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Review Article

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TOXIC ALKALOIDS IN *GLORIOSA SUPERBA*

Nubla M.*, Sajeena C.H.[1], Shiji Kumar P.S.[2] and Sirajudheen M.K.[3]

[1]Department of Pharmacognosy, Jamia Salafiya Pharmacy College, Malappuram, India- 673637.
[2]Department of Pharmaceutics, Jamia Salafiya Pharmacy College, Malappuram, India- 673637.
[3]Department of Pharmaceutical Analysis, Jamia Salafiya Pharmacy College, Malappuram, India-673637.

***Corresponding Author: Nubla M.**

Department of Pharmacognosy, Jamia Salafiya Pharmacy College, Malappuram, India- 673637.

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ABSTRACT

Aim of the review is to update the information regarding the toxic alkaloids in Gloriosa superba and their effect on body Gloriosa superba is a perennial creeper in the Liliaceae family It is widely used as a medicinal. plant and it has two toxic alkaloid namely colchicines and gloriosine are used in the treatment of gout and rhumatism G superba has long history. of use in folk medicine Whole plant of G superba keeps several biological. activities such as antioxidant antibacterial, antimicrobial, antihelminthic properties Fatal ingestion of the tubers of Gloriosa superba with an. intention of dibrate self harm,leading to systemic coagulopathy and progressive multiple organ dysfunction. Colchicine is known to cause alopecia The plant can be dangerous for cats,dogs,horses In chronic diseases that require life long treatment with. medications, adverse effects can arise with long periods of use This review include most update information about toxic alkaloids in G superba and their human use and poisonous effect.

KEYWORD: Pharmacological activities, Clinical effects in poisoning, Case reports.

INTRODUCTION

Herbal medicines are also on high demand in the developed world for primary health care, due to their effectiveness, safety and minor side effects. Plant origin herbal medication is considered a safe alternative to synthetic drugs.[1] According to the ancient proverbs "there is no plant on earth which has no medicinal property". A large numbers of plants used from ancient times as medicine.In recent times there is uplifting of interest and focus on the importance of medicinal plants ans traditional health systems.[2] The modern medicine has been developed so much improves to useful in curing many horrible diseases,but they are expensive.

Due to increase in population adequate supply of drug and high cost of treatment, side effects along with drug resistance has been encountered in synthetic drugs, which leads to elevated use of plants to treat human diseases.[3] The World Health Organization(WHO) has previously recognized to re-establish the tradinal knowledge of Medicine among our conventional theaters. Tradinal Knowledge since 200 B.C.in Ayurveda is very well Recognized especially in India among tribal people. In India the population of tribal people is around 53 million along With 555 tribal groups or communities, which are reside in Forest and surroundings. These people have enormous Indigenous knowledge which is possible tool to explore for novel cost-effective plants for medicine.[4]

Gloriosa superba Linn is an important medicinal plant belonging to the family *Liliaceae*. Which is the endangered species among the medicinal plants.[5] The medicinal important of *G.superba* is due to the presence of alkaloids in all part of the plant. It contain highly active alkaloids like colchicine, gloriosine, superbin chelidoric acid. Tuber part of plant is extremely poisonous. The colchicine is the major component in *G.superba* responsible for the toxic effect. It have inhibitory action on cell division(antimiotic) and also depress the action on the bone marrow. The noxious effect of colchicine include gasteroenteritis with nausea, diarrhoea with blood leading to dehydration, bone marrow toxicity with pancytopenia, hepatic and renal failure, hypovoluminous shock, hypo ventilation, muscleweakness, ascending polyneuropathy, cardiotoxicity, hypotension and alopecia. Severe poisoning causes death due to shock or respiratory failure. The alkaloid colchicine also responsible for the antigoutility and antirheumatic property of *G. superba*. The other pharmacological effects include anti inflammatory activity, antifungal activity, enzyme inhibitory activity, antitumor activity, antiprotozoal and anticoagulant activity.[6]

TAXONOMIC CLASSIFICATION

Kingdom : Plantae
Division : Magnoliophyta
Class : Liliopsida
Order : Liliales
Family : Liliaceae
Genus : *Gloriosa*
Species : *Superba*

