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THE CONSTITUENTS AND PHARMACOLOGICAL PROPERTIES OF *CALOTROPIS PROCERA* - AN OVERVIEW

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ABSTRACT

Calotropis procera contained many biological active chemical groups including, cardenolides, steroids, tannins, glycosides, phenols, terpenoids, sugars, flavonoids, alkaloids and saponins. It exerted many pharmacological effects such as antimicrobial, anthelmintic, anti-inflammatory, analgesic and antipyretic, anticancer, anti-angiogenic, immunological, antidiabetic, cardiovascular, hypolipidemic, gastroprotective, hepatic protective, renal protective, antidiarrheal, antioxidant, anticonvulsant, enhancement of wound healing, antifertility and smooth muscle relaxant effect. The present review will highlight the chemical constituents and the pharmacological and therapeutic effects of *Calotropis procera*.

Key words: *Calotropis procera* , pharmacology, constituents **.**

INTRODUCTION

Medicinal plants are the oldest form of healthcare known to mankind. Medicinal plants had been used by all cultures throughout history. The World Health Organization (WHO) estimates that 80 percent of the world population, presently use herbal medicine for some aspect of primary health care. The major pharmaceutical companies are currently conducting extensive research on plant materials to introduced new drugs in the medical practice [1-46]. *Calotropis procera* contained many biological active chemical groups including, cardenolides, steroids, tannins, glycosides, phenols, terpenoids, sugars, flavonoids, alkaloids and saponins. It exerted many pharmacological effects such as antimicrobial, anthelmintic, anti-inflammatory, analgesic and antipyretic, anticancer, anti-angiogenic, immunological, antidiabetic, cardiovascular, hypolipidemic, gastroprotective, hepatic protective, renal protective, antidiarrheal, antioxidant, anticonvulsant, enhancement of wound healing, antifertility and smooth muscle relaxant effect. The present review was designed to highlight the chemical constituents and the pharmacological and therapeutic effects of *Calotropis procera*.

Synonyms

Calotropis gigantea var. *procera* (Aiton) P.T.Li, *Calotropis heterophylla* Wall. ex Wight, *Calotropis heterophylla* Wall., *Calotropis inflexa* Chiov, *Calotropis persica* Gand, *Calotropis syriaca* Woodson, *Calotropis wallichii* Wight, *Madorius procerus* (Aiton) Kuntze, *Apocynum syriacum* Garsault, *Asclepias patula* Decne, *Asclepias procera* Aiton *and Calotropis busseana* K. Schum [47].

Common names

Arabic: dead sea plant, debaj, usher, oshar, kisher; **English:** calotrope, calotropis, dead Sea fruit, desert wick, giant milkweed, swallow-wort, mudar fibre, rubber bush, rubber tree, sodom apple; **French:** pomme de Sodome, algodón de seda, arbre á soie, coton soie, arbre a soie du Senegal; **German:** wahre mudarpflanzer, gomeiner; **Hindi**: madar, akada, akdo,aak; **Italian**: calotropo; **Marathi**: rui, mandara; **Punjabi**: aK; **Sanskrit**: arka, alaka, ravi**; Somali:** boah, bo'ah; **Spanish:** bomba, algodón extranjero, cazuela; **Swahili:** mpamba mwitu; **Tamil:** vellerukku; **Telgu**: jilledu; **Turkish:** ipekag and **Urdu:** madar, aak [48-50].

Taxonomic classification

Kingdom: Plantae, **Subkingdom**: Tracheobionta, **Superdivision**: Spermatophyta, **Division**: Magnoliophyta, **Class**: Magnoliopsida, **Subclass**: Asteridae, **Order**: Gentianales, **Family**: Asclepiadaceae, **Genus**: Calotropis, **Species**: *Calotropis procera* [51-52].

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Distribution

Calotropis procera is a plant widely distributed in Asia, Africa, and America [53]. It is native to West Africa as far south as Angola, North and East Africa, Madagascar, India, Pakistan, Nepal, Afghanistan, Algeria, Iran, Iraq, Palestine, Kuwait, Oman, Saudi Arabia, United Arab Emirates, Yemen, Vietnam, Niger, Nigeria, Kenya, Zimbabwe, southern Asia, and Indochina to Malaysia. The species is now naturalized in Australia, many Pacific islands, Mexico, Central and South America, and the Caribbean islands [54-57].

Description

Calotropis procera is a soft-wooded, evergreen, perennial shrub. It has one or a few stems, few branches, and relatively few leaves, mostly concentrated near the growing tip. The bark is corky, furrowed, and light gray. A copious white sap flows whenever stems or leaves are cut. Giant milkweed has a very deep, stout taproot with few or no near-surface lateral roots. Giant milkweed roots were found to have few branches and reach depths of 1.7 to 3.0 m in Indian sandy desert soils. The opposite leaves are oblongobovate to nearly orbicular, short-pointed to blunt at the apex and have very short petioles below a nearly clasping, heart-shaped base. The leaf blades are light to dark green with nearly white veins. They are 7 to 18 cm long and 5 to 13 cm broad, slightly leathery, and have a fine coat of soft hairs that rub off. The flower clusters are umbelliform cymes that grow at or near the ends of twigs. The flowers are shallowly campanulate with five sepals that are 4 to 5 mm long, fleshy and variable in color from white to pink, often spotted or tinged with purple. The fruits are inflated, obliquely ovoid follicles that split and invert when mature to release flat, brown seeds with a tuft of white hairs at one end [50, 58-69].

Traditional uses

Whole plant was used to treat common diseases such as fever, rheumatism, indigestion, cold, eczema, diarrohea, for the treatment of boils, to remove thorn from body and for the treatment of jaundice. The root was used for the treatment of eczema, leprosy, elephantiasis, asthma, cough, rheumatism, diarrhoea and dysentery. In case of diarrhoea it changed the faecal matter into a semisolid mass within the first day of treatment. The stem was used for the treatment of skin diseases, enlargements of abdominal viscera, intestinal worms, leprosy and cure leucoderma [62, 70].

The plant was recommended in leprosy, hepatic and splenic enlargements, dropsy and worms. The latex is applied to painful joints and swelling, fresh leaves were also use for the same purpose. Oil of the leaves was applied to paralyzed part. The milky juice was used in India as purgative, while flowers were considered as digestive, stomachic, tonic and useful in cough, asthma catarrh and loss of appetite. The root bark was said to promote secretion and to be useful in treating skin disease, enlargement of abdominal viscera, intestinal worms, ascites and anasarca [69,71-73].

Parts used

The latex, fresh or dried leaves, the roots and root bark, and the flowers were used medicinally [74].

Physicochemical properties

Plant contained 18.3-23.38% ash, 1.6-5.08 % acid insoluble ash, 1.9% water soluble ash, 33.38% water soluble extractive and 6.66% alcohol soluble extractive [55,75-80].

Chemical constituents

The preliminary phytochemical screening of leaf powder of *Calotropis procera* showed that the leaves contained cardenolides, steroids, tannins, glycosides, phenols, terpenoids, sugars, flavonoids, alkaloids and saponins [75,81-82].

The leaves also contained bitter compound (mudarine) and many glycosides, calotropin, uscharin, calotoxin and calactin[83-84]. Procesterol, a new steroidal hdroxy ketone was isolated from the fresh and undried flowers of *C. procera* [85].

Leaf and stem of *Calotropis procera,* gave 0.133% and 0.09% essential oils**.** Leaf oil is dominated by tyranton (54.4%), 1- pentadecene (9.5%) and 1-heptadecene (8.2%). Most abundant compounds in stem oil are Z-13-docosenamide (31.8%), isobutyl nonane (13.7%) and 2,7,10-trimethyldodecane (12.3%). Both leaf and stem volatile oils contain octadecenamide and its saturated form in appreciable amounts. Also characterized by the presence of long chain fatty acids, amides, sulfurate, halogen compounds and carbonyls like ketones [86].

