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SYSTEMATIC REVIEW ON THE EFFICACY AND SAFETY CALOTROPIS GIGANTEA

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Abstract:
Ancient sage monks had very vast knowledge of traditional Indian medicine. But this knowledge is obsolete in the present scenario due to lack of clinical evidences. Calotropis gigantea is one of them, which is found in description of many traditional Indian books such as the Shiva Purana. It is an easily available Indian medicinal herb that is applied in numerous conventional medicines to manage many chronic diseases. Traditionally, it is very good anthelmintic and carminative and capable to cure cough, leprosy, and asthma. To compile this review article, we carried out a rigorous exercise to search literature related to safety and efficacy of Calotropis gigantea on PsychInfo, PubMed, Science Direct, and PLOS databases. Currently traditional and botanical application of herbal bioactive, mainly which are derived from natural source, had acquired substantial interest because of their therapeutic values and minimal toxicity to human health. Herbal flora have been described to have therapeutic potential attributable to their bioactive such as terpenes, steroid, glycosides, tannins, saponins, flavonoids, alkaloids, and many more. This review examines efficacy and safety Calotropis gigantea along with their phytoconstituents.

Key-words: Calotropis gigantea, efficacy, safety, toxicity, phytoconstituents

Key Messages: A combination of traditional knowledge with current research findings open a new perspective to treat the patient with minimum toxicity. Calotropis gigantea is a folk Indian herb that can certainly be helpful in the management of many incurable diseases if clinical safety and efficacy are well established.

INTRODUCTION

From the start of civilizations plants were utilized for the management of various chronic disease.[1] Conventional therapeutic system based use of plants phytoconstituents having crucial role in health management system. In the last few decades, plants phytoconstituents have been obtaining much popular social acceptance due to belief that these phytoconstituents have fewer toxic effects and high therapeutic property than their synthetic equivalent.[2, 3] Nowadays, around 80% of global inhabitants depends on Conventional medicine as a main source of their primary health care.[4] Traditional herbal medicines is well known for its local anesthetic, antimicrobial, diuretics, sedative, antiulcer, analgesic and anti-inflammatory activity. Some of them also have bactericidal, virucidal, fungicidal potential.[5, 6] These pharmacological activities have been reported due to medicinal constituents such as tannins, terpenes, steroids, saponins, glycosides, flavonoids and alkaloids.[4] Currently, herbal

phytoconstituents have been mentioned as a essential source of searching new drug molecule that has been utilized to manage serious disorder. These known phytoconstituents have been taken as prominent leading moiety in the search for novel and pharmacologically active drugs.[5] Calotropis (C.) gigantea, is a laticiferous shrub, belongs to the apocynaceae family. It is very popular by various vernacular names such as crown flower or gaint milkweed in English, Madar or Safed aak in Hindi , and Svetarka in Sanskrit.[7]

Botanical Features

Calotropis species are erect, tall, vast evergreen perennial shrub that reaches to a height of 4-5 m, with milky latex like substance throughout. The bark is yellowish white with soft corky texture. Woolly pubescence is present on stout and terete plant branches. Leaves of C. gigantea are opposite, sub sessile, decussate, ovate-oblong, green, thick and surrounded by wooly pubescent hair. Flower have radial symmetry, light green to purple, bisexual with faint odor, 4 -5 cm diameter. Periodicals are much wider than the flower in umbellate cymes.[8] The stalks are overspread with wooly texture, bud ovoid, calyx differentiated to base. In umbellate lateral cymes; flowers are shorter than the periodicals, cottony wool are overspread with pedicels, calyx divided to the base, ovate, acute, cottony, buds ovoid, consist 5 white sepals 4mm, 1.3 cm length of lobes in corona, lower than the column, apex surrounded with obtuse auricles and slightly thickened margin. Follicles are green, wide, abundant, 9-10 cm in length, plump ventricose. Seeds are brown, Ovate, flattened, coma 2.5-3.2 cm long, minutely tomentose 6 x 5 mm in length, hairs at one end and situated in green color spongy fruit.[9]

