falciparum* caused 40%, *Plasmodium vivax* caused 50%, *Plasmodium malariae* caused 7-8% and *Plasmodium ovale* caused 2-3% of malaria infection all over the world (Anonymous, 2000(a)). In India 60 to 65% of the infections are due to *P. vivax* and 35 to 40% due to *P. falciparum*. Only few cases of *P. malariae* have been reported from Orissa and Karnataka. As per National Malaria Eradication Program (NMEP) survey report, the "National Average" of *P. falciparum* malaria has increased to 35.5% from a meager 9.34% in 1972. The state wise malarial epidemic is presented in Figure 1 in which Gujarat has 5% malaria. Figure 2 shows the percentage of *P. falciparum* (P.f.) among the states out of them Gujarat is having 2% P.f. infections (Shiv et al., 2000). Figure 3 & 4 show the total cases of malaria and deaths due to P.f. in India from 1947 to 2005. There were 75 million malaria cases and 0.8 million deaths in 1947 (Figure 3). It was gradually brought down to 0.1 million cases annually without any deaths by 1965 (Figure 3) (Anonymous, 2003). But gradually cases...
had again increased with increased death and from year 2000 it has not significantly decreased (Figure 4) (Anonymous, 2007(b)).

The work days lost due to malaria cause high impact on the economic setup of the country. In addition to the working days the cost of free treatment of malaria, preventive steps like insecticide, impregnated bed-nets and awareness programmes increase the additional burden on the country. None of the countries can achieve its desirable economic status with such additional burden of expenses. It increases burden on the poor families for the treatment of repeated bouts of malaria either by relapse or re-infection.

Still full proof protection against malaria has not been achieved. The vaccination trials by the collaborative efforts of the University of Oxford, Walter Reed Army Institute of Research (US), Glaxo-SmithKline Biologicals and regional Medical Research Councils (across Africa and Europe) resulted in only 30% protection against P. falciparum infection and still are not good enough for mass vaccination programmes. WHO has documented 94 malaria vaccines in 2005 comprising of proteins, out of these, 33 had reached up to clinical trials. This indicates that malaria vaccines are still not efficacious enough to protect the mankind, in spite of enormous labor, efforts and investment of millions of dollars. As preventive, now WHO recommends reverting to DDT even if it is harmful for population and environment both, because it is the best preventive although not protective but fundamentally the only solution available. DDT was most popular in the early 1950s as an insecticide to control malaria transmission but was then banned due to its many harmful consequences like derangement of central nervous system and various vital organs like liver and kidney. In addition to that spraying of DDT results in human sterility. In the absence of effective vaccines, drug is the best way to prevent disease and treat patients with malaria.
Introduction

Figure 1: State-wise distribution of malaria cases in India (Shiv et al., 2000).

![Pie chart showing state-wise distribution of malaria cases in India.]

- West Bengal: 6%
- Others: 10%
- Maharashtra: 8%
- Madhya Pradesh: 21%
- N.E. States: 9%
- Rajasthan: 4%
- Gujarat: 5%
- Orissa: 22%
- Andhra Pradesh: 5%
- Uttar Pradesh: 5%
- Bihar: 5%
- N.E. States: 9%
- Gujarat: 2%
- Madhya Pradesh: 23%
- West Bengal: 3%
- Andhra Pradesh: 6%
- Karnataka: 3%

Figure 2: State-wise distribution of *P. falciparum* cases in India (Shiv et al., 2000).

![Pie chart showing state-wise distribution of *P. falciparum* cases in India.]

- Others: 3%
- Bihar: 5%
- Maharashtra: 5%
- Orissa: 41%
- N.E. States: 9%
- Gujarat: 2%
- Madhya Pradesh: 23%
- West Bengal: 3%
- Andhra Pradesh: 6%
- Karnataka: 3%
Figure 3: Annual malarial death rate in India (Anonymous, 2003).

Figure 4: Total Malaria cases and deaths reported from India (Anonymous, 2007(a)).
2. Life Cycle of Malaria Parasite

The malaria parasite life cycle involves two hosts (Figure 5). During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host. Sporozoites infect liver cells and mature into schizonts, which rupture and release merozoites. (Of note, in *P. vivax* and *P. ovale* a dormant stage [hypnozoites] can persist in the liver and cause relapses by invading the bloodstream weeks, or even years later.) After this initial replication in the liver (exo-erythrocytic schizogony), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony). Merozoites infect red blood cells. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites. Some parasites differentiate into sexual erythrocytic stages (gametocytes). Blood stage parasites are responsible for the clinical manifestations of the disease.

The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal. The parasites' multiplication in the mosquito is known as the sporogonic cycle. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes. The zygotes in turn become motile and elongated (ookinetes) which invade the midgut wall of the mosquito where they develop into oocysts. The oocysts grow, rupture, and release sporozoites, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle.
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Trophozoid

P.v.

Erythrocytic Schizogony in man

P.V. P*

Ring Form in RBCs

Penetrat in RBCs

Merozoites

Exo-erythrocytic Schizont P.f.

Merozoites Released

Microgametocyte

Megametocyte

Anopheles mosquitoes

Exflagellating Microgametocyte

Macrogametocyte P.v.

Sporozoites Oocyst of Stomach Wall

P.f. Containing Sporozoits

Figure 5
3. Available chemotherapy, side effects and resistance

The available anti-malarial drugs in the market with their possible side effects are listed below (Anonymous, 2007(b)). Threats of the drugs used can be easily reflected from these data. They may cause permanent or temporary adverse effects and many of them are not advisable during pregnancy.

1. Chloroquine:
   - Acute side effects: Nausea, vomiting, stomach upset, cramps, loss of appetite, diarrhoea, tiredness, weakness or headache.
   - Chronic side effects: Blurred vision, trouble seeing at night or problems focusing clearly, ringing in the ears, impaired hearing, seizures, mood changes, fainting, irregular heartbeat, persistent sore throat or fever, easy bleeding or bruising. Allergic reactions include: rash, itching, swelling, dizziness and trouble breathing.

2. Halofantrine:
   - Acute side effects: Diarrhoea, nausea, vomiting, stomach pain, loss of appetite, dizziness and headache.
   - Chronic side effects: Cough, muscle pain, tremors, chest pain, mental/mood changes, convulsions, irregular heartbeat, fainting. Allergic reactions include: rash, itching, swelling, severe dizziness, trouble breathing.

3. Hydroxychloroquine:
   - Acute side effects: Nausea, vomiting, stomach upset, cramps, loss of appetite, diarrhoea, tiredness, weakness or headache.
   - Chronic side effects: Vision changes (such as blurred vision, trouble seeing at night or problems focusing clearly), ringing in the ears, impaired hearing. Allergic reactions include: rash, itching, swelling, dizziness, breathing trouble.

4. Mefloquine:
   - Acute side effects: Anxiety, mood change, nausea, vomiting, diarrhoea, loss of appetite, muscle aches, weakness, ringing in the ears, a rash or itching, blurred vision, insomnia, abnormal dreams, headache, dizziness or drowsiness.
   - Chronic side effects: Serious mental side effects: anxieties, depression, restlessness, feelings that people are against you, hallucinations (seeing or hearing things that are not there), psychotic behavior, thoughts
Introduction of suicide, or confusion, irregular heartbeats, seizure or psychosis, danger to liver and eyes. Allergic reaction: swelling of the lips, face, or tongue; shortness of breath; difficult breathing; or chocking of the throat.

5. Primaquine:

Acute side effects: Stomach upset, stomach cramps, nausea, vomiting, loss of appetite or muscle weakness.
Chronic side effects: Rash, rapid heart rate, changes in vision, hearing trouble, ringing in the ears, dark urine.