Flowers

Seeds

Tubers

TAXONOMIC DESCRIPTION

Morphologically as enlisted in (Figure 1), *Gloriosa superba* is erect perennial, tuberous, scandent or climbing herbs with tendrils formed at the tip of the leaves. Stem is soft, leaves are sessile, spirally arranged or sub-opposite (6-7 x 1.5-1.8 cm) in dimension, lanceolate, acuminate, entire, glabrous; the upper ones with cirrhose tips. Flowers are axillary, solitary, large, borne on long, spreading pedicels, actinomorphic, hermaphrodite; lanceolate, keeled within at base, long persistent, yellow in lower half, red in upper half; stamens are spreading, hypogenous; anthers are extrose, medifixed, versatile, opening by longitudinal slits; ovary is superior, 3-celled; ovules are numerous; style is deflected at base, projecting from the flower more or less horizontally. The fruit is oblong containing about 20 globose red colored seeds in each valve.[7]

OCCURRENCE

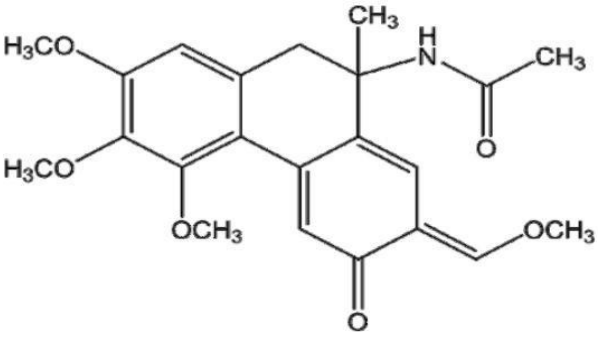
G. superba (Liliaceae) is a semi-woody herbaceous climber found throughout India upto an altitude of 6000 ft. It is a native of tropical Africa and is now growing in many parts of tropical Asia including India, Burma, Malaysia and Srilanka. It is now widely distributed throughout the tropics, and worldwide as a pot plant. In Africa, its distribution is from Senegal east to Ethopia and Somalia, and to South Africa. Gloriosa is national flflower emblem of Zambia. The altitudinal range of species is upto 2100 m above mean sea level and in India it is spread from hotter southern parts to the milder mid hill zones of Himachal Pradesh, Jammu Kashmir and Uttar Pradesh. It is known as 'Malabar glory lily' in English, in Hindi as 'Kalihari', in Sanskrit as 'Agnisikha' and its trade name is 'Glory lily'. Glory lily is an industrial medicinal crop in South India, for its high colchicine content, which is still collected from wild. Due to its over-exploitation in wild as well as problems faced during fifield cultivation, it was on the verge of extinction and was one of the endangered species among the most valued medicinal plants. Both its tuber and seeds have similar medicinal properties. Pharmacies and drug manufacturers often fulfifill upto 75% of their raw material demand from wild. Presently it is been cultivated in Tamil Nadu and other parts of South India. Various Botanical Research Institutes and Nurseries are also propagating this important plant. The fondness for flower beauty has also placed Kalihari as a pot plant in gardens.[8]

Phytochemistry

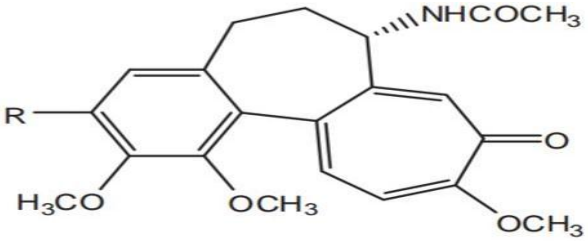
Photochemical studies show that all parts of the plant, especially the tubers are extremely toxic due to the presence of a highly active alkaloid, Colchicine. The species also structurally heterogeneous class of secondary biomolecules derived from basically five amino acids ornithine, lysine, phenylalanine, tyrosine and tryptophan.[9] Along with these two important alkaloids the other compounds such as lumicolchicine, 3-demethyl-N- deformyl-N- deacetylcolchicine, 3-demethylcolchicine, N-formyl deacetylcolchcine have been isolated from the plant.[10] *G. superba* seeds contain new colchicine glycoside, 3-O- demethylcolchicine-3-O-alpha-D- glucopyranoside. Colchicin, b-siltosterol, long chain fatty acids, b and g- lumiccolchicines, 2-hydroxy-6-methoxy benzoic acid from tubers and root while luterlin, N-formyl-deacetyl colchicines from flower have been isolated. Isolated, purified 3-monomeric monocot mannose-binding lectins from *G. superba* evaluated for antipoxviral activity.[11] *G. superba* is also known for its