Geographic Distribution:

C. gigantea, a laticiferous shrub found profusely in Indian subcontinent, northern Africa, Arabia, tropical Asia, Himalayan tract. Generally air and animal pollination takes place. Sandy soils with low rain fall is favorable for its growth. This noncultivable weed grows very easily on lagoon edge, in overgrazed native pastures, and roadside. [10, 11]

Chemical Constituents

The bioactive molecule of C. gigantea that are isolated from different parts of the plant include various calotroxin, sterols, uscharin, uschridin, and so forth, such as, proceroside, cardiac glycosides, triterpene esters, alkaloids, flavones, terpene and tannins.[11] Besides, various cardenolides, pregnanes, proteinases, flavonoids, terpenes, flavonol, , nonprotein amino acid and aromatic product have been isolated.[12-13]

Traditional Uses

C. gigantean have potential to treat bronchitis, dyspepsia, spleen, bronchial asthma, pain, Hansen's disease, tumors, liver, ulcers, haemorrhoids and; it is often employed for diarrhea, rheumatic diseases, dyspepsia, atopic eczema, cold, flu and jaundice. Each and every parts of plants is useful in conventional system of medicine for management of various disorder such as stem for Hansen's disease, vitiligo, parasitic gut worm, skin disease; the roots are utilized for the management of Hansen's disease, bronchial asthma, cough cold, filariasis, rheumatism and watery diarrhea; leaves and milky white latex are used in inflammation, myalgia and joint pain; juice of C. gigantean was incorporated for purgation, oil massage shows effectiveness on paralyzed body part;.

PHARMACOLOGICAL ASPECTS

Antibacterial and Antifungal Potentials:

Anhydrosophoradiol-3-acetate and 1Di-(2-ethylhexyl) phthalate were extracted from ethyl acetate fraction of C. gigantea. For elucidation of antibacterial activity isolated molecule, Kanamycin and Nystatin disc utilized in experimental study as positive control. Antibacterial activity of Anhydrosophoradiol-3-acetate and 1Di-(2-ethylhexyl) phthalate were assessed by utilizing 30, 60 and 90 µg/disc while antifungal potential of both compound assessed by using 100, 200 and 400 µg/ disc. Growth of gram positive (B. subtilis, S. aureus and S. lutea) as well as gram negative (S. sonnei, E.

coli, S. shiga and S. dysenteriae) bacteria was effectively suppressed by 1Di-(2-ethylhexyl) phthalate.[21]
1Di-(2-ethylhexyl) phthalate was ineffective against B.megaterium while Anhydrosophoradiol-3-acetate moderate efficacy against E.Coli, S. aureus and S.lutea.

Antitumor
Antitumor effect of C. gigantea was assessed in mice by using Ehrlich's ascites carcinoma model. NMR and mass spectral analysis was performed to evaluate characteristic of Anhydrosophoradiol-3-acetate. The potential of Anhydrosophoradiol-3-acetate was evaluated for body weight changes, survival time and viable cell count at 20 and 10 mg/kg dose. After C. gigantea treatment, significant increase life span and decrease in viable tumor cell count of mice was also observed.[22]
Methanolic root fraction of C. gigantea and its chloroform soluble extract have potential to suppress tumor growth in Ehrlich ascites carcinoma model in mice. Chloroform fraction at 20, 40 mg/kg, Petroleum ether fraction at 80 mg/kg and methanolic extract 10 and 20 mg/kg significantly exhibited cell viability. Methanolic and chloroform fraction potentially restored SALP and SGOT levels to normal.[23]

Anti-Hyperglycemic Effect
Leaves extract of C. Gigantea was evaluated against streptozotocin induced hypoglycemia in wistar rats. VLDL, LDL, TG and total Cholesterol (TC) decreased as well as HDL increased after 21 days continuous C. Gigantea extract treatments. There was an increase in kidney weight in streptozotocin treated rodents due to proliferation of glomerular cell while kidney weight was maintain at normal values after extract treatments.[25] Urea and creatinine level sustained at normal values in diabetic rat. Chloroform fraction of C. gigantea flowers and leave possess significant anti-diabetic activity.[24]