6. Pyrimethamine:

Acute side effects: Nausea, stomach upset or loss of appetite
Chronic side effects: Headache, lightheadedness, dry mouth, diarrhoea, trouble sleeping, sore throat, unusual bruising, pale skin, swelling of the tongue, depression, irregular heartbeat, blood disorders. Allergic reactions include: rash, itching, swelling, dizziness, trouble breathing

7. Quinine Sulfate:

Acute side effects: Headache, vasodilation and sweating, nausea, hearing impairment, vertigo or dizziness, blurred vision, and disturbance in color perception.
Chronic side effects: Vomiting, diarrhoea, abdominal pain, deafness, blindness, and disturbances in cardiac rhythm or conduction

8. Sulfadoxine and pyrimethamine:

Acute side effects: Severe peeling skin rash, blood disorders, liver damage, or lung injury.
Chronic side effects: Signs of infection (such as persistent sore throat or fever), paleness, joint pain/aches, persistent cough, trouble breathing, easy bleeding/bruising, yellowing eyes or skin, persistent nausea/vomiting, unusual fatigue, dark urine.

9. Proguanil (chloroguanide):

Acute side effects: Stomach upset or mouth sores, vomiting, moderate or severe stomach pain, pink-colored urine
Chronic side effects: Rash, fever, persistent sore throat, unusual bleeding or bruising, hair loss

Drug resistant malaria means malaria caused by a plasmodium, resistant to usual anti-malarial drugs. Drug resistant malaria and in particular chloroquine resistance is a major
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public health problem of the world. Table 1 shows the list of major anti-malarial drugs with mechanisms of action and the mechanisms by which organisms develop resistance to those drugs. In India, the first confirmed report of chloroquine resistance in *P. falciparum* was reported in Diphu area of Karbianglong district of Assam in 1973 (Sehgal *et al.*, 1973). Chloroquine-resistant *P. falciparum* has been reported in all malarious areas except Central America and west of the Panama Canal, the island of Hispanola (Haiti and the Dominican Republic), and certain areas of the Middle East (Anonymous, 1999). Mefloquine-resistant *P. falciparum* infection has been observed in the western provinces of Cambodia, the eastern provinces of Myanmar (Burma), and the Thailand–Myanmar and Thailand–Cambodia border areas (Anonymous, 1999). Widespread resistance of *P. falciparum* to sulfadoxine-pyrimethamine has been documented in many areas of the world, including sub-Saharan Africa, Southeast Asia, South Asia, Oceania, and the Amazon basin (Milhous and Kyle, 1998). Chloroquine-resistant *P. vivax* is reported in areas of Oceania, including Indonesia, Papua-New Guinea, Vanuatu and the Solomon Islands, as well as India, Thailand, Myanmar, and South America, including Brazil, Guyana, and Peru (Anonymous, 2000(b)). Strains of *P. vivax* that is tolerant, or even resistant, to primaquine are found in areas of Southeast Asia and East Africa, including Somalia (Petruccelli *et al.*, 1992). For chloroquine resistant strains sulfadoxine-pyrimethamine combination was used. Inspite of that some strains of *P. falciparum* was found to be resistant to this combination also.
Table 1 Major anti-malarial drugs with their mechanisms of action and resistance mechanism of parasite:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of Action</th>
<th>Mechanism of Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroquine</td>
<td>Inhibition of heme metabolism of parasite</td>
<td>Decreased intraparasite accumulation of chloroquine</td>
</tr>
<tr>
<td></td>
<td>(Warhurst, 2001)</td>
<td></td>
</tr>
<tr>
<td>Mefloquine, Halofantrine,</td>
<td>Probably inhibition of heme</td>
<td>Mefloquine and halofantrine resistance developed by increased</td>
</tr>
<tr>
<td>Quinine</td>
<td>metabolism</td>
<td>efflux of drug (Wilson et al., 1989).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The details of quinine resistance have not been established,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>although quinine and mefloquine resistance often correlate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(White, 1998).</td>
</tr>
<tr>
<td>Cycloguanil,</td>
<td>Block folate synthesis via</td>
<td>Resistance mediated through point</td>
</tr>
<tr>
<td>Chlorcycloguanil, Pyrimethamine</td>
<td>inhibition of dihydrofolate</td>
<td>mutations in <em>dhfr</em> and <em>dhps</em> genes,</td>
</tr>
<tr>
<td></td>
<td>reductase (DHFR)</td>
<td>although more efficient use of available folate may also</td>
</tr>
<tr>
<td></td>
<td></td>
<td>contribute (White, 1998).</td>
</tr>
<tr>
<td>Sulfonamides and sulfones</td>
<td>Block folate synthesis via</td>
<td></td>
</tr>
<tr>
<td>(sulfadoxine dapsone)</td>
<td>inhibition of dihydropteroate</td>
<td>Stable resistance not yet identified</td>
</tr>
<tr>
<td></td>
<td>synthase (DHPS)</td>
<td>in clinical isolates</td>
</tr>
<tr>
<td>Artemisinin</td>
<td>Damage of intraparasitic</td>
<td></td>
</tr>
<tr>
<td></td>
<td>organelles and alkylation of parasite</td>
<td></td>
</tr>
<tr>
<td></td>
<td>proteins via intraparasite heme-catalyzed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>production of carbon-centered free radicals</td>
<td></td>
</tr>
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<td></td>
<td>(White, 1998)</td>
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</tbody>
</table>

Out of 250,000 compounds synthesized since the 1970's only mefloquin and halofantrine were eventually developed into anti-malarial drug at the cost of over 150 Million $ (US).

Moreover the increasing resistance by the malarial parasite to chemotherapeutics created a high demand for the discovery of new anti-malarial drug. A search for safe and economic malaria treatment that can easily be made available to every class of people was felt necessary. In the present work an attempt has been made towards this need of the humanity.
4. Scope and advantage of the herbal anti-malarial drug

The past experience shows that a single drug therapy has resulted into malarial parasite resistance soon, which outdated the drug in less than ten years. The isolated Quinine molecule from the bark of \textit{Cinchona officinalis} is no longer effective on \textit{P. falciparum} and same is true for its derivative Chloroquine. The latest drug artemisinin, derived from the \textit{Artemisia annua} herb (qinghaosu), also met with the same fate. Ruebush \textit{et al.}, (1995) reported that 60% people from the rural area of Western Kenya (Sub-Saharan Africa) depend on herbal remedies to cure malaria. Out of the totally discovered 122 anti-malarial compounds which were used as a drug have been derived from 94 plant species having ethnobotanical support (Fabricant and Farnsworth, 2001). These examples indicate the potentiality of anti-malarial therapy derived from Mother Nature.

The principle of Ayurveda (The Indian System of Medicine) maintains “treat a man as a whole and use a drug as a whole”. When a single molecule is used for the treatment, the parasite develops resistance soon because its resistance mechanism is not much complex to change against a single compound but when several compounds enter in to the cell the resistance mechanism of parasite could not recognize a specific molecule for developing resistance and they die soon without producing any progeny with little bit resistance. This is the reason for the success of multiple component drug therapy against the chloroquine resistant parasite. In the whole drug consumption the synergistic effect of wholesome drug promises it as a better drug. Resistant \textit{Plasmodia}, which are very common in isolated single drug regimen, can be eliminated fast by this approach. The same principle applies with the herbal formulation which has been used for malarial treatment in the present research work. In addition to that, side effects of single molecule are minimized.
by the synergistic effects of herbal regimen at the same efficacy level. The acceptability of herbal medicine for the modern drug practitioners needs standardization, molecular information for used plant components, knowledge of drug mechanism and scientifically proved efficacy instead of exaggerated claims of local practitioners. The present work has been aimed to take care of all the scientific components for the herbal anti-malarial drug.