colchicines content which finds use to treat arthritis.[12] Biosynthetic enhancement of colchicines production on the root culture of *G. superba* by aluminium chloride as an elictor was successfully observed25 Colchicine is synthesized using mainly aromatic amino acids such as tryptophan, phenylalanine and tyrosine.

Biosynthesis of colchicine studied using in vitro supply of exogenous precursor from *G. superba* and B5 medium.[13]

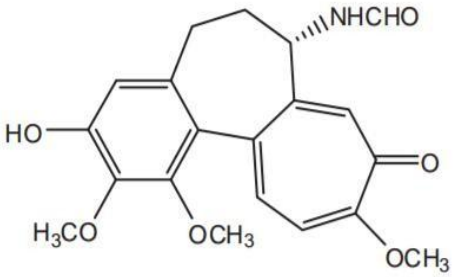


Colchicine

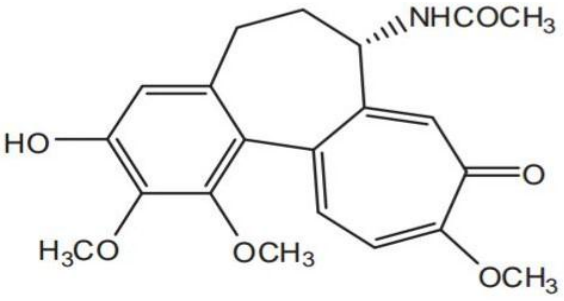


$R=OC_6H_{11}O_5$

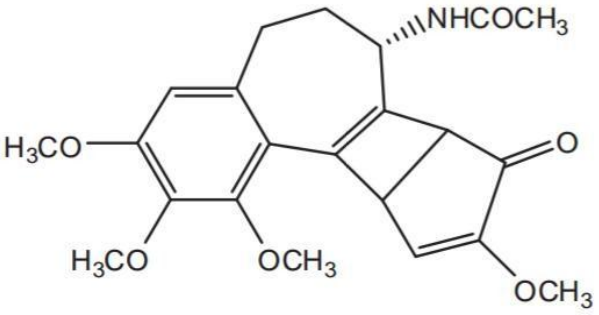
Colchicoside



3-demethyl-N-formyl-Ndeacetylcolchicine



3-demethylcolchicine



Lumicolchicine

Colchicine

It is conventional drug for gout obtained from corms of *G. superba* and *Colchicum autumnale* (Thakur et al., 1975; Sivakumar and Krishnamurthy, 2002). The term "colchicine" is derived from areaknown as Colchis near black sea. *C. autumnale* grows wild in Europe and Africa. Thomson was the first who proposed early idea of action of colchicines in gout treatment. Gout and uric acid metabolism is same way linked and colchicines might act on this and it is caused by deposition of microcrystals of uric acid in joints and may be due to defective regulatory mechanism for endogenous purine synthesis but contradictory result for the action of colchicine on synthesis and extraction of urates have been recorded, colchicines interrupt, the cycle of new deposition which seem to be indispensable for the persistence of acute gout. Distressing side effect has also been recorded sporadically but colchicines remain the drug for acute gout.

Modification of the side chain of rings does not abolish anti-gout activity as long as the configuration of C-ring confirms to that of colchicines. It also acts as anti-mitotic and anti-gout agent. It blocks or suppresses cell division by inhibiting mitosis. It inhibits the development of spindles as the nuclei are dividing (spindles are formed by the polymerization of tubuline) from a pool of subunit during a detached phase of cell-cycle and then depolymerized during other phase. It is also used to induce polyploidy initiation, occasionally other mutations also occur like chlorophyll mutations, but frequency is low.