Anti-asthmatic activity
Traditionally stem bark C. Gigantea is very effective to treat pneumonia and its flower extract is use to manage asthma.[26] The methanolic root fraction of C. Gigantea was studied mast cell stabilizing and anti-anaphylactic property by egg albumin generated passive paw anaphylaxis where molecule 48/80 produce degranulation of mast cell in rats. C. Gigantea were evaluated for antiasthmatic activity at doses of 400, 200, 100 mg/kg, p.o. At mention doses, plant root extract showed significant increase in percentage suppression of paw edema, whereas degranulation of mast cell was protected at 400 mg/kg, p.o. which confirm that plant root extract has is effective against asthma.[27]

Analgesic activity
The root juice of plants has been conventionally used to control labour pain, latex of plant effectively controls teeth ache and leaves were beneficial to manage rheumatic pain.[28] Hydroalcoholic fraction of aerial part of C. Gigantea was effective to control pain in mice by acetic acid induced writhing, tail flick and eddy's hot plate methods. The hydroalcoholic fraction developed significant rise in thermal latency of rodents. C. Gigantea fraction at 400 mg/kg dose induced significant ($p<0.05$) analgesic action between 1 and 2 h of experiment and remarkable growth in latency response was noted from 3 to 5h. Intraperitoneal administration 400, 200 and 100 mg/kg dose of C. Gigantea showed significant increase in tail flick test response through the study. Hydroalcoholic fraction (1:1) fraction at 200 and 100 mg/kg dose produced 48% and 37% protection while 400 mg/kg was sufficient to produced 69% of writhing response.[29]

Anti-arthritic activity
Ethyl acetate, petroleum ether, alcohol and aqueous fraction of C. gigantea leaves was evaluated for anti-arthritic activity by Freud's complete adjuvant test at 300, 200 and 100 mg/kg dose.[30] Aqueous fraction, petroleum ether and diclofenac sodium significantly ($p < 0.01$) decrease WBC count. These fraction significantly ($p < 0.01$) decrease lymphocyte count when compare to control. The red blood cell (RBC) count was insignificant ($p > 0.05$) because of iron deficiency. The rise in ESR content

(10±0.68) in control batch was reduced to normal in other batch significantly ($p < 0.01$). Petroleum ether as well as aqueous extract produced a significant effect in all parameters which was considerable in arthritis.[31]

Anti-convulsant activity

Family member of C. Gigantea were employed in conventional system of medicine that flower faction is having potential to manage epilepsy while stem bark is anticonvulsant in nature.[30] The chloroform, aqueous, benzene, petroleum ether and methanol isolated fraction of C. gigantea were evaluated to suppress pentylenetetrazole as well as maximal electroshock induced seizures (MES) induced convulsion in wistar rat. Methanolic extract significantly ($p < 0.01$) increase MES seizure. 180 mg/kg p.o, dose of methanolic extract exhibited 68% protection and 32% mortality in convulsion induced by MES. Methanolic fraction at 180 mg/kg p.o, suppressed convulsion significantly ($p < 0.01$) and 45% of mortality with 55% protection was observed in PTZ model. The experimental study revealed that methanolic fraction of C. gigantea leaves exhibited antiepileptic potential against seizure developed by MES and PTZ.

Anti-diabetic activity

Traditionally very popular to control rise in blood glucose, therapeutic efficacy of chloroform fraction of flower and leaves of C. Gigantea was studied at 50, 20 and 10 mg/kg dose against streptozotocin diabetic model. Flower and leaves fraction significantly suppressed ($p < 0.001$) streptozotocin induced blood glucose level in rat and better recovery was observed on 21st and 27th days of treatment. Both the chloroform fraction of C. gigantea exhibited antidiabetic activity in rat on 27th day of the study when compare to control and result was significant ($p < 0.001$). Likewise petroleum and chloroform fraction (10, 20 and 50 mg/kg) of leaves and flower of C. Gigantea studied for insulin resistance potential of high fructose diet in rat.[24] Petroleum and chloroform fraction significantly risen ($p < 0.01$, $p < 0.001$) insulin and blood glucose count in rat treated with fructose rich diet. 50 mg/kg dose of C. gigantea leaves fraction significantly suppress ($p < 0.01$, $p < 0.05$) cholesterol and triglyceride when contrasted with control group.