5. *Calotropis procera*: A herbal source to cure malaria

*Calotropis procera* is well known in India and Indian System of Medicine. The ethnic claim says that only one dose of the preparation from cut twig of *Calotropis* boiled with milk gives protection from malaria. The present research was under taken to develop efficient and dispensable drug from this plant. The research deals with the standardization of herbal anti-malarial formulation developed from a single plant *Calotropis procera* (Ait.) R.Br.. *Calotropis* (Asclepiadaceae) is a well-known genus throughout the tropics, commonly called as Giant Milk Weed or Swallow-Wort. In India it is commonly called as *Akado*. The genus has twelve species named *Calotropis acia* Buch.-Ham., *Calotropis busseana* K.Schum., *Calotropis gigantea* (L.) R. Br., *Calotropis hamiltoni* Wight, *Calotropis herbacea* Wight, *Calotropis heterophylla* Wall., *Calotropis inflexa* Chiov., *Calotropis persica* Gand., *Calotropis procera* (Ait.) R.Br., *Calotropis sussuela* G.Don, *Calotropis syriaca* (S.G.Gmelin) Woodson and *Calotropis wallichii* Wight. Out of that India has two species *Calotropis procera* (Ait.) R.Br. and *Calotropis gigantea* (L.) R. Br. (Shah, 1978). Both are common wasteland weeds in India (Singh et al., 1996). It is interwoven with traditional rituals of Indian culture that white flowers of *Calotropis gigantea* are offered to Lord Hanuman on Saturdays.
6. Literature survey

6.1 Botany and ecology

In Greek *kalos* means beautiful and *tropics* means ship, keel or turning- a curved structure; the corona of flower is very beautiful and on which the name *Calotropis* is based. *C. gigantea* has ovoid corolla-buds, corona-lobes shorter than the staminal column, with two obtuse auricles just below the apex while *C. procera* has corolla-buds hemispherical, corona lobes equaling or longer than staminal column and without auricles below the apex (Shah, 1978). Flower colour varies from deep violet to white (Figure 6 b,c,d). It is a magnificent shrub, reaching 6-15 feet (Figure 6a), with large silver-green leaves, densely covered with white hairs. It has umbel inflorescence with clusters of waxy purple-tipped flowers, and inflated pale green seedpods (Figure 6e). The pods split open when ripe to release silk-tufted seed to the wind. Roots have characteristic ridges and furrows on bark (Figure 6f). They are very sturdy species and grow wild up to 900 meters throughout the country (Sastry and Kavathekar, 1990) on a variety of soils in different climates, sometimes where nothing else grows. It is never found in forest; surprisingly it grows only on wastelands and therefore with the urbanization its population is gradually decreasing.
Figure 6: a. Whole plant of *Calotropis procera*, b. Flower of *C. procera* in violet-white colour, c. Flower of *Calotropis gigantia* in light violet-white colour, d. Flower of *Calotropis gigantia* in white colour; Inset: Flower of *Calotropis gigantia* in deep violet colour. e. Fruits of *C. procera*; Inset: Ripen and dehisced fruit with the aril of seeds, f. Root of *C. procera*. 
Calotropis is highly appreciated in Ayurveda, for its therapeutic potential; therefore all the Sanskrit names of the Sun, given to Calotropis are listed below (Raghunathan and Mitra, 1982).

6.2 *Sanskrit synonyms of C. procera*


Balarka, Bimbeeva, Tanuphala, Keera, Bimbora, Tapan, Toolaphala, Dasheela, Keeratanuphala, Pikasu, Pushpee, Rakarka, Raktapushpa, Kharjhghna, Himarathe, Haridashwa, Ushmarashmi.
Leaves are recommended in Ayurveda for fever, constipation (as purgative and flatulence), leprosy, cold, asthma, bronchitis, earache, boils, joint pain due to melancholy (Vat), headache, oedema, ascitis, skin psoriasis, scabies and syphilis (Sushruta, 1964; Sharangdhar, 1955; Vrunda, 1894; Pade, 1931; Beheramji, 1952). Ash of the leaves was used to cure spleen dysfunction (Vrunda, 1894) and splenomegaly (Pade, 1931). Leaves and latex both were reported for wound healing (Sharangdhar, 1955; Charaka, 1952; Beheramji, 1952). Sushruta (1964) held *Calotropis* for its bitter principle and hot property and recommended it as rabies antidote. The combined preparation from tomentum (hairs of leaves) with latex is claimed to be the antidote for snake poison (Vaidya, 1965). Charaka (1952), Bhavprakash (1981), Shodhal (1978) and Chakradatta (1928) have reported latex as antidote for scorpion bite. Charaka (1952) has highly held this plant for its purgative, laxative, sudorific, emetic and stimulatory properties (Charaka, 1952; Vaidya, 1965). Latex was mentioned to cure vaginal infection, skin diseases, otorrhoea, scabies, black spots of face, cough, tuberculosis, dandruff and *Tinea capitis* (Shodhal, 1978; Sharangdhar, 1955; Bangsen, 1893; Kalidas, 1913; Vaidya, 1965). Vaghbhata (1939) had used latex and stem with stem bark for dental caries. Brushing teeth with chawed finger thick stem was also recommended by them. Leaves and flowers were used to treat tuberculosis. Flowers were used for haematoma, bronchitis, splenomegaly, colic, stomachache and ascitis. Fruits are anthelmintic and applied for cough, and asthma. Seed cotton was applied on bleeding wounds for blood clotting. Stem and stem bark were used for hydrocoeile, elephantiasis, gout, eye problems, hepatomegaly and splenomegaly (Chakradatta, 1928; Bangsen, 1893). Root and root bark were used for piles, boils, jaundice, elephantiasis, scorpion bite antidote, oedema, joint pain, bronchitis,
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sore throat, leprosy, syphilis, blood purification, renal dysfunction, delirium, epilepsy, tetanus, gas, severe cold, whooping cough after pregnancy, ulcer and oedema and to facilitate parturition. Inhalation of root (smoke) was effective on migraine.

6.4 Calotropis in ethnomedicine

The taxon is widely used by tribes and the villagers in India for varied ailments. The whole plant is useful for fever, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, blotches of the skin, as tonic, expectorant, depurative, anthelmintic, blood circulation enhancer and mucous membrane regeneration for ulcers (Das, 1996; Vaidyaratnam, 1994; Ghosh, 1988; Ferrington, 1990). Terminal leaves are diuretic, digestive, antidiarrhoeal, abortifacient, analgesic, antidyserteric, anticongestant, antihepatitic and are recommended on headache, lice, jaundice, sore gums, toothache, swellings and ulcers (Anonymous, 2004). Flower is ethnomedicinally reported for its analgesic activity (Mascola et al., 1988). In India latex of this plant is used for treating severe skin disorders like ringworm, guinea worm, blisters, boils, dermatitis, infected wounds and parasitic skin infestations; moreover it is also used for asthma, anorexia, inflammations, tumors, cardioactive disorder, scorpion stings, venereal sores, ophthalmic disorders and depilatory (Bown, 2003; Behl et al., 1966; Morton, 1962; Vaidyaratnam, 1994; Mascola et al., 1988; Duke, 1992). In Malawi it is reported as rubefacient, strongly purgative, violent emetic, deodorant and caustic (Williamson, 1956). In West Tropical Africa it is used on the conjunctiva in conjunctivitis, epiphora and as local anaesthesia (Dalziel, 1937). Stem bark is used for leprosy, elephantiasis and to stimulate lactation in cattle. Root and root bark are tonic, anthelmintic, antispasmodic, febrifuge, depurative, expectorant, laxative, purgative, to treat elephantiasis, leprosy, chronic eczema, asthma,
bronchitis, dyspepsia, malaria fever, menorrhagia, snake bite and it promotes gastric secretion and in a large doses emetic (Grieve, 1971; Parrotta, 2001). Homoeopathy has used *Calotropis* to treat tuberculosis, syphilis, elephantiasis, leprosy, acute dysentery, pneumonic phthisis and as a sudorific.