It can solve an important problem of fuchsia breeding. Most of the fuchsia species are diploid or tetraploid, a crossing between diploid and tetraploid result often in a triploid, which is mostly sterile because the process of meiosis (cell division for reproduction) requires the coupling of similar chromosomes and there is no mechanism allowing for the alignment of three similar chromosomes, triploid plants are not able to produce fertile reproductive cells. They are, therefore, sterile and unusable as parents. A special problem of colchicines induced ploidy, particularly in vegetatively propagated crops, is the chimerism caused by the instantaneous presence of tissue of different ploidy levels in one plant or plant parts.

Colchicine is mostly used in its freshly prepared aqueous form. The range of concentration of colchicine applied varies from 0.006- 3%, concentration of about 0.05% is the most commonly used (Milne and Meek, 1998). Kannan et al. (2007) studied optimization of solvents for efficient isolation of colchicines from *G. superba*. The maximum yield of colchicine was obtained when it is extracted with water and alcohol in the ratio of 50:50. Bellet and Gaignault (1985) reported the production of colchicinic substance from *G. superba*. The colchicines-like activity of *G. superba*-extracted for mosquito (Diptera: Culicide) in which four fractions i.e. hexane fractions, dichloromethane fraction-1, dichloromethane fraction-2 and methanol fraction were investigated. The latter three fractions yielded hopefully high colchicine like activity, whereas hexane fraction yielded very low activity.Ghosh et al. (2002) studied the root culture of *G. superba* by using direct and indirect precursor of the biosynthetic pathway for the enhancement of colchicine production. They successfully used aluminium chloride as an elicitor, in which they have used root cultures of *G. superba* treated with 5 mM methyl jasmonate and 125 µM AlCl3. The enhancement of intracellular colchicine content was observed in the roots by 50-fold and 63-fold respectively.

Ghosh et al. (2006) reported that colchicine can also be applied in the lanolin paste or as a solution, for instance, on a cotton dot, placed in a leaf axil. Khan et al. (2007) evaluated the enzyme inhibition activities of *G. superba* rhizomes extract against lipoxygenase, actylcholinesterase, butyrycholinesterase and urease in which wonderful inhibition was observed on lipoxygenease. Further, Khan et al. (2008) reported antimicrobial potential of *G. superba* extracts in which excellent antifungal activity was confirmed against *Candida albicans*, *C. glabrata*, *Trichophyton longifusus*, *Microsporum canis* and *Staphylococcus aureus.*

Colchicine is synthesized using mainly aromatic amino acids such as tryptophan, phenylalanine and tyrosine. Key enzymes involved in colchicine metabolism are tyrosine ammonia lyase (TAL) and phenylalanine ammonia lyase (PAL). Sivakumar and Krishnamurthy (2004) reported the biosynthesis of colchicine, the *in vitro* supply of exogenous precursor using B5 medium from *G. superba* calluses. The maximum amount of colchicine i.e. 9.0 mg was detected in the medium fed with 30 µM tyrosine. The activity of TAL was higher than that of PAL and a low frequency of tracheary elements was observed.[14]

Medicinal importance of *G. superba* according to the different communities.

Communities	Plant Parts	Uses
Santal	(i) Tuberous Root (ii) Plant (iii) Leaf	Abortifacient, intermittent fever, wound infections. Syphilis, tumors, Spleen complaints, Asthma.
Munda and Oraon	Tuber	Antifertility purpose.
Ethnic Communities of North-East India	Root	Gout, stomachache, as a tonic.
Ethnic Communities of Bihar	Root	Cholera, to facilitate childbirth.
Ethnic Communities of Dehradun and Siwalik	Root	Anthelmintic.
Ethnic Communities of Garhwal	Tuberous root	Abortion.
Tribes of Varanasi	Root	Gout, rheumatisms.
Tribes of Pithoragarh	Tuber	Gonorrhea, leprosy, piles.