C. Gigantea significantly increased ($p < 0.001$) the insulin, insulin resistance, fasting serum glucose, and study outcome show that a low dose of plant extracts will restore functional activity beta cells and as well as prevent insulin resistance and improve action.[33]

Chloroform fraction of C. Gigantea flower and leaves were studied at 50, 20 and 10 mg/kg diabetic potential in Streptozotocin induced hypoglycaemia. 5% tween 80 suspension was used as negative control and Glibenclamide was considered as positive control. The leaves and flower fraction at 10 mg/kg dose reduced in Streptozotocin induced hypoglycemia significantly ($p < 0.001$) at 27th day of the study.[24]

Anti-diarrhoeal activity

Cow milk along with C. Gigantea powder can be employed for dysentery and therapeutic potential was evaluated based on conventional knowledge. Anti-diarrhoeal activity of hydro ethanolic stem fraction was studied against diarrhoea induced by Castor oil, electrolyte secretion and enteropooling in Wistar albino mice and rat of both sex at 500 mg/kg, 250 mg/kg dose. In dose dependent manner Defecation affected by C. gigantea and reduced by four hour but at 500 mg/kg dose, frequency of stools was reduced by maximum at first 2 h time period and inhibited to 68% by 3h and significant ($p < 0.01$) reduction was noticed in frequency of defecation. 500 mg/kg dose of C. gigantea fraction significantly suppressed ($p < 0.05$) propulsion of charcoal meal in the gastrointestinal tract and when given one hour prior to castor oil administration significantly reduced weight of intestinal content (2.3±0.6 g) and enteropooling (1.5±0.2 ml; $p<0.05$).

Anti-histamine activity

C. Gigantea is conventionally employed for treatment of asthma therefore evaluated for antihistaminic activity which is responsible to cause allergies. Histamine is important contributor in pathogenesis of

asthma therefore methanolic root fraction of C. gigantea was evaluated for clonidine Pheritima posthuma and haloperidol induced catalepsy in mice. The experiment was performed at of 400, 200 and 100 mg/kg dose and significant ($p<0.01$) reduction was noted in the haloperidol induced catalepsy when compared to that of the clonidine. This experiment proves antihistamine activity of the C. gigantea.

Anti-helminthic activity

Traditionally, C. Gigantea flower was employed for intestinal worms besides many synthetic compounds available for management of worm infestations have their own constraints therefore natural molecule need to be explored. Aqueous and alcoholic fraction of peeled roots of C. Gigantea were employed for anthelmintic potentials at 100, 80, 40 and 20 mg/kg dose for both both paralysis and deathtime in a Pheritima posthuma (earthworm). Water soluble fraction of C. Gigantea at 100 mg/kg dose showed potent Anti-helminthic activity comparable with 80 mg/kg of Albendazole.

Anti-oxidant activity

Different part of C. gigantea was used in many disease condition which clearly demonstrates that it possess anti-oxidants which are capable to suppress oxidative stress via various mechanism. Ethanolic fraction of C. gigantea was evaluated for free radical scavenging activity by hydrogen peroxide radical assay and hydroxyl radical scavenging activity at various concentration 100, 80, 60, 40 and 20 mg/ml. Plant showed concentration dependent antioxidant activity comparable to ascorbic acid due to the presence of terpenoids and flavonoids. [38]