6.5 *Pharmacologically relevant constituents of C. procera*

6.5.1 *Constituents of latex*

Seiber *et al.* (1982) separated the cardiac glycosides from the latex (Figure 7) which are calotropagenin (1), calotropin (2), uscharin (3), calotoxin (4), calactin (5), uscharidin and voruscharin. The glycoside calotopin are of different types viz. calotropin D I, calotropin D II, calotropin F I and calotropin F II. The glycoside calactin is toxic and insecticidal. Brüschweiler *et al.* (1969(a)&(b)) isolated proceroside, uzarigenin and syriogenin. Latex contains a powerful bacteriolytic enzyme lyses *Micrococcus lysodekticus* (Shukla and Krishnamurthy, 1961). Thakur *et al.* (1984) isolated two new triterpene esters, viz. 3'-methylbutanoates of α-amyrin and Ψ-taraxasterol, besides the known 3'-methylbutanoates of three triterpene alcohols from the hexane and methanol soluble extract of the latex of *C. gigantea*. *C. gigantea* latex contains two proteinase containing carbohydrate- calotropain-F I and calotropain-F II; their properties are like chymopapain and papain respectively. They both are homogeneous in nature (Abraham and Joshi, 1979(a)&(b)). Procerain, a stable cysteine protease was isolated from the latex of *C. procera*. Procerain contains 8 tryptophan, 20 tyrosine and 7 cysteine residues. (Kumar *et al.*, 2003). Two kinds of esterases, E61 and E62, were isolated from the latex of *C. procera*. They were monomers with molecular mass of 27.1 kDa and 18.3 kDa,
respectively. Esterase is known as a useful enzyme in the field of dairy food processing, fat and oil industry, and related biotechnology industry (Yoon et al., 2003).

6.5.2 Constituents of stem

The four different cardenolides have been isolated from the stem of *C. procera* viz. uzarigenone (6), uzarigenine (7), deglucouzarin (8) and frugoside (9) (Figure 7) (Elgamal et al., 1999).

6.5.3 Constituents of root

The major chemical constituents of the roots are mudarol, akundarol, usharidin, calotropin, frugoside, and a yellow bitter resin, a black acid resin, a crystalline colourless substance (madaralban), amber-coloured viscid substance (madarfluavil), and caoutchouc (Anonymous, 1911). Three cardenolides glycosides named (Figure 7) frugoside (9), coroglaucegenin (10) and 4-β-D-glucofrugoside (11) were isolated from roots of *C. gigantea* (Kiuchi et al., 1998). They are toxic to human cell lines but not for mouse cell line at 2μg/ml level. Two new oxypregnane-oligoglycosides (Figure 7), calotroposides-A (12) and B (13), were isolated from root of *C. gigantea* (Kitagawa et al., 1992). Isoursane pentacyclic triterpene C-18 (14) (Figure 7) was isolated from the root bark of *C. procera* (Bhutani et al., 1992). Four new chemical constituents, one naphthalene derivative, named calotropnaphthalene, two terpene derivatives, namely calotropisesquiterpenol and calotropisesterterpenol and an aromatic product calotrophenzofuranone have been isolated from the roots of *C. gigantea* (Gupta, 2000).
6.5.4 Constituents of aerial part

Compounds isolated from aerial parts of *C. gigantea* are (Figure 7) isorhamnetin-3-O-rutinoside (15), isorhamnetin-3-O-glucoside (16) and a flavonols tri-saccharide named isorhamnetin rhamnoglucoside (17) (Sen *et al.*, 1992). In general, both the species contain (Figure 7) β-sitosterol (18), taraxasterol (19), α-amyrin (20) and β-amyrin (21). *C. gigantea* contains additional gigantin, giganteol and isogiganteol. Akhtar *et al.* (1992) isolated a new cardenolide named proceragenin (22) having antibacterial activity, the structure shows three different moieties designated as 1, 1a and 1b in Figure 7. Asclepin was identified by Singh and Rastogi (1972).

In leaves, mudarine is isolated as principal active constituent with a yellow bitter acid, resin and glycosides calotropin, uscharin and calotoxin. Flower contains major compounds viz. α-calotropeol, β-calotropeol, rutin, β-amyrin and hyperoside. Procesterol (23), a new steroidal hydroxy ketone was isolated from the fresh and undried flowers of *C. procera* (Figure 7) (Khan and Malik, 1989). The seeds of *C. procera* contain frugoside, coroglaucigenin and corotoxigenin (Brüschweiler *et al.*, 1969(a)).
Cardenolides isolated from latex of *C. procera* (after Seiber et al., 1982).

![Cardenolides Structure](image)

**Introduction**

**Figure 7**

Cardenolides isolated from latex of *C. procera* (after Seiber et al., 1982).

<table>
<thead>
<tr>
<th>Structure No.</th>
<th>Nomenclature</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Calotropin</td>
<td>α-OH, β-H</td>
<td>H</td>
</tr>
<tr>
<td>3</td>
<td>Uscharin</td>
<td>S-CH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>H</td>
</tr>
<tr>
<td>4</td>
<td>Calotoxin</td>
<td>γ-H, γ-OH</td>
<td>H</td>
</tr>
<tr>
<td>5</td>
<td>Calactin</td>
<td>α-H, β-OH</td>
<td>H</td>
</tr>
</tbody>
</table>

Structure of four cardenolides isolated from stem (6,7,8,9) of *C. procera* (After Elgamal et al., 1999) and three from root (9,10,11) of *C. gigantea* (After Kiuchi et al., 1998).

<table>
<thead>
<tr>
<th>Structure No.</th>
<th>Nomenclature</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Uzarigenone</td>
<td>=O</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
</tr>
<tr>
<td>7</td>
<td>Uzarigenine</td>
<td>OH</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
</tr>
<tr>
<td>8</td>
<td>Deglucouzarin</td>
<td>Glc</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;</td>
</tr>
<tr>
<td>9</td>
<td>Frugoside</td>
<td>O-(6-desoxyallosyl)</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;OH</td>
</tr>
<tr>
<td>10</td>
<td>Coroglaucegenin</td>
<td>-OH</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;OH</td>
</tr>
<tr>
<td>11</td>
<td>4β-D-glucofrugoside</td>
<td>O-bis(6-desoxyallosyl)</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;OH</td>
</tr>
</tbody>
</table>
Calotroposides A (12) and B (13) isolated from root of *C. gigantea*.

Isoursane pentacyclic triterpene (14) isolated from the root bark of *C. procera* (after Bhutani *et al.*, 1992).

Compounds isolated from aerial parts of *C. gigantea* (after Sen *et al.*, 1992).

<table>
<thead>
<tr>
<th>Structure No.</th>
<th>Nomenclature</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Isorhamnetin-3-O-rutinoside</td>
<td>Glu-ORha Glu Gal</td>
</tr>
<tr>
<td>16</td>
<td>Isorhamnetin-3-O-glucoside</td>
<td>Glucoside</td>
</tr>
<tr>
<td>17</td>
<td>isorhamnetin rhamnoglucoside</td>
<td>Glucose-O-Rhamnose</td>
</tr>
</tbody>
</table>
Compounds isolated from aerial part of *Calotorpis*: β-Sitosterol (18), taraxasterol (19), α-amyrin (20), β-amyrin (21).