Medicinal importance of G. Superba according to the sources of literatures

Sources of Literature	Plant Parts	Uses
Charak Samhita	Plant	Useful in itching, skin diseases and ailments caused by kapha and vata.
Sushruta Samhita	Root	To relive from postnatal complaints.
Rajanighantu	Plant parts	Pungent, thermogenic, eliminates deranged kapha (phlegm) and vata (wind), terminates pregnancy.
Dhanvantari Nighantu	Plant parts	Leprosy, labor pain, wound infections, purgative.
Maudanani Nighant	Plant parts	Bitter, pungent, thermogenic, abortifacient, skin infections.
Bhavaprakasha	Plant parts	Aperient, alkaline, astringent, pungent, bitter, highly potent light abortifacient, excites pitta (bile), cures dropsy, piles, wounds, acute spasmodic pain, removes warms.
Chakradatta	Root-paste	If smeared over the palms and feet of pregnant women, delivery of child becomes easier.
Ayurveda	(i) Roots	Abortifacient, acrid, anthelmintic, antipyretic, bitter, depurative, digestive, emetic, expectorant, purgative, stomachic, tonic, thermogenic, promoting labor pain, expulsion of placenta.
	(ii) Leaf juice	Effective against paralysis, rheumatism, snakebite, insect bites, asthma.
Siddha	Root & Tuber	Various skin diseases.

Ethnomedicinal / Traditional Uses

Gloriosa superba is a well known ethnomedicinal plant which is used in Ayurveda. Its use in the Indian traditional folk medicine is also well documented. Plant pacifies vitiated kapha, indigestion, fever, arthritis, obstructed labor, cardio-myopathy, skin diseases, in higher dose or without purification, it is highly poisonous. In Ayurveda and yunani systems of medicine, the tuber of plant is well known due to its pungent, bitter, acrid, heating, anthelmintic, laxative, alexiteric and abortifacient nature. It is widely used in the treatment of ulcers, leprosy, piles, inflammations, abdominal pains, intestinal worms, thirst, bruises, infertility and skin problem. However, ingestion of all parts of the plant is extremely poisonous and can be fatal. The tuber also claims antidotal properties to snake-bite and in India it is commonly placed on window sills to deter snakes. Many cultures believe the species to have various magical properties. Tribals crush roots of the plant in water and apply on head for curing baldness. To avoid painful delivery, Gonds and Bharias of Patalkot, apply rhizome extract over the navel and vagina. It induces labour pain and performs normal delivery. According to Bhumkas of Patalkot, this dose may lead to abortion if given to a lady with pregnancy of 1 or 2 months. Since the rhizome is having abortive action, this is prescribed for normal delivery.[2]

The current status

The leaf juice of G*loriosa* is used to kill-lice in hair, tubers contain the bitter principles, superbine and gloriosine, which in large doses are fatal; however, in small doses they are used as tonic, antiabortive, and purgatives. The white flour prepared from the tubers is bitter and used as stimulant. It is given with honey in gonorrhea, leprosy, colic and intestinal worms and for promoting labor pains, a paste of tubers is applied over the suprapubic region and vagina. Its warm- poultice is locally applied in rheumatism and neuralgic pains (Samy et al., 2008). Medicinally, the tuber is used as abortifacient, and in small dose it acts as a tonic, stomachic and anthelmintic. It is also used in the treatment of gout because it contains colchicine. Paste of the tuber is externally applied for parasitic skin diseases.