Anti-ulcer activity

Decoction and flower of C. gigantea are employed for management of ulcer. Ulcer is severe side effects of many allopathic medicine hence plant is evaluated for antiulcer activity. Ethanol and chloroform fraction of plant was evaluated for antiulcer activity by Aspirin as well as pylorus ligation induced ulcer where Ranitidine, aspirin were employed as positive control for the experiments. Test group treated with C. gigantea fraction significantly ($p > 0.01$) suppressed ulcer produced due to aspirin and pylorus ligation.[37]

Cardio protective studies

Search for safer and economical cardio protective drugs is quite essential currently due to increased incidences of stroke. The methanolic root fraction of C. gigantea was evaluated in male adult Wistar rats for Isoprenaline induced myocardial infarction and parameter like as endogenous enzymatic, non-enzymatic antioxidants, plasma marker enzymes, ATPases activity, protein levels, lipid peroxidation and heart weight were estimated. In 28 days study, wistar rats were treated with C. Gigantea fraction at doses of 400 and 200 mg/kg p.o and except control all other groups administered Isoprenaline of 85 mg/kg s.c on 29th and 30th days. The animal group received isoprenaline exhibited significant ($p < 0.001$) increase in plasma marker enzymes of cardiac lipid peroxidation, intercellular calcium levels, heart weight, protein levels, cardiac damage and significant decrease ($p < 0.001$) in non-enzymatic and enzymatic endogenous antioxidants Ca^{2+} ATPase, Mg^{+2} ATPase and Na^{+}–K^{+} ATPase. Animal study clearly indicated that C. Gigantea effectively suppress isoprenaline induced myocardial infarction.[31]

Contraceptive activity

Latex and pulp of C. Gigantea fruit is employed as abortive agent whereas entire plant is utilized to induce labour during pregnancy. Ethanolic root fraction of C. gigantea and its extracts such as n-butanol, hexane, chloroform insoluble and soluble fraction were studied for oestrogen agonist and antagonist property and postcoital contraception efficacy.Fraction of C. gigantea at a dose of 12.5 mg/kg showed maximum efficacy when given between days 1-5 days and 1-7 postcoitum. Plant extract in Chloroform at a dose of 200 mg/kg exhibit maximum action in single day but all other fraction were not therapeutically active at 100 mg/kg. Approximate 6-25 % loss in body weight was

observed for minimum active contraceptive dose in the batch treated with fraction and chloroform extract when correlate to that of control batch
Likewise ethanolic and crude root fraction of C. Gigantea at a dose of 300 mg/kg and 150 mg/kg were evaluated for ovaro-utero toxic effects and antifertility potentials. These fraction decrease alkaline phosphatase, glycogen, and total protein which show anti-estrogenic potentials. Biochemical estimation indicates that C. Gigantea possess strong antiprogestagenic potential.[22]

Cytotoxic studies
Major portion of cytotoxic drugs are originated from natural origin. Methanolic roots extracts of C. Gigantea were studied for the cytotoxicity using human gastric cancer SGC-7901 cell lines and MTT assay on human chronic myelogenous leukaemia K562 cell lines. C. gigantea fraction (IC_{50}–3.3, 7.0 µg/ml), Frugoside (IC_{50}–3.4, 6.5 µg/ml) and Coroglaucigenin (IC_{50}–4.7, 14.1 µg/ml) exhibited significant cytotoxic activity against SGC7901 and K562 cell lines. Deoxy sugar at C-3 position was responsible for cytotoxic action.[19]

Likewise, Calotropone (pregnanone) tested for cytotoxicity and study revealed that C. gigantea fraction was influential at an IC_{50} values of 9.7, 5.7 mg/ml against SGC-7901, K562 cell lines and molecule I, II were influential at IC_{50} values of 9.2, 4.7 and 91.3, 14.1 mg/ml respectively.[20]