Proceragenin (22) (cardenolide) isolated from aerial part of *C. procera* having three different moieties designated as 1, 1a and 1b in figure (After Akhtar et al., 1992). Procesterol (23), a steroidal hydroxy ketone, is isolated from the fresh flowers of *C. procera* with three moieties designated as 1,1a and 1b. (After Khan and Malik 1989).
6.5.5 Compounds reported from Calotropis spp. (Duke, 1992)

1. 3-O-ACETYL-CALOTROPIN- Bark
2. ALPHA-AMYRIN-BENZOATE- Plant
3. ALPHA-CALOTROPEOL- Plant
4. ALPHA-LACTUCEROL- Latex Exudate
5. ALPHA-LACTUCERYL-ACETATE- Latex Exudate
6. ALPHA-LACTUCERYL-ISOVALERATE- Latex Exudate
7. ARABINOSE- Leaf
8. ASCLEPIN- Bark
9. BENZOYLISOLINEOLONE- Root
10. BENZOYLLINEOLONE- Root
11. BETA-AMYRIN- Bark
12. BETA-AMYRIN-BENZOATE- Plant
13. BETA-CALOTROPEOL- Plant
14. BETA-SITOSTEROL- Bark
15. CALACTIN- Latex Exudate
16. CALOTOXIN- Latex Exudate
17. CALOTROPAGENIN- Leaf
18. CALOTROPIN- Latex Exudate
19. CALOTROPINE- Seed (94 ppm)
20. CAOUTCHOUC- Latex Exudate (8,000 - 25,000 ppm)
21. COROGLAUCOGENIN- Seed (4,370 ppm)
22. COROTOXIGENIN- Seed (66 ppm)
23. D-GLUCOSAMINE- Leaf
24. FRUGOSIDE- Seed (224 - 2,310 ppm)
25. GIGANTEOL- Plant
26. GIGANTIN- Plant
27. GLUCOSE- Leaf
28. HISTAMINE- Latex Exudate
29. ISOGIGANTEOL- Plant
30. ISOLACTUCEROL- Latex Exudate
31. ISOLINEOLONE- Root
32. LAURANE- Seed
33. LINEOLONE- Root
34. LINOLEIC-ACID- Seed (86,000 - 117,000 ppm)
35. LINOLENIC-ACID- Seed (2,400 - 3,300 ppm)
36. MELISSYL-ALCOHOL- Seed
37. MUDARINE- Leaf
38. OLEIC-ACID- Seed (139,000 - 190,000 ppm)
39. PALMITIC-ACID- Seed (39,900 - 54,900 ppm)
40. PROCEROSIDE- Latex Exudate
41. PSEUDOCALOTROPAGENIN- Plant
42. RHAMNOSE- Leaf
43. STIGMASTEROL- Seed
44. SYRIOGENIN- Latex Exudate
45. TARAXASTEROL- Bark
46. TARAXASTEROL-BENZOATE- Plant
47. TRYPsin- Latex Exudate
48. USCHARIDIN- Latex Exudate
Numbers of activities reported from the compounds isolated from *Calotropis spp* are 114 listed as under. The number in the bracket with activity indicates the total number of compounds reported for that activity from *Calotropis spp* and the names of compounds narrated with that.

5-Alpha-Reductase-Inhibitor (3): LINOLEIC-ACID, OLEIC-ACID, PALMITIC-ACID
Acetylcholinergic (1): GLUCOSE
Allergenic (2): HISTAMINE, OLEIC-ACID
Alpha-Reductase-Inhibitor (1): OLEIC-ACID
Analgesic (2): ALPHA-AMYRIN, BETA-AMYRIN
Androgenic (1): BETA-SITOSTEROL
Anemiagenic (1): OLEIC-ACID
Angiogenic (1): BETA-SITOSTEROL
Anorexic (1): BETA-SITOSTEROL
AntiMS (1): LINOLEIC-ACID
AntiMeniere's (1)
Antiacne (1): LINOLEIC-ACID
Antidiabetic (1): BETA-SITOSTEROL
Antialopecic (3): LINOLEIC-ACID, OLEIC-ACID, PALMITIC-ACID
Antianaphylactic (1): LINOLEIC-ACID
Antiandrogenic (4): BETA-SITOSTEROL, LINOLEIC-ACID, OLEIC-ACID, PALMITIC-ACID
Antiarteriosclerotic (1): LINOLEIC-ACID
Antiarthritic (1): LINOLEIC-ACID
Antibacterial (1): BETA-SITOSTEROL
Anticancer (Breast) (1): BETA-SITOSTEROL
Anticancer (Cervix) (1): BETA-SITOSTEROL
Anticancer (Lung) (1): BETA-SITOSTEROL
Antichoilblain (1): HISTAMINE
Anticoronal (1): LINOLEIC-ACID
Antieczemic (1): LINOLEIC-ACID
Antiedemic (5): ALPHA-AMYRIN, BETA-AMYRIN, BETA-SITOSTEROL, GLUCOSE, TARAXASTEROL
Antiestrogenic (1): BETA-SITOSTEROL
Antifeedant (1): BETA-SITOSTEROL
Antifertility (1): BETA-SITOSTEROL
Antifibrinolytic (2): LINOLEIC-ACID, PALMITIC-ACID
Antiflu (1): D-GLUCOSAMINE
Antigondotrophic (1): D-GLUCOSAMINE
Antigranular (1): LINOLEIC-ACID
Antihematotoxic (2): GLUCOSE, STIGMASTEROL
Antihyperlipoproteinaemic (1): BETA-SITOSTEROL
Introduction