The fresh *C. perociera* leaves produced volatile organic compounds that included thioacetic acid, 2,3-dihydro-3,5-dihydroxy-6- mehtyl-4H-pyran-4-one, and 5-hydroxymethyl-2- urancarboxaldehyde [82].

Phytochemical screening of the stem bark of *C. procera* indicated the presence of various secondary metabolites (%), such as polyphenols (4.78 gm), teriterpene glycosides (5.98 gm), flavonoids (5.36 gm), steroids (3.42 gm), tannins (7.15 gm), coumarins, anthraquinones, saponins, cardiac glycosides, sterols, and alkaloids [87].

Phytochemical studies indicated that extracts of roots of *C. procera* contained alkaloids, flavanoids, glycosides, saponins and terpenes [88].

The root bark contained benzoyllineolone, benzoylisolineolone, alotropterpenyl ester, calotropursenyl acetate and calotropfriedelenyl acetate. The root and root bark also reported to contain α-amyrin, β- amyrin, taraxasterol and its four J-isomer, taraxasteryl isovalerate, taraxasteryl acetate, β-sitosterol and quercetin-3-rutinoside [89-91]. A new norditerpenyl ester, named Calotropterpenyl ester, and two unknown pentacyclic triterpinoids, namely calotropursenyl acetate and calotropfriedelenyl acetate, akundarol isovalerate, mundarol isovalerate and quercetin -3- rutinoside [92-93].

However, all parts of the plant have shown the highest protein content during summer season except apical bud in which slightly higher content was found

during winter. The protein content was found lowest in the winter sample of flowers (6.50g%) and the maximum was reported in the summer sample of mature leaf (19.99g%). Pronounced seasonal variations in protein content were observed for mature leaves and flowers. Winter was found to be less in protein content except apical bud, where the protein content was almost identical in summer and winter. Carbohydrate content was next to protein in concentration. Modest seasonal variations were noted in the carbohydrate content of apical bud, mature leaves and whole plant. Apical bud and mature leaf both exhibited higher carbohydrate content during summer while rests of the plant parts were at highest during winter. There was a drastic decrease in carbohydrate content of flower in the summer sample (9.45g%) than in winter (18.46g%). Thus the variation ranges up to almost 50%. The same holds true for stem monsoon sample. Tannin was found less in the taxon. Stem and whole plant possess lesser amount of tannins compared to other parts whereas the terminal plant parts viz. apical bud and flowers showed high tannin content. Except stem and whole plant, all the parts showed higher tannin content during monsoon. Apical buds showed higher fixed oil content among all the plant parts followed by mature leaves, whole plant, stem and flower. Seasonal variation was not pronounced in the fixed oil content of *C. procera*. The taxon was found low on phenol too. All the plant parts were more or less similar in having phenol content. However the winter sample of apical bud did show slightly higher phenol content and it was found almost nil in the monsoon sample of stem. The apical bud, mature leaf and stem samples of winter had shown the maximum phenol content [94].

Trace element (mg/kg) in the latex, leaves and bark of *Calotropis procera* were found as follow: Al :200, not detected, and not detected, Ca 340, 3694 and 1650, Cd 120, 2.10 and 2.12, Co 80.00, 2.54 and 2.52, Cu 0.00, 6.01 and 7.23, Fe 50, 72.69 and 38.32, Mg 39400, 11.32 and 529.00, Mn 17, 8.07 and not detected, Ni 50, not detected and not detected, Pb 0.00, 0.36 and 0.33, Zn 23.00, 11.62 and 8.93, K(%) not detected , 3.82 and 4.29, N(%) not detected, 4.46 and 1.44, P(%) not detected, 0.58 and 0.29 respectively[(95)].

The total phenolics in crude extracts of *C. procera* flowers were determined and expressed as gallic acid equivalent, flavonoids and tannins were estimated as quercetin equivalent. The estimated amount of phenols, flavonoids and tannins present in methanolic extract of *C. procera* flowers were 5.2 mg/g, 7.8 mg/g and 4.2 mg/g respectively [96].

Kumar *et al.,* found that the total phenol and flavonoid contents in root extract were 15.67 ± 1.52 mg propyl gallate equivalent/g and 1.62 ± 0.05 mg quercetin equivalent/g, respectively. UV-visual spectroscopic scanning of the extract indicated the presence of glycoside-linked tannins or flavonoids [97].

However, Bouratoua *et al.,* found that ethyl acetate extract and butanol extract of *Calotropis procera* showed nearly the same percentages of total polyphenols, 14.21 ± 0.02 and 10.77 ± 0.02 (g/100 g equiv. Pyrogallol) respectively. The IC_{50} values showed a moderate antioxidant activity (390 and 440, DPPH mg/ml) respectively [98].

Four flavonoid glycosides including 3-O-rutinosides of quercetin, kaempferol and isorhamnetin, besides the flavonoid 5-hydroxy-3,7-dimethoxyflavone-4'-O-β-glucopyranoside, were isolated from the crude methanolic extract of *Calotropis procera* [99] . However, n-butanol and ethyl acetate extracts of *Calotropis procera*, proceeded two flavonoides, isorhamnetin-3-O-rutinoside and isorhamnetin-3-O-rubinobioside [98].

The latex of *C. procera* was acidic in nature, with specific gravity of 1.021 and contains 14.8% solids. A nontoxic proteolytic ezyme, calotropin (2-3%) and a powerful bacteriolytic agent were isolated from the latex [100]. Latex composition depends on season, environment, soil and the maturity of the lactifier. It contained water and water soluble 88.4 to 93% , coagulate 0.8 to 2.5, calactin, calotropagenin, calotropin, calotropin, calotoxin, L-lactucerol, rpoceroid, syriogenin, tetraxasterol, uscharin, uscharidin, uzarigenin, voruscharin, β-amyrin, calotropeol, 3-epimoretenol lupeol, trypson, active labenzyme and a heart poison traces of orthohydroxy phenol [101]. 2,6 dimethyl tetra-1,5-decaene and 3, 7,11-Trimethyl-2,6,10,12-pentadecatrien-1-ol were also isolated from the latex [102].

The latex contained two distinct cysteine peptidase, procerain and procerain B. However, a new cysteine peptidases were purified from *C. procera* latex. The purified enzymes exhibited plasma-clotting activity mediated by a thrombin-like mechanism [103].

The amino acid composition of the dialyzable fraction of crude latex produced by the green parts of the plant was included: aspartic acid, glutamic acid, serine, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, isoleucine, leucine, phenylalanine and lysine [53].Various cardiac glycosides including calotropin, calactin, calotoxin, usharin, usharidin and voruscharin were isolated from the latex of the plant. Latex also contained proteases calotropin DI and DII and calotropin FI and FII, an enzyme with invertase activity and trypsin [89].

PHARMACOLOGICAL EFFECTS

Antimicrobial effects

The antimicrobial activity of aqueous and ethanolic extract of roots and leaves of *Calotropis procera* against *Staphylococcus aureus, Streptococcus pyogen, Escherichia coli* and *Pseudomonas aeruginosa* was studied on disc method. Both ethanolic and aqueous extracts of *Calotropis procera* had inhibitory effect on the growth of isolates. The effect exhibited by ethanolic extract of leaves and roots was significantly greater than that of the aqueous extract of leaves and roots [104].

The petroleum ether extract of *Calotropis procera* exhibited the best antibacterial activity against *Pseudomonas aeruginosa* ATCC and *Klebsiella pneumonia* while the chloroform extract was more potent antibacterial against *Pseudomonas aeruginosa* ATCC with 19 mm, 16 mm and 17 mm inhibition zone diameters respectively [98].

The methanolic and aqueous extract of leaves of *Calotropis procera* were subjected to the potential antibacterial against both Gram-positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus* and *Streptococcus pyogenes*) and Gram-negative bacteria (*Plesiomonas shigelloides, Shigella dysenteriae, Vibrio cholerae, Salmonella typhi, Shigella flexneri, Shigella boydii, Shigella sonnei and Pseudomonas aeruginosa*) in agar diffusion method. It was evident that both extracts are active against the bacteria at low concentrations [105].