Fibrinolytic activity
The polyacrylamide gel electrophoresis method was to evaluate the fibrinolytic activity of C. gigantea latex under basic as well as acidic environment. Caseinolytic activity was studied at various doses of 100, 80, 60, 40 and 20 µg/ml. Human fibringenolytic activity was performed at 40, 30, 20 and 10 µg/ml doses. Fibrinolytic activity was studied at doses of 100, 80, 60, 40 and 20 µg/ ml. Human plasma and blood clot hydrolyzing potential was evaluated at 75µg and haemorrhagic property was studied at 1–6 µg dose ranges (human plasma SDS-PAGE pattern for clot hydrolyzing property). The electrophoresis experimentation showed that the crude faction constitute natured basic proteins. Plant latex exhibited twice potential of papain as compare to caseinolytic activity. The C. gigantea latex potentially hydrolysed all the fibrinogen subunit in the order of Aα>Bβ>γ. This activity showed a plateau path at a 30 µg/ml concentration and from 150 s to 47 s it lowered significantly. Greatest hydrolysis of plasma and blood clot was manifested by C. gigantea latex when evaluated against to papain and trypsin. The crude latex also showed haemorrhagic activity at a concentration of >75 mg.[15]

Hepatoprotective activity
C. gigantea popularly used in digestive system related ailments. Alcoholic flower fraction at exhibited hepatoprotective activity 200 mg/kg. Serum parameters such as BLN, ALP, SGPT, SGOT, histopathology sections and weight of the liver were analysed. liver weight and serum parameters was Significantly decrease ($p<0.001$) when compared to CCl4. The alcoholic fraction was found to be more pharmacologically active than that of Silymarin. Histopathological outcome confirmed that batches treated with alcoholic fraction exhibited minimum injury when compare to that of toxin batch thereby supporting physical, functional and biological parameters. In-vivo studies shows alcoholic fraction have potent hepatoprotective potentials.[16]

Mosquitocidal activity
Various species of Calotropis genus such as C. procera was evaluated against larvae of Culex quinquefasciatus, Anopheles stephensi and Aedes aegypti. C. gigantea was also studied for same plasmodium strains. The leaf fraction of plants was evaluated for Mosquitocidal activity on pupae and larvae of Culex quinquefasciatus, Aedes aegypti and Anopheles stephensi. The rate of mortality followed dose dependent relationship i.e. for 250 ppm it was 100% and for 50 ppm it was 40% and for pupil mortality for 250 ppm it was 56% and for 50 ppm it was 22%. The LC90 value was 509.57 ppm and LC50 value for pupae was 213.79 ppm. Pupal and Larval mortalities of Bacillus thuringiensis, Culex quinquefasciatus and Aedes aegypti after treatment with methanolic fraction was

increased to 93% at 100 ppm and 91% at 250 ppm concentration. The pupal and larval mortalities after the treatment for Aedes aegypti and Bacillus thuringiensis pupae; 10% (20 ppm) to 38% (100 ppm)); (larvae; 31% (20 ppm) to 86% (100 ppm)) and Culex quinquefasciatus (larvae; 81% (100 ppm) and 27% (20 ppm)) followed dose dependent relationship. The experiment showed that methanolic fraction of C. gigantea is having mosquitocidal activity.[33]

Nerve muscle activity

Herbal biosphere serve diverse medicinal needs of the human, latex of C. procera was documented for lysis of Micrococcus lysodeikticus. Latex of C. gigantea also evaluated for nerve muscle potential. Rana hexadactyla was used to evaluate Nerve muscle activity after diluting milky latex in water (1:10 and 1:100 concentrations). Paralytic effect was showed in isolated nerve preparation and d-Tubocurarine and Acetylcholine was used as standard drug to compare contraction and relaxation. In isolated nerve preparation the nerve impulses were destroyed by plant latex quickly in reaction with nerve stimulation. Transmission with neuromuscular junction was stopped but still nerve muscle responded to the direct stimulation that was evident from contraction. Experiment confirmed that C. gigantea latex changed the permeability of membrane which was responsible to inward movement of K^+ and paralysis was happened due to stoppage of transmission through neuromuscular junction which confirmed that C. gigantea latex potential to block nerve muscle activity.[20]