Samy et al. (2008) conducted ethno botanical survey of folk plants for the treatment of snakebites in southern part of Tamilnadu, India, in which traditional approach was evaluated methodically with some selected plant extracts which showed potent neutralizing effect against the venom. The conventional method of propagation is through corms, since poor seed germination restricts their use in multiplication. Therefore, propagation by tissue culture technique is necessary (Finnie and van Staden, 1989, 1991). *G. superba* contains about 0.1- 0.8% colchicine in bulb which is used in plant breeding to induce mutation and polyploidy and also can solve an important problem in fuchsia breeding. *G. superba* also produces another alkaloid gloriosine. Colchicines affect cell membrane structure indirectly by inhibiting the synthesis of membrane constituent. It binds to tubelin preventing its polymerization into microtubules. This anti-mitotic property disrupts the spindle apparatus that separate chromosomes throughout metaphase, cells with high metabolic rates are most implicated by the arrest of mitosis. Kumar (1953) studied doubling of chromosomes induced by gloriosine isolated from *G. superba.*

Jitpakdi et al. (1999) screened ten plant species including *G. superba* for metaphase chromosome preparation in adult mosquitoes (Diptera: Culicidae) using an inoculation technique for colchicine like substances using a mosquito cytogenetic assay have shown the increased metaphase chromosome. Due to over exploitation for its diverse medicinal applications *G. superba* has been endangered, therefore, there is urgent need to conserve the plant by biotechnological approaches like tissue culture. (Rajgopalan and Khader, 1994). This approach

has been very important because it provides complete sterile and virus-free plants by rapid multiplication. *G. superba* is now promising as an industrial medicinal crop in Asia particularly in South India for its high colchicine content. For commercial production of colchicine and its derivatives, natural production from *in vitro* methods of the source plant are thus of great attention. In the past two decades, focus has been on plant biotechnology as a potential alternative production method, using cultured cells rather than plants.[15]

Source of precious alkaloids

Gloriosa superba produces two important alkaloid colchicine and gloriosine, which are present in seeds and tubers while the other compounds such as lumi-colchicine, 3-demethyl-N-deformyl-N-deacetylcolchicine, 3- demethylcolchicine, N-formyl deacetylcolchcine have been isolated from the plant (Sugandhi, 2000; Suri et al., 2001). Suri et al. (2001) reported new colchicine glycoside, 3-O-demethylcolchicine-3-O-alpha-D-glucopyranoside in *G. superba* seeds. Kaur et al. (2007) studied purification of 3-monomeric monocot mannose-binding lectins and their evaluation for antipoxviral activity isolated from *G. superba.* Alkaloids are structurally heterogeneous class of secondary biomolecules derived from basically five amino acids ornithine, lysine, phenylalanine, tyrosine and tryptophan (Thakur et al., 1975). Thakur et al. (1975) reported the substances from plant of the sub family Wurmbaeoideae and their derivatives along with alkaloids from the *G. superba*.[16]

Conservation by means of in vitro propagation

Gloriosa superba usually multiply by corm and seeds but due to low germination capability it restricts for the regeneration. Therefore, in order to safeguard and preserve this important plant biotechnological approaches would be very useful (Sivakumar and Krishnamurthy, 2002). The conventional method of propagation has many disadvantages as 50% of the yield has to be set aside for raising the next crop, transmittance of soil-borne diseases from one crop to the next, and from one location to another and during the 2-3 month storage period between harvest and the raising of next crop (Mrudul et al., 2001).[17]

Kala et al. (2004) studied the prioritization of medicinal plants on the basis of available knowledge, existing practices and use value status in Uttaranchal, India in order to understand the pattern of indigenous uses of medicinal plants available in the Uttaranchal state, India and documented 300 species including *G. superba*. Hassan and Roy (2005) reported 92% of the cultures of apical and axillary buds of young sprout from naturally grown *G. superba* plants regenerate four shoots per culture in MS basal medium fortified with 1.5 mg/L BA + 0.5 mg/L NAA.[18]

Custers and Bergervoet (1994) reported micropropagation of *G. superba* by shoot cuttings and explants from node, internode, leaves, flowers, pedicels and tubers. *G. rothschildiana* (duphur) vs. *G. rothschildiana* (new accession) and *G. rothschildiana* vs. *G. superba* were cultured on MS basal medium with 3% w/v sucrose, 0-10 mg/L Benzyl Adenine (BA) and 0.1 mg lndole Acetic Acid (IAA) and maintained at 24 days under 16 hours photoperiod. Addition of low level of Benzyl Adenine (BA)(1 mg/L) improved plant growth, whereas the high level of BA (10 mg/L) caused proliferation of multiple shoots, from rhizome meristem, by applying alternatively high and low BA level, a method of continued propagation was achieved which resulted in a 4-7 fold multiplication of qualitatively good plantlets every 18 week. The resulting shoots were incubated on MS medium, with 3% sucrose and 0-1 mg/L IAA or NAA. Transplantation into soil was only possible after the plants had formed.[19]