Antimicrobial activity of solvent extracts of *Calotropis procera* growing wild in Saudi Arabia was evaluated against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*Pseudomonas aeruginosa* and *Salmonella enteritidis*) using agar well-diffusion method. A bioassay-guided fractionation of the crude flavonoid fraction (Cf3) of methanol extract which showed the highest antimicrobial activity led to the isolation of four flavonoid glycosides as the bioactive constituents. Most of the isolated extracts showed antimicrobial activity against the test microorganisms, where the crude flavonoid fraction was the most active, diameter of inhibition zones ranged between 15.5 and 28.5 mm against the tested bacterial strains, while reached 30 mm against *Candida albicans*. The minimal inhibitory concentrations varied from 0.04 to 0.32 mg/ml against all of the tested microorganisms in case of the crude flavonoid fraction. Quercetin-3-O-rutinoside showed superior activity over the remainder flavonoids. The Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) were more susceptible than the Gram-negative (*Pseudomonas aeruginosa* and *Salmonella enteritidis*), and the yeast species were more susceptible than the filamentous fungi. Calo-protein was purified from the most-active aqueous extracts of *C. procera* and showed broad-spectrum antibacterial activity. Calo-protein inhibited the growth of *S. aureus* and *E. aerogenes* effectively at 25µg/ml concentration. Ethyl acetate, methanol, and aqueous extracts (20µL of the extracts, containing 100 µg of residues), displayed high antimicrobial activity against *E. coli E. aerogenes P. vulgaris P. mirabilis P. aeruginosa* and *S. aureus.* Methanolic extract appeared as the most potent antimicrobial extract, with a diameter of inhibition zone (mm) of 14±0.31, 19±0.2, 23±0.4, 20±0.6, 8±0.12 and 27±0.06 against *E. coli, E. aerogenes, P. vulgaris, P. mirabilis, P. aeruginosa* and *S. aureus* respectively [87,99].

The differential antimycoses activities of chloroform, methanol and ethyl acetate extracts of *Calotropis procera* (50,100 and 150 mg/ml) were studied against *Trichophyton rubrum, Trichophyton tonsurans, Trichophyton mentagrophyte, Epidermophyton floccosum* and *Aspergillus.* Ethyl lactate extract produced the potent activity followed by chloroform extract, while methanol extract had no antifungal activity in all concentrations used in the study [106].

The osmotin purified from *Calotropis procera* latex, inhibited the spore germination of *Fusarium solani*. Osmotin interacted with the negatively charged large unilamellar vesicles (LUVs) of 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-rac-1-glycerol (POPG), inducing vesicle permeabilization by the leakage of calcein. Osmotin induced the membrane permeabilization of spores and hyphae from *Fusarium solani*, allowing for propidium iodide uptake [107].

Anthelmintic effects

Different extracts of *Calotropis procera* leaves were evaluated for *in-vitro* anthelmintic activity against Indian earthworms *Pheritima posthuma.* The perusal of the anthelmintic activity data reveals that 70% hydroethanolic extract at the concentration of 12.5 mg/ml showed paralysis and death in 18.58 and 29.05m. respectively. Similarly *n*-butanol and chloroform extract at the concentration of 12.5 mg/ml showed both paralysis in 21.03 and 48.26 and death in 26.53 and 51.25m. respectively. The effect was positively correlated with concentration [108].

The anthelmintic effect of crude aqueous (CAE) and methanolic extracts (CME) of *Calotropis procera* flowers was evaluated by *in vitro* and *in vivo* models in comparison with levamisole. The *in vitro* studies demonstrated the anthelmintic effects ($P<0.05$) of (CAE) and (CME) of *Calotropis procera* flowers on *Haemonchus contortus* as evaluated by mortality or temporary paralysis. For the *in vivo* studies, *Calotropis procera* flowers were administered as a crude powder (CP), CAE and CME to sheep naturally infected with a mixed sample of gastrointestinal nematodes. The percentage reduction in egg count (ECR) was recorded as 88.4 and 77.8 % in sheep treated with CAE and CP at a dose of 3000 mg/kg body weight respectively. CME was the least effective producing only a 20.9 % reduction in ECR on day 7 after the treatment. The anthelmintic activity of *Calotropis procera* against nematodes, was less than that exhibited by levamisole (97.8 %-100 %) [109].

The antischistosomal activity of *Calotropis procera* extracts was evaluated gainst *Schistosoma mansoni* in mice exposed to 80 ± 10 cercariae per mouse. They were treated orally (250-500 mg/kg for three consecutive days) by aqueous stem latex and flowers of *C. procera*, *Calotropis procera* latex and flower extracts were toxic (50–70% mortality) even in a small dose (250 mg/kg). *C. procera* (stem latex and flowers) extracts revealed significant *S. mansoni* worm reductions by 45.31 and 53.7% respectively. Extracts also produced significant reductions in tissue egg load (~34–38.5%) and positively affected oogram pattern [110].

The ethanolic extracts of the different parts of *Calotropis procera* showed IC_{50} values ranging from 0.11 to 0.47 mg/ml against *P. falciparum* MRC20-chloroquine-sensitive, and from 0.52 to 1.22 mg/ml against MRC76-chloroquine -resistant strains, flower and bud extracts being the most active. Although 220-440 times less effective than chloroquine [111].

The saponins-rich fraction of *Calotropis procera* (cpsf) did not demonstrate an *in vitro* antitrypanosomal activity. Furthermore, the (cpsf) treatments did not

significantly (P>0.05) keep the parasites lower than the infected untreated rat groups. At the end of the experiment, all *T. evansi* infected rats developed anemia whose severity was not significantly (P>0.05) and was ameliorated by the cpsf treatment [112].

The efficacy of *Calotropis procera* against *Theileria annulata* infection in cattle was investigated. The efficacy of *C. procera* against *Theileria annulata* infection was higher (92.5%), compared with 75% of buparvaquone on 21 day post treatment. The result of liver and kidney function tests after treatment with *C. procera* showed no toxicity at the dose rate of 0.3 mg/Kg orally (8 doses on alternate days) [113].

Ethanolic extract of the leaves of *Calotropis procera* was fractionated using aqueous methanol with petroleum ether, chloroform and ethyl acetate. The residue of ethanol extract (marc) was extracted with 5M HCl, basified and then extracted with chloroform. These were labeled as CP1-01 to CP1-05 for the plant. The fractions obtained from the plant were found to be selectively active against brine shrimp larvae. These fractions were also subjected to antimalaria parasites bioassay. Fractions CP1-01, CP1-04 and CP1-05 were found to be active against the tested organisms, withCP1-04 being the most active [114].

The latex of *C.procera* (0-1-1%) has shown larvicidal efficacy against all three important vector species *viz,*. *Ae. aegypti*, *An. stephensi* and *Cx. quinqefaciatus*, vectors of dengue, malaria and Lymphatic filariasis respectively. The latex dissolved in methyl alcohol and acetonitrile showed highest larvicidal efficacy among all the experimental solvents [115].

In studying the comparative effectiveness of larvicidal potential of methanol extracted latex of *Calotropis procera* with temephos, a synthetic larvicide which is widely used in all vector control programme against *Aedes aegypti* mosquitoes, it appeared that methanolic extracted latex gave 100% mortality after 1 hour exposure, while water extracted latex gave 60% mortality after 3 hours exposure [116].