Antiinflammatory (7): ALPHA-AMYRIN, BETA-AMYRIN, BETA-SITOSTEROL, LINOLEIC-ACID, OLEIC-ACID, STIGMASTEROL, TARAXASTEROL
Antiketotic (1): GLUCOSE
Antileukotic (1): BETA-SITOSTEROL
Antileukotriene-D4 (2): LINOLEIC-ACID, OLEIC-ACID
Antilymphemic (1): BETA-SITOSTEROL
Antimenorrhagic (1): LINOLEIC-ACID
Antimigraine (1): HISTAMINE
Antimutagenic (1): BETA-SITOSTEROL
Antinociceptive (3): ALPHA-AMYRIN, BETA-AMYRIN, STIGMASTEROL
Antiphagic (2): BETA-SITOSTEROL, STIGMASTEROL
Antioxidant (3): BETA-SITOSTEROL, PALMITIC-ACID, STIGMASTEROL
Antiprogestational (1): BETA-SITOSTEROL
Antiprostaglandin (1): BETA-SITOSTEROL
Antiprostataadonic (1): BETA-SITOSTEROL
Antiprostatite (2): BETA-SITOSTEROL, LINOLEIC-ACID
Antipyretic (1): BETA-SITOSTEROL
Antitumor (3): ALPHA-AMYRIN, CALOTROPIN, UZARIGENIN
Antitumor (Breast) (1): BETA-SITOSTEROL
Antitumor (Cervix) (1): BETA-SITOSTEROL
Antitumor (Lung) (1): BETA-SITOSTEROL
Antiulcer (2): ALPHA-AMYRIN, BETA-AMYRIN
Antivaricose (1): GLUCOSE
Antiviral (3): BETA-SITOSTEROL, D-GLUCOSAMINE, STIGMASTEROL
Arrow-Poison (1): USCHARIDIN
Artemicide (2): BETA-SITOSTEROL, STIGMASTEROL
Bronchoconstrictor (1): HISTAMINE
Bronchostimulant (1): HISTAMINE
Cancer-Preventive (4): BETA-SITOSTEROL, LINOLEIC-ACID, OLEIC-ACID, STIGMASTEROL
Candidicide (1): BETA-SITOSTEROL
Carcinogenic (1): LINOLEIC-ACID
Cardioactive (5): CALACTIN, CALOTOXIN, CALOTROPIN, USCHARIDIN, USCHARIN
Cardiovascular (1): HISTAMINE
Choleretic (1): OLEIC-ACID
Comedolytic (1): LINOLEIC-ACID
Cytotoxic (2): ALPHA-AMYRIN, UZARIGENIN
Dermatitigenic (1): OLEIC-ACID
Estrogenic (2): BETA-SITOSTEROL, STIGMASTEROL
FLavor (2): OLEIC-ACID, PALMITIC-ACID
Febriuge (1): BETA-SITOSTEROL
Gastroprotective (2): ALPHA-AMYRIN, BETA-AMYRIN
Gastrostimulant (1): HISTAMINE
Gonadotrophic (1): BETA-SITOSTEROL
Hemolytic (1): PALMITIC-ACID
Hepatoprotective (4): ALPHA-AMYRIN, BETA-AMYRIN, BETA-SITOSTEROL, LINOLEIC-ACID
Histaminic (1): HISTAMINE
Hypercholesterolemic (1): PALMITIC-ACID
Hyperglycemic (1): GLUCOSE
Hypocholesterolemic (4): BETA-SITOSTEROL, LINOLEIC-ACID, OLEIC-ACID, STIGMASTEROL
Hypoglycemic (1): BETA-SITOSTEROL
Hypolipidemic (1): BETA-SITOSTEROL
Hypotensive (1): HISTAMINE
Immunomodulator (1): LINOLEIC-ACID
Insectifuge (3): ALPHA-AMYRIN, LINOLEIC-ACID, OLEIC-ACID
Irritant (2): HISTAMINE, OLEIC-ACID
Larvicide (1): BETA-AMYRIN
Lubricant (1): PALMITIC-ACID
Memory-Enhancer (1): GLUCOSE
Metastatic (1): LINOLEIC-ACID
Mosquitocide (1): BETA-AMYRIN
Myostimulant (1): HISTAMINE
Nematicide (2): LINOLEIC-ACID, PALMITIC-ACID
Ovulant (1): STIGMASTEROL
Percutaneostimulant (1): OLEIC-ACID
Perfumery (1): OLEIC-ACID
Pesticide (2): BETA-SITOSTEROL, PALMITIC-ACID
Propionic (3): LINOLEIC-ACID, OLEIC-ACID, PALMITIC-ACID
Proteolytic (1): CALOTROPIN
Radioprotective (1): HISTAMINE
Secretogogue (1): HISTAMINE
Sedative (1): STIGMASTEROL
Soap (1): PALMITIC-ACID
Spasmogenic (1): HISTAMINE
Spermicide (1): BETA-SITOSTEROL
Tachycardic (1): HISTAMINE
Ubiquiot (1): BETA-SITOSTEROL
Ulcereogenic (2): BETA-SITOSTEROL, HISTAMINE
Vasodilator (1): HISTAMINE
6.6 Pharmacological profile of C. procera

Out of the above mentioned Ayurvedic and ethnopharmacological activities only few are pharmacologically evaluated.

6.6.1 Anti-ulcer activity

The antiulcer activity of the chloroform fraction of C. procera root extract was reported on in vivo ulcer models named pyloric-ligated rats. It significantly inhibited aspirin, reserpine, absolute alcohol and serotonin-induced gastric ulcerations in rats and also protected the gastric mucosa from aspirin-induced ulceration in rats. It gave significant protection in histamine-induced duodenal ulcers in guinea-pigs (Basu et al., 1997). The use of root and root bark on ulcer is found in Ayurveda (Beheramji, 1952) and ethnopharmacology (Ghosh, 1988).

6.6.2 Anti-pyretic activity

The antipyretic effect of dry latex was evaluated on male albino rats. The fever was induced by 20% Baker’s yeast suspension. Administration of yeast increases rectal temperature from 97.32 °F to maximum 100.02 °F in 4 h. After four hours of yeast administration, dry latex (250 or 500mg/kg) was administered orally in saline solution. Aspirin (200mg/kg) was used for comparison. Administration of dry latex 250 mg/kg and 500 mg/kg resulted in to decline of rectal temperature to 98.50°F and 98.45°F respectively in 2h. Aspirin brought down the temperature to 96.9 °F in 2h (Kumar, 2000). The antipyretic activity of different extracts from C. procera was investigated on rats by
Larhsini et al. (2002). All extracts have shown good effect in comparison to the standard drug acetylsalicylic acid. The roots of *C. gigantea* have also shown the same antipyretic effect (Chitme, 2005). In Ayurveda, leaves (Beheramji, 1952) and in ethnopharmacology the whole plant (Das, 1996) is used for fever.

6.6.3 Anti-diarrhoeal activity

The anti-diarrhoeal effect of *C. gigantea* was evaluated against castor oil-induced diarrhoea model. The hydroalcoholic (50:50) extract of aerial part of *C. gigantea* was administered in 100, 200 and 400 mg/kg doses to rats. The plant extract in all tried doses significantly retarded the castor-oil induced enteropooling and intestinal transit. The effect is equivalent to atropine of 3mg/kg (Chitme et al., 2004). A single oral dose of dry latex (500 mg/kg) also produced a significant decrease in frequency of defecation, severity of diarrhoea and afforded protection from diarrhoea in 80% rats treated with castor oil. Dry latex produced a decrease in intestinal transit (27–37%) as compared to both normal and castor oil treated animals. Unlike atropine, dry latex significantly inhibited castor oil induced enteropooling (Kumar et al., 2001).

The normal saline soluble part of dry latex of *C. procera* was evaluated on smooth muscle function, both in *in vivo* and *in vitro* on rat and rabbit. Oral administration to rats (50–1000 mg/kg) produced a dose-dependent decrease in intestinal transit along with a decrease in intestinal content as compared to control group. At lower doses of dry latex saline fraction produced dose-dependent contractions of gastrointestinal smooth muscles in *in vitro* (rabbit ileum and fundus of rat stomach) that was followed by desensitization at higher doses (Kumar and Shivkar, 2004). The anti-diarrhoeal effect is contradicted
with report of latex as strongly purgative in Ayurveda (Charaka, 1952) and the ethnopharmacological uses (Williamson, 1956). However, flowers were mentioned for colic and root bark for renal dysfunction (Beheramji, 1952) in Ayurveda. Moreover, root bark (Grieve 1971) and terminal leaves (Anonymous, 2004) were mentioned for diarrhoea and dysentery in ethnopharmacology. Jain et al. (1985) reported cure of the patients of diarrhoea and dysentery by root preparation. This indicates that the plant may have both types of compounds but in the experimental extract only anti-diarrhoeal compounds were extracted from latex while in whole latex administration the purgative compounds have shown to overcome the effect.

6.6.4 Anti-coccidial activity

The anticoccidial activity of *C. procera* latex was examined in experimental *Eimeria ovinoidalis* infection in Najdi lambs. The symptoms observed for coccidiosis were bloody diarrhoea, tenesmus, anorexia, moderate dehydration, denudation of intestinal epithelium, presence of schizonts and cellular infiltration, decreases in the values of packed cell volume, haemoglobin and serum sodium concentration and increase in serum potassium levels in Najdi lambs. The lambs were treated with single oral doses of 0.02 ml/kg body weight of *C. procera* latex. Oocysts production was considerably suppressed for 4 days post-treatment and faeces were completely free from oocysts between 7 and 17 days after treatment with *C. procera* latex. These resulted into a return of normal appetite and activity, regular pelleted faeces and markedly reduced number of schizonts in intestinal cells. The concentration of serum sodium returned to normal but that of potassium remained high after therapy with *C. procera* latex, for that the dosing lambs with *C. procera* latex and sulfadimidine was suggested by the authors (Mahmoud et al., 2001).
6.6.5 Anti-inflammatory and Analgesic activity