Samarajeewa et al. (1993) studied clonal propagation of *G. superba* from apical bud and node segment of shoot tip, cultured on solidified agar (0.8% w/v) Gamborg's B5 medium containing BA, IAA, Kinetin, NAA, IBA or 2,4-D. The cultures were maintained under fluorescent light at 25-27ºC. Primary cultures were initiated in solid B5 medium containing 0.5 to 1 mg/L BA and 0.01-0.5 mg/L IAA, IBA, NAA when shoot tip of primary cultures were transferred to shoot multiplication media, shoot proliferation occurred via adventitious bud formation within 4-8 weeks.[20]

Somani et al. (1989) reported *in vitro* propagation and corm formation in *G. superba*. The fresh sproutswere excised from corms of *G. superba* and dissected propagules with shoot and root primordia were placed on MS basal medium (Murashige and Skoog, 1962) containing 3% sucrose and 0.6% agar. Explant germinated on the MS medium producing shoot and root, which formed new corm within one month. For shoot and cormlet regeneration, 1-4 mg/L kinetin was added to the medium. Cultures were maintained at 25ºC in white fluorescent light (2500 lux) with an 8- h/day photoperiod.[21]

Sivakumar and Krishnamurthy (2002) reported *in vitro* organogenetic responses of *G. superba.* They used MS medium supplemented with ADS and BA, 98%. The callus induction occurred in non-dormant corm bud explants. The maximum number of multiple shoot (57%) was observed in corm-derived calluses.[22]

Gupta (1999) compared the production of different colchicinic substances from *G. superba* and *C. autumnale.* He reported extensive range of these colchicinic compounds like colchicines (0.9%), dimethyl-3-colchicine (0.19%), colchicoside (0.82%) and their formyl derivatives from *G. superba*. While these values were found to be less in case of *C. autumnale* which were reported as 0.62%, 0.9%, and 0.39% respectively. Sivakumar and Krishnamurthy (2002, 2004) studied induction of embryoids from leaf tissue of *G. superba*.

The nodular calli were observed on S.H. medium supplemented with 2,4-D and 1 isopentyldene. Gupta et al. (1999) found hepatoprotective activity of *G. superba*.[23]

Jha et al. (2005) reported production of forskolin, withanolides, colchicine and tylophorine from plant source by using biotechnological approach.[24]

PHARMACOLOGICAL ACTIVITIES

Antimicrobial activity

Haroon *et al.*, reported antibacterial and antifungal activity of methanolic extract and its subsequent fractions in different solvent systems. The study claimed that n-butanol fraction showed excellent antifungal potential against *candida albicans* and *candida glaberata* (up to 90%) and against *trichophyton longifusus* (78%) followed by chloroform fraction against *microsporum canis* (80%). The chloroform fraction demonstrated highest antibacterial activity against *staphylococcus aureus* (69.4%).[25]

Enzyme inhibition activity

Haroon *et al.*, reported the enzyme inhibition activity of alcoholic extract of G. superba Linn rhizomes. The alcoholic extract and its subsequent fractions in chloroform, ethyl acetate, n-butanol, and water were investigated against lipoxygenase, acetylcholinesterase, butyrylcholinesterase and urease. The chloroform extract represented maximum inhibition potency (90%) on lipoxygenase and 29.10% inhibition potency on butyrylcholinesterase. The ethyl acetate fraction showed highest inhibition potency (83.50%) on acetylcholinesterase. However, urease was not inhibited by any of the tested fractions.[26]

Treatment of snakebite

Ramar Perumal Samy *et al.*, claimed the use of *G. superba* tubers paste in the treatment of snakebite. The study reported that purified fraction (2.4 mg/kg, body weight) significantly inhibited the toxic effects of snake venom induced changes in serum SOD and LPx levels in mice.[27]