The toxic effects of *Calotropis procera* were evaluate upon egg hatching and larval development of *Aedes aegypti*. The whole latex was shown to cause 100% mortality of 3rd instars within 5 min. It was fractionated into water-soluble dialyzable (DF) and non-dialyzable (NDF) rubber-free materials. Both fractions were partially effective to prevent egg hatching and most of individuals growing under experimental conditions died before reaching 2nd instars or stayed in 1st instars. On the other hand, the fractions were very toxic to 3rd instars causing 100% mortality within 24h. When both fractions were submitted to heat-treatment, the toxic effects were diminished considerably suggesting low thermostability of the toxic compounds. Polyacrylamide gel electrophoresis of both fractions showed the presence of proteins in both materials. When submitted to protease digestion prior to larvicidal assays NDF lost most of its toxicity but DF was still strongly active. It may be possible that the toxicity involved protein and non protein molecules [53].

The crude ethanol extract of *Calotropis procera* leaves have been screened for its larvicidal activities against *Musca domestica*. The third instar larvae of housefly were treated with the different concentrations of the extract by dipping method for 48 h. The LC_{50} values of the extract of *C. procera* leaves was found to be 282.5 mg/l [81].

Antiinflammatory, analgesic and antipyretic effects:

The anti-inflammatory effect of the chloroform (CH) and hydroalcoholic extract (HE) of the stem bark of *Calotropis procera* against carrageenan-induced paw oedema has been studied by using two acute models, aspirin (100 mg/kg, po) and ethanol (96%) in albino rats. CH and HE extracts showed significant anti-inflammatory activity at 200 and 400 mg/kg. As part of investigations to obtain compounds with anti-inflammatory effects, a bioassay was carried out with fractions obtained from the CH extract with n-hexane (NF1), 1-butanol (BF1), ethyl acetate (EF1) and chloroform (CF1). The HE extract of the stem bark was fractionated with n-hexane (NF2), 1-butanol (BF2), ethyl acetate (EF2), chloroform (CF2) and water (WF2). The fractions were evaluated for their anti-inflammatory effects. Fractions NF1, CF1, BF2 and EF2 (20 mg/kg) showed significant anti-inflammatory activity [117]. The latex of *Calotropis procera*, ethanol extract of its flowers and the chloroform soluble fraction of its roots possessed significant anti-inflammatory activity [118].

The methanolic extract of plant *Calotropis procera* roots has been reported to exhibit potent anti-inflammatory activity against carrageenan induced paw oedema and cotton pellet induced granuloma in albino Wistar rats. The different extracts of the roots of *C. procera* and standard anti-inflammatory drugs were administered orally 1 hour before inducing of inflammation. The methanolic extracts (180mg/kg, po) of roots of *C. Procera* has potential to inhibit sub-acute inflammation by interruption of the arachidonic acid metabolism in both paw oedema as well as cotton pellet model and showed inhibition of inflammation ($p<0.01$ and $p<0.001$) very close to the inhibitory effect of diclofenac sodium (25 mg/kg, ip) [119].

The ethanolic extract of root bark of *Calotropis procera* was investigated for its anti-inflammatory activity at different dose in the different animal models. The experimental paradigms used were complete Freunds adjuvant (CFA) induced arthritis (chronic inflammation), acetic acid induced vascular permeability model in mice for anti-inflammatory activity. The extract of *Calotropis procera* (CPE) exhibited significant anti-inflammatory effect at the dose 100 and 200 mg/kg. The extract showed 21.6 and 71.6% inhibition against CFA induced arthritis at the dose of 100 and 200 mg/kg after drug treatment, as compared to standard drug dexamethasone which produced 99% inhibition. The extract also exhibited significant inhibition in polyarthritic index in rats caused by CFA induced arthritic inflammation. In the acetic acid induced vascular permeability the CPE (100 and 200 mg/kg), significantly reduced dye leaking by 45.4% and 61.5% ($p<0.001$) respectiviley as compared to standard

drug dexamethasone and ibuprofen 23.7% and 67.4% respectively [62].

Laticifer proteins (LP) of *Calotropis procera* were fractionated by ion-exchange chromatography, and the influence of a sub-fraction LP(PI)) on the inflammatory response of Swiss mice challenged by *Salmonella enterica Ser. Typhimurium* was investigated. The survival rate reached 100 % in mice treated with LP (PI) (30 or 60 mg/kg as a single inoculum by the intraperitoneal route 24 h before infection), whereas, the phosphate-buffered saline treated group died 1-3 days after infection. The neutrophil infiltration into the peritoneal cavity of pretreated mice was enhanced and accompanied by high bacterial clearance from the bloodstream. Tumor necrosis factor-alpha mRNA transcripts, but not interferon-gamma, were detected early in spleen cells of pretreated mice after infection; however, the nitric oxide contents in the bloodstream were decreased in comparison to the phosphate-buffered saline treated group [120].

The protective effect of latex of *Calotropis procera* in complete Freund's adjuvant (FCA) induced monoarticular arthritis was evaluated in rats. Arthritis was induced by a single intra-articular injection of 0.1 ml of 0.1% FCA in the right ankle joint. The effect of dried latex (DL, 200 and 400 mg/kg) and its methanol extract (MeDL, 50 and 500 mg/kg) following oral administration was evaluated on joint inflammation, hyperalgesia, locomotor function and histology at the time of peak inflammation. The effects of DL and MeDL were compared with antiinflammatory drugs phenylbutazone (100 mg/kg), prednisolone (20 mg/kg), rofecoxib (20 and 100 mg/kg) and immuno-suppressant methotrexate (0.3 mg/kg). Daily oral administration of DL and its methanol extract (MeDL) produced a significant reduction in joint inflammation (about 50% and 80% inhibition) and associated hyperalgesia. The antihyperalgesic effect of MeDL was comparable to that of rofecoxib. Both DL and MeDL produced a marked improvement in the motility and stair climbing ability of the rats. The histological analysis of the arthritic joint also revealed significant reduction in oedema and cellular infiltration by MeDL that was comparable to that of rofecoxib [121].

Oral mucositis is an important dose-limiting side effect of cancer chemotherapy. Soluble proteins of the latex of *Calotropis procera*, phytomodulatory laticifer proteins (LP) were challenged to regress the inflammatory events associated with 5-fluorouracil-induced oral mucositis. Oral mucositis was induced in hamsters by two injections of 5-fluorouracil (5-FU; 60 and 40 mg/kg, ip, on experimental days 1 and 2, respectively). LP (5 mg/kg, ip) was injected 24 h before and 24 h after mechanical trauma of the cheek pouches. The expression of pro-inflammatory cytokines and inducible enzymes, such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) were studied. On day 10, the cheek pouches were excised for macroscopic and histopathological analysis and immune histochemical assessment of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), iNOS, and COX-2. Proteins of the latex of *Calotropis procera* were significantly inhibited macroscopic histopathological scores and myeloperoxidase activity compared with the 5-FU control group. 5-Fluorouracil also induced marked immunostaining of TNF-α, IL-1β, iNOS, and COX-2 on inflamed conjunctive and epithelial tissue compared with the normal control group. Such damage was also significantly inhibited ($p < 0.05$) by LP treatment compared with the 5-FU group [122].

The non-dialysable protein fraction isolated from the latex (LP) of *Calotropis procera* was evaluated for its efficacy against inflammation in rats where paw edema was induced by sub-plantar injection of carrageenin and monoarthritis was induced by intra-articular injection of Freund's complete adjuvant (FCA). The effect of LP was evaluated on edema volume in the paw model and on joint diameter, stair climbing ability, motility, dorsal flexion pain, levels of oxidative stress markers and joint histology in arthritis model. The protection afforded by LP was compared with that of standard antiinflammatory drug, diclofenac (5 mg/kg). LP exhibited a dose-dependent antiinflammatory effect and produced 32% and 60% inhibition of paw edema at 10 and 25 mg/kg doses and 12% and 36% inhibition of joint inflammation at 50 and 150 mg/kg doses. The protective effect of LP was associated with normalization of joint functions, histology and levels of oxidative stress markers in joint tissue [123].