Skeletal muscle activity

Latex of C. gigantea was diluted with water at ratio (1:1000, 1:500 and 1:100) and at different concentration it was tested in Rana hexadactyla for skeletal muscle activity by using Acetylcholine as standard drug. The outcome showed that either Acetylcholine or C. gigantea latex did not show any significant action whereas Acetylcholine in combination with C. gigantea (0.8 ml+0.8 ml) revealed better results and action is directly related to dilution of latex. The Skeletal muscle relaxant activity was observed to be 23 mm and 22 mm respectively at 1:1000, 1:100 dilution of the dose of 16 µg and at dilution of 1:500 of the dose 2 µg it was 13mm.[26]

Vasodilation activity

For evaluation of vasodilation activity latex of C. gigantea was diluted with water in proportion (1:10 and 1:100). Vasodilation activity was shown in dose dependent manner such as Cardiac output was 66% and 50% at 1:100 and 1:10 respectively. Study outcome confirmed that C. gigantea was vasodilator in nature.[27]

Wound healing activity

C. gigantea was employed traditionally for curing wounds. Dead space wound healing, excision and incision models used to evaluate wound healing activity at different doses of 400, 200 and 100 mg/kg p.o. All the parameters such as hydroxyproline content, granuloma breaking strength and wound breaking strength were statistical significant ($p<0.05$) as compare to Povidone Iodine and plant showed dose dependent Wound healing property. C. gigantea extract when tropically applied it significantly increased ($p<0.05$) wound contraction and healed completely by the end of 20th day as compare to standard Povidone Iodine. The scar area was reduced to 40.87 mm^2 ($p<0.05$) and the epithelization time was reduced to 18.67 days ($p<0.05$) compared to that of the standard. Plant extract also significantly increased ($p<0.05$) Hydroxyproline level. Dose dependent pattern was observed in dead space wound study and outcome was significant as compare to control and standard. [28]

Toxicity studies

C. gigantea is very popular in various traditional medicine but proper toxicity profiling is required to increase market value of formulations. Here is a glance of toxicity profiling of the plant. Sub-acute and acute study of C. gigantea flower extract was performed by in-vivo method in rats. Biochemical parameters (BUN, TG, TP, TB, ALBN, ALP, ALT, AST, creatinine and glucose) and Haematological parameters (NEUT, LYM, PLT, MCHC, MCH, MCV, HCT, HGB, RBC and WBC), organ weight

and total body weight were checked. 1000, 500 and 250 mg/kg doses were evaluated for sub-acute toxicity studies whereas 2000 mg/kg dose was checked for acute toxicity studies for a period of 30 days. Sub-acute (1000, 500 and 250 mg/kg) and acute toxicity (2000 mg/kg dose) study did not revealed any signs of mortality when evaluated with time bound activity. All biochemical and haematological parameters were normal throughout study.[30,31]

Acute toxicity study was also performed in antipyretic animal models and at 4600 mg/kg extract dose, 50% mortality was observed hence experiment was performed at 100,200 and 400 mg/kg dose levels. No mortality and toxic sign were observed for chloroform flower extract at 2000 mg/kg dose. So, Chloroform flower fraction at 2000 mg/kg proved to be safe.[32]

Acute toxicity and lethality (LD50) test was performed during expectorant, anti-asthmatic and anti-tussive study 500 and 250 mg/kg found safer dose for various water and ethanol extract

No signs of mortality and toxicity were noticed for acute dermal toxicity at 2000 mg/kg latex extract during wound healing experiments. No abnormal conditions were observed with ethanolic root extract hence doses were fixed as 400, 200 and 100 mg/kg respectively for wound healing study. [29]

Acute toxicity of various leaves fraction (methanol, chloroform, acetone and Pet ether) were evaluated and there was no lethal effects was observed, hence dose was optimised as 450 mg/kg of each extract for hepatoprotective study. In an experiment for Erlich′s ascites carcinoma for methanol, chloroform and pet ether fraction of C. gigantea root bark acute toxicity studies were performed at doses of 1600, 800, 400, 200 and 100 mg/kg of b.w. Doses were confirmed as 20, 40 mg/kg (chloroform fraction), 40, 80 mg/kg (pet ether fraction) and 10, 20 mg/kg (methanolic extract) respectively as there were no signs of toxicity.[25,20]