Dry latex of *C. procera* was evaluated against various induced acute and chronic inflammations, like, carrageenan-induced oedema, Freund's adjuvant-induced oedema, cotton pellet granuloma, carrageenan air pouch inflammation, vascular permeability and UV-induced erythema. Oral administration of dry latex significantly inhibited oedema formation induced by carrageenan and Freund's adjuvant. It also prevented granuloma formation induced by cotton pellet and carrageenan. In addition to that it also inhibited fluid exudation, possibly due to its effect on vascular permeability. Moreover, it delayed the onset and intensity of UV induced erythema. The anti-inflammatory action of dry latex was also compared with standard antiinflammatory drugs (Sangraula *et al*., 2001). A single dose of the aqueous suspension of the dried latex was also effective against the acute inflammatory response in carrageenin and formalin induced rat paw oedema model (Kumar and Basu, 1994). Further purification from dry latex by methanol and aqueous extract was evaluated for anti-inflammatory action on the rat paw oedema model by Arya and Kumar (2005). The histological analysis revealed that the antiinflammatory effect of aqueous and methanolic extracts was more pronounced than phenylbutazone (PBZ) standard drug against carrageenin induced cellular infiltration and subcutaneous oedema. The effect was comparable to chlorpheniramine and PBZ standard drugs against histamine and prostaglandin (PGE$_2$) induced inflammation, respectively. Both extracts produced about 80%, 40%, and 30% inhibition of inflammation induced by bradykinin (BK), compound 48/80 and serotonin respectively. The extracts are effective mainly by inhibiting histamine and BK and partly by inhibiting PGE$_2$ (Arya and Kumar, 2005).
The dry latex of *C. procera* has significant analgesic effect against acetic acid induced writhings in mice by a single oral dose ranging from 165 to 830 mg/kg. The effect of dry latex at a dose of 415 mg/kg was more pronounced as compared to a 100 mg/kg oral dose of aspirin. Moreover, the oral dose of dry latex did not produce toxic effects in mice and the LD$_{50}$ was found to be 3 g/kg (Dewan *et al.*, 2000). The anti-inflammatory effect of latex (Mascola *et al.*, 1988) and analgesic effect of latex, flowers (Mascola *et al.*, 1988) and terminal leaves (Anonymous, 2004) were reported in ethnopharmacological works.

### 6.6.6 Anti-cancer activity

Ethanolic (70% v/v) flower extract of *C. procera* was tested for cytotoxicity on COLO 320 tumour cells responsible for cancer. This proved to have strongest cytotoxic effect with IC$_{50}$-values of 1.4 µg/ml. It’s potential for cancer treatment compared with the standard anticancer drug cisplatin is more advantageous (Smit *et al.*, 1995). A wide variety of anti-cancer drugs exhibit cytotoxic effect by interfering with cell-cycle kinetics. These drugs are effective either by damaging the DNA during the S-phase of the cell cycle or by blocking the formation of the mitotic spindle in M-phase (Gali-Muhtasib and Bakkar, 2002). The cytotoxic and anti-mitotic activities of dry latex of *C. procera* were evaluated on root tip meristem of *Allium cepa*. Dry latex significantly inhibited the growth of roots and mitotic activity in comparison to standard cytotoxic drug cyclophosphamide and non-cytotoxic drugs cyproheptadine and aspirin, which served as controls (Sehgal *et al.*, 2006). *C. procera* contain characteristic cardioactive glycoside, calotropine, which has shown an antitumor effect *in vitro* on human epidermoid carcinoma cells of the rhinopharynx (Hansel *et al.*, 1993). It also acts as expectorant and diuretic. Latex is reported to cure tumours in ethnopharmacology (Vaidyaratnam, 1994).
6.6.7 Wound healing activity

The latex was partitioned with chloroform and water and the aqueous fraction was evaluated for wound healing activity after evaporating water. The excisional wounds of 8.0 mm diameter were inflicted on the back of male guinea pigs of Swiss strain bred as an experimental model. Topical application of 20 μl of 1.0% sterile solution of the residue of aqueous latex partition of *C. procera* (twice daily) was followed for 7 days. It significantly augmented the healing process by markedly increasing collagen, DNA and protein synthesis and epithelisation leading to reduction in wound area. Chloroform fraction did not show healing activity of wound. Wounds treated for 7 days with *C. procera* exhibited marked dryness and there was no visual sign of inflammation in the wounds. Further, there was no sign of any pathological fluid oozing out from the wound edges. Rasik *et al.* (1999) scientifically proved the traditional use of this plant in the management of wound healing. The latex was used for wound healing as mentioned in Ayurveda (Sharangdhar, 1955; Charaka, 1952) and ethnopharmacology (Bown, 2003). Moreover, leaves and fruits (Beheramji, 1952) have also been reported as wound healer in Ayurveda.

6.6.8 Anthelmintic activity

The crude latex of *C. procera* was evaluated for anthelmintic activity using adult earthworms. Both, fresh as well as aqueous extracts of dried latex exhibited a dose-dependent inhibition of spontaneous motility (paralysis) and evoked responses to pin-prick. With higher doses (100 mg/ml of aqueous extract of dry latex and 100% fresh
latex) the effects were comparable with that of 3% piperazine. However, there was no
final recovery in the case of worms treated with latex in contrast to piperazine in which
the paralysis was reversible and the worms recovered completely within six hours. The
results show that latex possesses wormicidal activity and thus, may be useful as an
anthelmintic (Shivkar and Kumar, 2003).

The anthelmintic activity is present in the latex of C. procera. Sheeps were infected by
Haemonchus contortus larvae. They were treated with a single oral dose of 0.01 ml or
0.02 ml/kg body weight with latex of C. procera, which resulted in to significant
reduction of egg production, and fewer adult Haemonchus worms were found in the
abomasum. The other beneficial symptom was improved appetite, but still with reduced
level of haemoglobin concentration in blood. Calotropis latex showed a concentration-
dependent larvicidal activity in vitro within 20 min of application. (Al-Qarawi et al.,
2001).

The cardiac glycosidal (cardenolide) extract of C. procera has contact and dipping LC50
values on adult stages of the camel tick, Hyalomma dromedarii Koch (Acari: Ixodidae)
which were respectively 9.63 µg cm⁻² and 1096 mg litre⁻¹. The risks and benefits both are
associated with the use of cardiac glycosides and to be considered in unison (Al-Rajhy et
al., 2003). This indicates that the anthelmintic property of the plant is due to the presence
of cardiac glycosides.

Soil amended with the leaves of C. procera with Paecilomyces lilacinus was most
effective to reduce the multiplication of root-knot nematode-Meloidogyne incognita in
soil. This treatment reduced root galling and improved plant growth (Ahmad and Khan,
2004). The anthelmintic property of flower (Beheramji, 1952) was reported in Ayurveda and that of whole plant (Vaidyaratnam, 1994), terminal leaves (Anonymous, 2004) and root/root bark (Grieve, 1971) have been reported in ethnopharmacology.

6.6.9 Hepatoprotective and antioxidant activity

Efficacy of *C. procera* flower extract in the restoration of liver function after carbon tetrachloride induced hepatic injury in albino rats and mice has been evaluated. The ethanolic (70%) extract of flowers resulted in reduction of the biochemical markers of hepatic injury. The extract protected the animals from CCl₄ induced liver damage as revealed by histopathological observations. In addition, the extract showed free radical scavenging activity. The antioxidant activity was confirmed on *in vitro* models. The results indicate that flowers of *C. procera* possess hepatoprotective property possibly because of its anti-oxidant activity. This property may be attributed to the quercetin related flavonoids present in the flowers of *C. procera* (Qureshi *et al.*, 2007). The same hepatoprotective activity of *C. procera* root extract was also observed by Basu *et al.* (1992). However, in Ayurveda the ash of leaves and flowers were used for spleen dysfunction (Vrunda, 1894) and splenomegaly (Pade, 1931). The former application indicates the effect of elements in ash on splenomegaly.