The effect of non-dialyzable protein (LP) sub-fractions on neutrophil functions and nociception in rodent models (the rat peritonitis model and on nociception in the mouse model) was investigated. LP sub-fractions exhibit distinct protein profile and produce a significant decrease in the carrageenan and DF induced neutrophil influx and exhibit anti-nociceptive property. The LP and its sub-fractions produced a marked reduction in the number of rolling and adherent leukocytes in the mesenteric microvasculature as revealed by intravital microscopy. The anti-inflammatory effect of LP (PI), the most potent anti-inflammatory fraction of LP, was accompanied by an increase in the serum levels of NO [124].

The latex protein fraction administered intraperitoneally to male mice at doses of 12.5, 25 and 50 mg/kg showed a dose-dependent antinociceptive effect compared with the controls. Inhibition of the acetic acid induced abdominal constrictions was observed at doses of 12.5 mg/kg (67.9 %), 25 mg/kg (85 %) and 50 mg/kg (99.5 %) compared with controls. Latex protein at doses of 25 mg/kg (39.8 %; 42 %) and 50 mg/kg (66.6 %; 99.3 %) reduced the nociception produced by formalin in the 1st and 2nd phases, respectively, and this effect was not reversed by pretreatment with naloxone (1 mg/kg). In the hot plate test, an increase in the reaction time was observed only at 60 min after treatment with latex at doses of 25 mg/kg (79.5 %) and 50 mg/kg (76.9 %), compared with controls. Naloxone was unable to reverse this effect. The antinociceptive effects of protein fraction of the latex of *Calotropis procera* didn't depend of the opioid system [125].

A single oral dose of dry latex ranging (165 to 830 mg/kg bw) produced significant dose-dependent analgesic effect against acetic acid-induced writhing. The

effect of a dose of 415 mg/kg was more pronounced than a 100 mg/kg oral dose of aspirin. Dry latex (830 mg/kg) produces marginal analgesia in a tail-flick model which was similar to that of aspirin [126].

The ethanol extract of *C. procera* produced significant reduction of yeast induced increase in body temperature. There was a significant increase in reaction time of the treated mice placed on hot plate confirming analgesic activity of the extract [127].

The ethanolic extract of the aerial parts also possessed antipyretic effect. Administration of yeast produced an increase in rectal temperature from 97.32 ± 0.19**o**F which reached to its maximum in 4 h (100.02 ± 0.27^{o}F). Administration of dry latex (DL)-250 mg/kg and 500 mg/kg at 4 h produced a significant (P <0.05) decline in rectal temperature to 98.50 ± 0.29^{o}F and 98.45 ± 0.60^{o}F respectively. The antipyretic effect was compared with that of aspirin, which was found to be more potent and brought down the temperature to 96.9 ±0.38 ^{o}F (P <0.001) [118].

Anticancer effects

The *Allium cepa* root tip meristem model was used to evaluate the cytotoxic and anti-mitotic activities of latex of *Calotropis procera*(DL). Both DL and cyclophosphamide arrested the root growth. The mitotic cells were counted in the root meristems in at 0, 48 and 96h of incubation. The mitotic index ranged between 60.7±0.7 and 63.0±2.3 in the control group over a period of 96h. DL produced a significant decrease in mitotic index that was dose and time dependent. The mitotic index at 10 mg/ml concentration of DL was 32.7±0.8 at 48h as compared to 57.6±0.4 at 0h, while at 96h, the cellular morphology was lost [128].

The cytotoxic activity of methanolic extract of *Calotropis procera* flowers was studied by MTT assay using Hep2 and Vero cell lines. The extract showed maximum activity on Hep 2 cells than Vero cells at higher concentration, and it exhibited toxicity only on Hep 2 cells at lower concentration. Following treatment with the extracts for 24h, the cells lost their morphology and showed cell aggregation, cell roundening and finally the 100 % inhibition was observed at the concentration of 50, 25 and 12.5 mg [96].

Different extracts of *Calotropis procera* leaves were evaluated for *in-vitro* cytotoxic activity against the Hep-2 cell line. The *n*-butanol extract had most pronounced cytotoxicity against the Hep-2 [108].

Cardiotonic steroid UNBS1450 01 (derived from 2-oxovoruscharin) from *C. procera* exerted anti-cancer activity. UNBS1450 01 has been proven to be a potent sodium pump inhibitor, showing anti-proliferative and cell death-inducing activities. This anti-cancer potential of UNBS1450 01 was achieved by disorganization of the actin cytoskeleton after binding to the sodium pump at the cellular membrane, by inducing autophagy-related cell death, by repressing NF-kB activation as well as by down-regulating c-Myc in cancer cells [129]. The root extract of *C. procera* has been found to produce a strong cytotoxic effect on COLO 320 tumor cells [130].

The hemi synthetic derivative of a cardenolide isolated from the root barks of *C. procera* showed a strong cytotoxic effect on several human cancer lines, a high *in vivo* tolerance to tumor growth and prolonged survival in the human xenograft models of nude mice [131].

The cytotoxic potential of stem organic extracts from *Calotropis procera* was evaluated against cancer cell lines by MTT assay. Subsequently, samples with cytotoxic effects were tested for antimitotic activity on sea urchin egg development and for *in vivo* antiproliferative activity in mice bearing Sarcoma 180 tumor. Among the five extracts (hexane, dichloromethane, ethyl acetate, acetone and methanol), ethyl acetate and acetone extracts displayed higher cytotoxic potential against tumor cells, with IC_{50} ranging from 0.8 to 4.4 µg/ml, while methanolic extract was weakly cytotoxic. Cytotoxic extracts also exhibited cell division inhibition capacity by antimitotic assay, revealing IC_{50} values lower than 5 µg/ml. In the *in vivo* antitumor assessments, ethyl acetate- and acetone extract-treated animals showed tumor growth inhibition ratios of 64.3 and 53.1%, respectively, with reversible toxic effects on liver and kidneys [132].

Dry latex of *C. procera* has the potential for anti-cancer effect due to its differentiable targets and non-interference with regular pathway of apoptosis. Dry latex treatment of mice showed a complete protection against hepato carcinogenesis. No adverse effect was observed in these animals. The serum vascular endothelial growth factor (VEGF) level was significantly lowered in the treated mice as compared to control animals. Cell culture studies revealed that the methanolic extract of dry latex as well as its fraction 8 induced extensive cell death in both hepatoma (Huh7) and non-hepatoma (COS-1) cell lines, while nontransformed hepatocytes (AML12) were spared. This effect was accompanied by extensive fragmentation of DNA in Huh-7 and COS-1 cells. No change in the levels of canonical markers of apoptosis such as Bcl2 and caspase 3 was observed [133].

The anti-tumor potential of the root extracts of *Calotropis procera* was investigated using the methanolic, hexane, aqueous and ethyl acetate extract against Hep2 cancer cells. Treatment with the extracts at different doses of 1, 5, 10 and 25 µg/ml revealed that methanolic, hexane and acetate extract possessed cytotoxicity, whereas aqueous extract had no cytotoxic effect. Acetate extract (10 µg/ml) showed strongest cytotoxic effect (96.3 %) on Hep2 at 48h exposure, whereas methanolic and hexane exhibited cytotoxicity of 72.7 and 60.5 %, respectively. The extract-treated cells exhibited typical morphological changes of apoptosis. The root extracts produced apoptosis of Hep2 cells through cell cycle arrest at the S phase, thus preventing cells from entering the G2/M phase [134].

The *in vitro* and *in vivo* antitumor activities of *Calotropis procera* protein (CP-P) isolated from root bark, was studied. CP-P protein inhibited the proliferation and induced apoptosis of breast cancer cells through the suppression of nuclear factor kappaB (NF-kB) activation. When CP-P was administered individually or in combination with cyclophosphamide (CYC, 0.2 mg/kg) to rats with 7, 12-dimethyl benz(a)anthracene (DMBA)-

induced breast cancer, it decreased tumor volume significantly without affecting the body weight. SOD, CAT, GST, GSH, vitamin E and C levels were high in combination-treated groups (CP-P+CYC) versus the CYC alone-treated groups. Also, the combination was more effective in down-regulating the expression of NF-kB-regulated gene products (cyclin D1 and Bcl-2) in breast tumor tissues [135].