Acute oral toxicity study was performed with ethanolic root fraction of C. gigantea at a single dose of 2000 mg/kg in antidiarrheal experiment and the doses were determined as 400, 200 and 100 mg/kg respectively. Similarly Crude ethyl acetate flower extract was tested in Ehrlich′s ascites carcinoma with doses 3200, 1600, 800, 400, 200 100 mg/ kg b.w where LD50 of 2225.0 mg/kg b.w was produced. Swiss albino mice was used for the subacute toxicity studies at doses of 200, 100 and 50 mg/kg b.w, there were no significant abnormal changes were noticed in biochemical and haematological parameter at dose of 100 and 50 mg/kg but 200 mg/kg dose exhibited significant changes in both the parameters.[34]

Acute toxicity study was carried out while testing for anti-convulsant action on the various stem bark extracts (Aqueous, Methanol, Chloroform, Benzene and Pet ether). All parameter were normal and no signs of toxicity were noticed and the doses were fixed as 200 mg/kg for chloroform, benzene, aqueous extract and pet ether whereas 180 mg/kg for methanolic fraction.

2000 mg/kg dose of methanolic root fraction of C. gigantea flower was studied for acute toxicity study in a cardio protective study. The final dose selected in cardio protective study were 400 and 200 mg/kg which were double of one-tenth and one-tenth doses.[35]

Till date most of the published literature related to sub-acute and acute toxicity study of C. gigantea were summarized here. Since toxicity related to C. gigantea fruit and latex is known, hence essential research lies in the fields of chronic toxic models, sub-acute and also toxicity profiling in appropriate organ system in accordance with the pathophysiology studied for the active metabolites. If therapeutic potential of single active moiety is established then toxicity profiling with respect to appropriate human organ system becomes essentials which is important steps in process of new drug discovery. If this space is bridged up then C. gigantea is having an enough potential to be potent drug for various pathophysiological condition and it increase its market value worldwide.

Clinical trails

Any clinical trial was not reported on gigantea species and neither on Calotropis genus nor on C. gigantea as per www.clinicaltrails.gov.

Conclusion

The pharmacological activity such as wound healing, vasodilation, skeletal muscle, nerve muscle, fibrinolytic, hepatoprotective, cardioprotective, anti-ulcer, anti-oxidant, anti-microbial, anti-pyretic,

anti-inflammatory, anti-histamine, anti-diarrhoeal, anti-diabetic, anti-asthmatic, antiarthritic, anti-helminthic and analgesic activities were confirmed in crude fraction of various parts of the plant along with their chemical constituents and toxicity in some events.[32] In parallel to above findings pure active moiety such as 4'-O-β-D-gluco pyranosyl frugoside, frugoside and calotropin were reported for cytotoxic action in cell lines. Almost all traditional uses of plants is validated, formulation development, clinical trials and isolation of active compounds could be considered as future directions along with opportunistic approach for those studies. [33]

The profound Systematic Review on efficacy and safety C. gigantea mirrors the gap in toxicity profiling of active moieties in authentication with the conventional findings. The factors such as seasonal variation and geographical distribution play a crucial role in the substantiation of the active compound behind the pharmacological activity which also can be a field of attentiveness. Activities such as anti-ulcer, anti-arthritic, analgesic, anti-rheumatism and anti-inflammatory co-relate with each other by means of interrelated mechanistic pathways which can provide a clear view in relating the mechanistic pathways useful for defining a disease in-specific. Although the Systematic review may have the setback in respect to scientific data reviewed from journals other than English.

Abbreviations

Serum alkaline phosphatase (SALP) serum glutamate oxaloacetate transaminase (SGOT) Triglycerides (TG), p.o.- ,Very Low Density Lipoprotein (VLDL), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), PTZ, MES

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