6.6.10 Schizonticidal activity

Sharma and Sharma (1999, 2000) have screened flower, bud and root of *C.procera* for *in vitro* antimalarial testing (schizonticidal activity) against Chloroquine sensitive (MRC 20) and Chloroquine resistance strain (MRC 76). The solvent used was ethanol and
purified with column chromatography on Si gel (60-120 mesh) with ethylacetate, acetone and methanol. The highest activity was found in acetone fraction of bud on MRC 20 with 0.1mg/ml IC$_{50}$ value; where as the ethylacetate fraction of bud has shown maximum effect on MRC 76 with 0.3mg/ml IC$_{30}$ value.

6.6.11 Reversible anti-ovulatory activity

Effects of ethanolic (90%) extract and aqueous decoction of roots of *C. procera* were studied on oestrous cycle and oestrogenic functionality of female Wistar rats. The extracts were administered orally for 5 days (one complete cycle) at the doses of 25, 50 and 100 mg/kg. The oestrous cycle showed temporary inhibition of ovulation. 80% and 70% rats showed ovulation inhibition in 100mg/kg oral dose of ethanolic and water extract respectively. Aqueous extract was more active than ethanolic extract. A gradual normalization of the cycle started 10 days after the end of the treatment; at the fourth cycle all the animals showed a normal cycle. Milvane was used as a positive control. It is a commercially available oestro-progestin that prevent the normal oestrous cycle in 100% rats. The water decoction did not alter body, uterine weight, histoarchitecture of the uterus and the induced vaginal opening significantly in comparison to control. This suggested that inhibitory effect of *C. procera* on ovulation is not of oestrogenic nature and other mechanisms should be involved (Circosta *et al*., 2001).

The effect of fresh leaf extract of *C. procera* on the reproductive organs of male Wistar rats was examined. The extract was orally given in 20mg/g body weight daily for varying number of days resulted in to potentially deleterious effect on the testes and accessory sex organs (Akinloye *et al*., 2002).
6.6.12 Larvicidal activity

Aqueous phase of the latex of *C. procera* has 50% mortality at a dose of 28 ppm on larvae of *Anopheles labranchiae*. Ethanolic phase of latex also presented interesting larvicidal activity with LC$_{50}$ 145 ppm. Ethanolic extract of roots was effective at LC$_{50}$ of 215 ppm. DDT was used as reference with LC$_{50}$ of 0.1 ppm for *Anopheles* larvae (Markouk *et al*., 2000).

6.6.13 Molluscicidal activity

The leaf and stem of *C. gigantea* were tried to control golden apple snail (*Pomacea canaliculata*) in lowland transplanted rice. It has consistent and very satisfactory molluscicidal activity in protecting the crop from snail damage. The leaves were more effective than the stems. The field requirement of leaf for snail control and crop protection was 200 kg/ha (Lobo and Llagas, 1991).

6.6.14 Antimicrobial activity

*C. procera* as antibiotic against microorganisms was evaluated for apical twig and latex (Parabia *et al*., 2007), leaves (Suresh and Chauhan, 1992), flowers (Larhsini *et al*., 2001), root and stem bark (Jain *et al*., 1996). Latex was reported for antifungal treatment of wheat seeds (Abdul *et al*., 1987). The solvents used in previously reported work of *C. procera* were water (Desta, 1993), n-butanol (Larhsini *et al*., 2001) and ethanol (Nand *et al*., 1997) for antimicrobial screening with significant inhibition in the growth of both gram-positive and gram-negative bacterial strains. In Ayurvada latex and leaves were
used for vaginal infection (Charaka, 1962), wound dressing (Beheramji, 1952), dental caries (Vaghbhata, 1939), dandruff (Shodhal, 1978) and Tinea capitis (Vrunda, 1894). These indicate its traditional application as antimicrobial agent.

6.7 Toxicity

This plant is alleged as a poisonous plant but in permissible dose it is harmless for humans and in large dose it may cause emesis and diarrhoea. Moreover, in experimental models the solvent extracts were used and injected in body which enhanced the adverse effect of plant, whereas in Ayurvedic or ethnotherapeutic use the whole plant is orally administrated which proved harmless treatment at judicious dose.

The daily collected fresh latex of *C. procera* was orally administered to rats and changes in homological parameters on days 7 and 14 were observed. It was concluded that the latex of *C. procera* has no significant effects on blood parameters but it readily caused loss of weight (Dada *et al.*, 2002). The same feature was also noted in leaves administered in sheep and cattle by Radunz *et al.* (1983, 1984).

7. The objectives covered under the research

*Calotropis* spp. possesses number of traditional medicinal properties out of which many have been confirmed on animal models under pharmacological aspect. Leaves, flowers, root, root-bark and latex are used in various medicinal preparations. In the present work the anti-malarial therapy of this plant has been mainly targeted. The herbal medicine was developed in the form of tablets/capsules through various validation processes. The anti-
malarial efficacy of the plant has been evaluated through schizonticidal assay against *in vitro* *Plasmodium falciparum* culture. Majority of the compounds identified were present in herbal components used for tablets/capsules preparation. Preclinical trials were conducted on mice model infected by *Plasmodium berghei*, for both acute and cerebral malaria. The phase-I clinical trial was conducted to prove its efficacy for malaria treatment. The parameters have been standardized for pharmaceutical production of herbal tablets/capsules with unique bio-molecules (Figure 8).

Figure 8 Present research work is a part of DST sponsored project between two universities:
The following milestones were achieved in the present research.

1. *In vitro* schizonticidal screening of plant extracts

   The extracts derived from *C. procera* have been evaluated and compared for their anti-malarial potentiality using *in vitro* testing known as schizonticidal assay. The best samples were selected and used for preclinical trial on mice model.

2. *In vivo* extract testing against *P. berghei* infected mice with toxicity study

   Preclinical trial is a very essential step to test the physiological efficacy of the proposed drug. It will helpful for dose determination, toxicity and side effects. Separate toxicological investigation was carried out on mice with large amount of plant parts used in medicine.

3. Chemical profile of active extracts

   The active phytochemicals that act on malaria has aimed to characterize and therefore the most active extracts were investigated for identification of compounds present in it and chemical profile has been ascertained before the drug was used for human consumption.

4. Pharmacognosy of the plant parts used in herbal formulation

   Pharmacognosy helps for the identification of raw material especially when dried and powdered. The traditional pharmacognosy tools are less significant in recent era (anatomy, histochemistry, biochemical essay, etc.); therefore it has not been covered in the present work (most of them have been already explored). Instead of that fingerprinting of the range of chemical compounds and elements present in the used material were carried out.
5. Clinical trial of herbal regimen

The open clinical trial was conducted for proposed herbal regimen to prove its anti-malarial efficacy. Majority of the victims were from rural areas reported as endemic for malaria. Many cases had the Chloroquine resistant infection which has been cured by this drug without any chronic side effects.

6. Commercial production of medicine with quality control standards

The prepared formulation was manufactured at pharmaceutical level to provide requirement for clinical assessment of the medicine. A good GMP was followed with necessary quality control standards during drug preparation.

7. Antibacterial testing of plant extracts

Most of the anti-malarial compounds have antibacterial effect, in the same way many antibacterial compounds have more or less schizonticidal effect. Therefore the extracts of *C. procera* were evaluated for anti bacterial testing.

8. Micro-propagation of *Calotropis procera* from node explants

Both the species of *Calotropis* are common wasteland weeds found on road sides and barren lands. Population of both the species is gradually decreasing due to the fast urbanization. Moreover the genus is not observed in forest area, and therefore the plant must be propagated and conserved if utilized in large quantity for the treatment of malaria. Therefore, the protocol has been established for *in vitro* micro-propagation for mass multiplication through tissue culture technique.