Normal human skin fibroblast (HEPK) cells were exposed to Calo-protein of *Calotropis procera* to assay for cytotoxicity. However, this protein did not exert any toxic effect on skin cells even at higher concentrations (1000µg/ml). Furthermore, the Calo-protein did not display any cytolytic effects at all the tested concentrations after 24h compared with control cells. Light microscopic images of human skin fibroblasts were exposed to the Calo-protein at varying doses (1000–0.001µg/ml). The dose of (100µg/ml) of protein did not affect the cell morphology, but the higher dose of protein (1000µg/ml) showed some changes on HEPK cells after 24h exposure [87].

Anti-angiogenic activity

Angiogenesis is controlled by number of growth factors, including vascularendothelial growth factor (VEGF). The methanolic (CM), n-hexane (CH), ethylacetate (CE) and water (CW) extracts of the roots of *Calotropis procera* were tested for anti-angiogenic activity in the chicken egg chorioallantoic membrane (CAM) assay. CM, CH and CE but not CW inhibited vascular endothelial growth factor (VEGF)-induced neovascularization in a dose-dependent manner. Of all the tested extracts, CM at the dose of 10, 5 and 2.5 ng most effectively inhibited over 83, 71 and 64%, of neovascularization induced by 10ng of VEGF, respectively. Sponge implantation assay in mice further showed that at the dose of 100ng, CM, CH and CE but not CW significantly inhibited neovascularization induced by VEGF (100 ng) [136].

Immunological effects

The immunological potential of the latex of *Calotropis procera* against sheep red blood cells (SRBC) as antigen was investigated in Wistar albino rats by studying cell-mediated, delayed type hypersensitivity reaction (DTH), humoral immune response, macrophage phagocytosis and *E. coli* induced bacteremia sepsis. The latex was fractionated according to water solubility and molecular size of its components. The fractions were named as non-dialyzable latex (NDL) which corresponding to the major latex proteins, dialyzable latex (DL) corresponding to low molecular size substances and rubber latex (RL) which was highly insoluble in water. The HA titer levels were quantified by primary and secondary humoral immune response in rats. The fractions induced production of antibodies titer level significantly ($p<0.05$) in response to SRBC. In addition immunostimulation was counteracted by up regulating macrophage phagocytosis in response to carbon particles. Rats received NDL fractions by oral route displayed considerable immunological response. Oral administration of NDL fractions, dose dependently increased immunostimlatory responses. DTH reaction was found to be augmented significantly ($p<0.05$) by increasing the mean foot pad thickness after 48h. In the survival study, control group I and negative control group II in *E. coli* induced peritonitis has shown 50% and 66.6% mortality, while pretreated groups with NDL has reduced mortality in rats injected with 1×10^8 *E. coli* intraperitoneally from 0.0% - 16.6% [137].

The immunomodulatory functions of the water-soluble *C. procera* extract (CPE) was investigated via determination of its ability to activate macrophages-effector cells in inflammatory and immune responses. Intraperitoneal injection of CPE in mice (2 mg/mouse) induced migration of macrophages to the intraperitoneal cavity. The direct effects of CPE on macrophages were then assessed by measuring the production of nitric oxide (NO) as an indicator for macrophage activation. Addition of CPE (1-10 microg/ml) to the culture medium of the murine monocyte/macrophage cell line RAW264.7 caused an increase in NO production in a time- and dose-dependent manner. CPE-elicited NO production was blocked by application of an inhibitor of inducible nitric oxide synthase (iNOS). Expression of iNOS mRNA was induced by treatment of cultured macrophages with CPE. Injection of CPE in mice also resulted in an increase in plasma NO level [138].

The hexane, ethyl acetate, and dichloromethane crude extracts of *C. procera* (250 and 500µg/mL), showed toxicity to human macrophages (U-937). However, methanol and aqueous extracts were less toxic up to >2000µg/ml. The lower concentrations (100-12.5µg/ml) were devoid of toxic effects and morphological changes of cells. However, various toxic effects were observed in the *C. procera* crude extracts in a dose-dependent manner compared to control cells [87].

Antidiabetic effects

The root extracts of *Calotropis procera* were investigated for its anti-hyperglycemic effect in Male Wister Albino rats. Glibenclamide 500 µg/kg, petrolium ether, methanol and aqueous extracts of roots of *C. procera* were administered to streptozotocin induced diabetic rats at a dose of 250 mg/kg bw as a single dose per day for 15 days. It appeared that methanol and aqueous extracts were the most effective hypoglycemic extracts [88].

The protection effects of the dried latex of *Calotropis procera* against alloxan induced changes in rat kidney was evaluated. Daily oral administration of the aqueous suspension (100 and 400 mg/kg) in diabetic rats produced anti-hyperglycemic effect that was comparable to that of glibenclamide (10 mg/kg). Unlike glibenclamide, the aqueous suspension did not increase the serum insulin levels in diabetic rats. However, it produced a marked reduction in the levels of urinary glucose and protein and normalized the renal tissue levels of thiobarbituric acid-reactive substances (TBARS) and glutathione (GSH) in diabetic rats and the effect was comparable to that of

glibenclamide. The protection afforded by the aqueous suspension was also evident from the histological analysis of the renal tissue [139].

Chronic administration of root methanol, stem methanol and leaf ethyl-acetate extracts of *Calotropis procera* for 2 weeks at 100 and 250 mg/kg doses were significantly ($p<0.01$) attenuated the diabetes induced mechanical hyperalgesia, thermal hyperalgesia, tactile allodynia and HbA1C% level in streptozotocin diabetic rats as compared to negative control rats. Furthermore, the root methanol extract of *Calotropis procera* in a dose of 100mg/kg enhanced the regeneration capability of β cells in the pancreas with significant ($p<0.01$) improvement in plasma insulin level in streptozotocin diabetic rats compared to untreated control rats [140].

The dry latex was evaluated for its antioxidant and antihyperglycemic effects in rats with alloxan-induced diabetes. Daily oral administration of dry latex at 100 and 400 mg/kg produced a dose-dependent decrease in blood glucose and an increase in hepatic glycogen. It also prevented the body weight loss in diabetic rats and reduced the daily water consumption to values comparable with those of normal rats [141].

Cardiovascular and hypolipidemic effects

Latex of *Calotropis procera* was evaluated for protection against isoproterenol (20 mg/100g) induced myocardial infarction in albino rats. The pretreatment with an ethanolic latex extract of *Calotropis procera* at a dose of 300 mg/kg body weight orally three times a day for 30 days, reduced significantly ($p<0.01$) the elevated markers enzyme levels in serum and heart homogenates in isoproterenol induced myocardial infarction [142].

The effects of ethanol, n-butanol, and ethyl acetate (EtOAc) extracts of the aerial parts of the plant, were evaluated on isolated toad heart. Their mechanisms of action were also studied. Perfusion with 2 µg/ml ethanol, 0.2 µg/mL butanol, and 0.2 µg/mL EtOAc extracts caused a significant decrease in heart rate (bradycardia), significant increase in the force of ventricular contraction, and increase in T-wave amplitude. The different extracts and latex of *C. procera* induced negative chronotropism and positive inotropism on isolated toad heart [143].

The proteins derived from the latex (LP) of *Calotropis procera* were evaluated for their efficacy in maintaining coagulation homeostasis in sepsis. Intraperitoneal injection of LP markedly reduced the procoagulation and thrombocytopenia observed in mice infected with Salmonella; while in normal mice, LP produced a procoagulant effect. In order to understand its mechanism of action, the LP was subjected to ion-exchange chromatography, and the three subfractions (LPPI, LPPII, and LPPIII) thus obtained were tested for their proteolytic effect and thrombin- and plasmin-like activities *in vitro*. Of the three subfractions tested, LPPII and LPPIII exhibited proteolytic effect on azocasein and exhibited procoagulant effect on human plasma in a concentration-dependent manner. Like trypsin and plasmin, these subfractions produced both fibrinogenolytic and fibrinolytic effects that were mediated through the hydrolysis of the Aα, Bβ, and γ chains of fibrinogen and α-polymer and γ-dimer of fibrin clot, respectively [144].

Serum lipid profile was measured in the diabetic rats. The extracts were significantly ($p<0.001$) decreased total cholesterol, triglycerides, phospholipids, LDL and VLDL cholesterol and significantly ($p<0.001$) increased HDL cholesterol [88].

Gastroprotective effects

The protective effect of methanolic extract of *Calotropis procera* latex was investigated on experimentally induced gastric ulcers in rats. The methanolic extract was found to inhibit mucosal damage in both ethanol (85-95%) and aspirin (70- 80%) model, with maintaining the tissue integrity and significant reduction in gastric hemorrhage. Oxidative stress markers (glutathione, thiobarbituric acid reactive substance and superoxide dismutase) were found to be regulated [145].

The gastromucosal protective effect of chloroform extract (CH) and hydroalcoholic extract (HE) of the stem bark of *Calotropis procera* was investigated in rats. CH extract at 400 mg/kg was found to have a significant gastromucosal protective effect. As part of investigations to obtain compounds with gastromucosal protective effects, a bioassay was carried out with fractions obtained from the CH extract with n-hexane (NF1), 1-butanol (BF1), ethyl acetate (EF1) and chloroform (CF1). The HE extract of the stem bark was fractionated with n-hexane (NF2), 1-butanol (BF2), ethyl acetate (EF2), chloroform (CF2) and water (WF2). The fractions were evaluated for their gastromucosal protective effects. Fractions NF1 and BF2 (20 mg/kg) showed gastromucosal protective effects which further supported by histopathological examination of the open excised rat stomach [117].

The chloroform fraction of *Calotropis procera* root extract demonstrated significant anti-ulcer activity against aspirin, indomethacin, ethanol, indomethacin + ethanol, or stress-induced ulcerations. Significant inhibition of gastric secretory volume and total acidity in pylorus ligated rats were observed to occur with the extract. It was also observed that the root extract significantly inhibited arachidonic acid metabolism induced by soyabeian lipoxygenase. The anti-ulcer activity of the extract might be attributable to the inhibition of 5-lipoxygenase [146].

The methanol and acetone extracts from *Calotropis procera* exhibited strong anti-H. pylori activity, almost comparable activity with tetracycline, but were found to be less potent thanamoxicillin and clarithromycin [147].

Antidiarrheal effects

The dry latex (DL) of *Calotropis procera* was evaluated for its anti-diarrhoeal activity. Like atropine, a single oral dose of DL (500 mg/kg) produced a significant decrease in the frequency of defecation and the severity of diarrhea as well as protecting from diarrhoea in 80 % of rats treated with castor oil. The effects of DL on intestinal transit, castor oil-induced intestinal fluid accumulation

(enteropooling) and electrolyte concentration in intestinal fluid were also evaluated . Dry latex produced a decrease in intestinal transit (27%–37%) compared with both normal and castor oil-treated animals. Unlike atropine, dry latex significantly inhibited castor oil induced enteropooling. However, it did not alter the electrolyte concentration in the intestinal fluid compared with castor oil- treated rats [148].

Antioxidant effects

Total phenol and flavonoid contents in extract were 15.67 ± 1.52 mg propyl gallate equivalent/g and 1.62±0.05 mg quercetin equivalent/g, respectively. UV-visual spectroscopic scanning of the extract indicated the presence of glycoside-linked tannins or flavonoids. The extract exhibited appreciable reducing power signifying hydrogen donating potential. DPPH radical scavenging assay revealed substantial free radical scavenging activity (42-90%) in the extracts. Concentration dependent response was observed in the metal ion chelating activity (16-95%). Extracts also provided protection against iron induced lipid peroxidation in rat tissue (liver, brain, and kidney) homogenates. Comparatively better protective efficacy against peroxidative damage was observed in liver (71%) followed by kidney (65%) and brain (60%) tissues. Positive correlation (r (2) = 0.756) was observed between DPPH free radical scavenging activity and reducing power of extract. Similarly strong positive correlation (r (2) ≈ 0.756) was observed between metal ion chelating ability and percentage lipid peroxidation inhibition in different tissues [97].

Free radical scavenging activity was estimated using *in vitro* models like 1,1,-diphenyl-2- picryl hydrazyl (DPPH), hydroxyl radical, hydrogen peroxide radical, reducing power and ferric thiocyanate method. *C. procera* at 500 µg/ml showed better scavenging activity in ferric thiocyanade method (83.63 %) with the lowest $IC_{50,}$ followed by hydrogen peroxide, hydroxyl radical scavenging and least activity was found to be present in DPPH assay (50.82 %). Flavonoids were found in greater amount than phenols and found to be correlated with antioxidant activities [96].

The methanolic and aqueous extracts of leaves of *Calotropis procera* were subjected to the potential antioxidant activities. The antioxidant potential of the methanolic extract was determined on the basis of their scavenging activity of the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical. IC_{50} of the methanol extract of *Calotropis procera* Linn. was 110.25 µg/ml which indicated the strong antioxidant activity of the plant. However the aqueous extract showed mild antioxidant activity [105].

The dry latex produced an increase in the hepatic levels of endogenous antioxidants (superoxide dismutase and catalase and glutathione), while it reduced the levels of thiobarbituric acid-reactive substances in alloxan-induced diabetic rats [141].

Effects on eczema and skin healing

Topical preparation of *C. procera* was used for the treatment of eczema in 94 patients. The trials were conducted for nine months. The result was found encouraging, complete cure of all the signs and symptoms have been noted in 14 (14.89%) patients, excellent response was noted in 24 (25.53%) patients, good response in 33 (35.10%) patients, fair response in 10 (10.63%) patients. Two (2.12%) patients showed poor response to the treatment and 2 (2.12%) patients exhibited worsened condition [149].

The wounds healing effect of the latex of *Calotropis procera* was evaluated in rabbits. Animals were treated daily for 21 days. The wounds' diameters were measured on the day of wound creation, thereafter on days 7, 14 and 21 post wound creation. Biopsies of the wounds were taken on days 3 and 21 and viewed histologically. The wounds were found to be significantly ($p<0.05$) reduced in groups treated with 50% latex in honey and triamcinolone, on day 7 post wound creation, while there was a significant ($p<0.05$) reduction in wound surface area in all treated groups on days 14 and 21 post wound creation. Histological findings in untreated group showed thick bundle of collagen fibres some of which had broad based configurations, reminiscent of keloid. The group treated with 2ml of Calotropis latex revealed the presence of florid granulation tissues on day 3, while there was a marked reduction in quantity and size of collagen fibres on day 21 post wound creation which was comparable with what was seen for the triamcinolone-treated group [150].

Mice topically treated with Calo-protein, purified from the aqueous extracts of *C. procera* revealed antibacterial activity and significant wound healing after 14 days comparable to fusidic acid as positive control. This protein was devoid of cytolytic effect even at higher concentrations on skin cells after 24 h [87].

Effects on Reproductive systems

The effects of ethanolic and aqueous extracts of *Calotropis procera* roots were studied on the oestrous cycle regularity. Both extracts were found to interrupt the normal oestrous cycle in 60 % and 80 % of female rats respectively. The extracts had no oestrogenic activity when tested in immature female bilaterally ovariectomized rats [151].

The antifertility effect of the ethanolic extract of roots of *Calotropis procera* was investigated in female rats. A strong antiimplantation (inhibition 100%) and uterotropic activity was observed at the dose level of 250 mg/kg (1/4 of LD_{50}) [152].

Calotropis procera was uterotonic drug, its aqueous extracts induced significant sustained increases in human myometrial smooth muscle cell contractility, with varying efficiencies, depending upon time of exposure and dose [153].

Effects on muscle contraction

The effects of ethanol, n-butanol, and ethyl acetate extracts of the plant on smooth muscle were also investigated. The different extracts increased the power of contraction of the duodenum and ileum. Pretreatment with

atropine sulfate abolished the stimulatory effect of the different plant extracts and latex of *C. procera*, which indicated that the stimulatory effect on smooth muscle was mediated by cholinergic effect. The extracts also produced relaxant action on skeletal muscle contraction [127, 143].

Anticonvulsant effects

The anticonvulsant activity of different root extracts of *Calotropis procera* was studied in rats using seizures induced by maximal electroshock seizures (MES), pentylenetetrazol (PTZ), lithium-pilocarpine and electrical kindling seizures. In the MES test, the chloroform extract of *Calotropis procera* roots showed the most significant ($P<0.01$) anticonvulsant effect, it decreased the duration of hind limb extension (extensor phase), clonus and also the duration of the stupor phase compared with the controls. In the PTZ test, the chloroform extract exhibited a highly significant ($P<0.001$) effect, and the aqueous extract had a significant ($P<0.01$) effect compared with the controls by delaying the onset of convulsions. The extracts also inhibited convulsions induced by lithium-pilocarpine and electrical kindling [154].

Hepatic and renal protective effects:

An aqueous ethanolic extract of *Calotropis procera* flowers was tested for its hepatoprotective effect against paracetamol-induced hepatitis in rats. Paracetamol (2000 mg/kg) has been reported to enhance SGPT, SGOT, ALP, bilirubin and cholesterol levels and reduce serum levels of HDL and the tissue level of GSH while treatment with an aqueous ethanolic extract of *C. procera* flowers (200 mg/kg and 400 mg/kg) restored the altered levels of biochemical markers to almost normal levels in a dose-dependent manner [155].

The possible hepatoprotective and nephroprotective activities of the ethanolic extract of *C. procera* root were investigated in female rats. Carbon tetrachloride (CCl_4) was used for induction of hepatotoxicity and nephrotoxicity with significant ($P<0.05$) increase in the level of serum enzyme markers of hepatotoxicity and nonenzyme markers of nephrotoxicity. Administration of 150 and 300 mg/kg body weight (bw) of the ethanolic extract of *C. procera* root did not protect the liver and kidney from CCl_4-induced toxicity. Pretreatment with the extract rather potentiated the toxicity induced by CCl_4. It is advised strongly that caution should be taken when ingesting alcoholic preparations of *C. procera* root [156].

The chloroform extract of *Calotropis procera* (100 and 200 mg/kg, po) showed remarkable hepatoprotective activity against paracetamol-induced hepatotoxicity as judged from biochemical parameters such as serum aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin, total protein, gamma glutamate transpeptidase (GGTP) and levels of lipid peroxides in liver, which was comparable to the activity exhibited by the reference standard Silymarin. Histopathological examination of the liver section of the rats treated with paracetamol showed intense centrilobular necrosis and vascuolisation. The rats treated with extracts with paracetamol showed sign of protection against paracetamol toxicity to considerable extent as evident from formation of normal hepatic cards and absence of necrosis and vascuoles [157].

Adverse effects and toxicity

Aqueous extract did not produce any significant changes in the behavioral or neurological responses up-to 2500 g/kg bw. Acute toxicity studies revealed the non-toxic nature of the petrolium ether, methanol and aqueous extracts of the roots of *C. procera* [88].

The safety evaluation studies revealed that the use of extract in single high doses (up to 3g/kg) didn't produce any visible toxic symptoms or mortality. However, prolong treatment (90 days) causes significantly higher mortality as compared to control group [127].

An 830 mg/kg oral dose of dry latex did not produce any toxic effects in mice and the LD50 was found to be 3000 mg/kg [126].

LD50 of choloroform extract of *Calotropis procera* in rats was 993 mg/ kg in rats [157] .

The plant is toxic and is one of the few plants not given to grazing animals due to its toxicity, the latex extracted from the stem was used traditionally to make poison arrows. The latex is highly toxic to human eyes and produces sudden painless
dimness of vision with photophobia [158].

The toxic effects of *Calotropis procera* latex was studied in rats and C. procera leaves in sheep. Male rats were subjected to an intra-peritoneal injection of fresh C. procera latex (without carrier solvent) at 1.0, 0.6, 0.3 or 0.1 ml of latex/kg of body weight. None of the rats died. The histological lesions were restricted to rats dosed with 1.0 ml of latex/kg body weight and included multi-focal coagulation necrosis of cardiac fibers and vacuolized hepatocytes. Sheep were treated with (1) a single dose of 30 g/kg, (2) a single dose of 60 g/kg or (3) 60 g/kg per day for 10 consecutive days. Exposure to the C. procera leaves caused tachycardia and transitory cardiac arrhythmias in sheep of all groups. Gross pathological analysis of sheep dosed with 60 g/kg per day for 10 days revealed mild ascites, exudates on the trachea, pulmonary edema, mild hemorrhage in the liver, hydropericardium, flaccid heart, ulcers on the abomasum and kidneys presenting pale juxtamedullary cortex. The histological findings of the rat and sheep studies were similar and included multi-focal coagulation necrosis of cardiac fibers and vacuolized hepatocytes. These findings indicate that C. procera is a cardiotoxic and hepatotoxic plant [159].

The toxic effects of ethanolic leave extract of *C. procera* on the liver was evaluated in adult male rabbits . Group A served as the control, which received distilled water only. Rabbits were treated with ethanolic extracts of *C. procera* at 250 and 500 mg/kg body weight respectively for two weeks(14 days). Histological observations of the liver showed that ethanolic leave extract of *C. procera* caused damage to the liver tissues of male rabbits at a high

dose as evident in necrotic tissue seen in treated groups [160].

Calotropis procera is part of the composition of FACA , drug developed by Institute for Research in Health Sciences, Burkina Faso and used in the treatment of sickle cell disease. The toxic effects at short and long term use of *Calotropis procera* root barks was evaluated in some rodents. In the acute test, the limit test dose of 2000 mg/kg of aqueous and hydroalcoholic extracts were administered orally to NMRI mice and then observed individually 2 h post-dosing and at least once daily for 14 days. Sub-chronic toxicity was evaluated after a daily oral administration of 20 mg/kg body weight of aqueous extract for 3 and 6 weeks to Wistar rats. The limit dose of 2000 mg/kg did not cause any mortality or signs of acute toxicity in the mice tested during the observation period. In the sub-chronic tests, the results did not show any treatment-related abnormalities in terms of physiological and hematological parameters. However, on biochemical parameters, a slight but not significant ($p>0.05$) elevation of ALT and AST were noticed in treated groups. These findings suggest that aqueous extract of *Calotropis procera* which contains many chemical compounds is relatively safe when administered orally and contribute to the safe use of this part of plant in pharmaceutical formulations [161].

The sap from C. procera is mildly toxic to humans and other animals, causing inflammation of the skin, vomiting, diarrhea, blindness, lowered blood pressure and even death [162].

As conclusion, the plant is highly toxic. Higher dosages cause vomiting, diarrhea, bradycardia and convulsions. Very high dosages may cause death. Following gastric lavage, the treatment for poisonings should proceed symptomatically [163].

Dose

It was used in a ground form, as a powder, and also topically. Daily dosage: as an expectorant and diaphoretic 200 mg to 600 mg; as an emetic 2 gm to 4 gm [163].

Conclusion

Calotropis procera is a plant with wide range of chemical constituents which exerted many pharmacological effects. There is a great promise for development of novel drugs from *Calotropis procera* to treat many human diseases as a result of its effectiveness and high LD_{50}.

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