Analgesic and anti-inflammatory activity

Jomy C. John *et al.*, reported the analgesic and anti-inflammatory activity of hydroalcoholic extract obtained from dried aerial parts of *G. superba* employing Eddy's hot plate method and acetic acid induced writhing method for determination of analgesic potential; cotton wool granuloma and carrageenan induced paw edema model for anti-inflammatory activity. The study claimed that the treatment of mice at 100, 200, and 400 mg/kg body weight exhibited significant ($P<0.01$) increase in reaction time. The maximum percentage protection was observed at 90 min for all the three doses. The % inhibition of writhes were 64.09%, 78.56% and 81.45% at dose of 100, 200, and 400 mg/kg body weight. The dose of 200 and 400 mg/kg exhibited significant results in carrageenan induced paw edema model ($P<0.05$) as compared to control. The rats exhibited 9.59%, 28.72% and 45.8% inhibition of granuloma mass formation after 7 days of treatment with doseof 100, 200, and 400 mg/kg body weight.[28]

Neuroprotective activity

V. Uma Rani *et al.*, reported neuroprotective activity of hydroalcoholic extract obtained from tubers of *G. superba*. The study revealed that the extract of *Gloriosa superba* Linn decreased the transfer latencies, strengthened its memory improvement action in drug treated rats. Hence showed decrease in muscle strength measured by rota-rod test whereas, in hydroalcoholic extract of *Gloriosa superba* treated group there was improvement in muscle strength. The locomotor activity assessed by actophotometer and open field test was decreased in lead nitrate group compared with hydroalcoholic extract of *Gloriosa superba Linn* treated group. Biochemical analysis of brain revealed that the chronic administration of lead nitrate significantly increased lipid peroxidation and decreased levels of catalase (CAT), reduced glutathione (GSH) and glutathione reductase (GR), an index of oxidative stress process. Administration of hydroalcoholic extract of *Gloriosa superba Linn* attenuated the lipid peroxidation and reversed the decreased brain CAT and GSH levels. Lead exposed rats showed increased levels of various serum parameters like glucose, ALT, ALP, TG and TC.[29]

Anti-arthritic activity

K.P. Latha *et al.*, reported the anti-arthritic activity of chloroform extract obtained from tubers of *G. superba* using Freund's complete adjuvant induced arthritis model in rats. The study demonstrated that chloroform extract of tubers of *G superba* has shown a dose dependent and significantly decreased paw edema and ankle diameter in treated groups as compared with arthritic group.[30]

Anticoagulant activity

Nalise Low Ah Kee *et al.*, reported anticoagulant/anti-thrombotic potential of methanolic extract obtained from leaves of *G. superba*. The study proclaimed that leaf extract of *G. superba* inhibited thrombin-induced with IC50 values of
2.97 mg/ml.[31]

Anticancer activity

Samson Eugin Simon *et al.*, reported the anticancer activity of phytochemical extract obtained from tubes of *G. superba* against Hep-G2 cancer cell line (Human liver cancer cells) employing MTT assay. The study revealed that concentration of 100µg of plant extract has maximum inhibition value of 54.3% against Hep-G2 cancer cell line.[32]

Toxicity/Poisoning

The colchicine which is major component of *G. superba* is mainly responsible for toxic effect.[21] The commonest clinical presentation of poisoning is severe gastroenteritis

with nausea, vomiting, diarrhoea with blood leading to dehydration, hypovolaemic shock and acute renal failure. Muscle weakness, hypoventilation, ascending polyneuropathy, bone marrow depression and coagulation disorders are the other features of poisoning. Death in severe poisoning occurs due to shock or respiratory failure although haemorrhagic or infective complications may cause death after the first day.[33]

CLINICAL EFFECTS IN POISONING

1. Cardiovascular: There is no direct effect on the heart, but fluid and electrolyte loss, often causes hypovolaemic shock manifested by hypotension and tachycardia.
2. Respiratory: Respiratory failure is thought to be due to the paralysis of intercostal muscles rather than the direct depression of the respiratory centre by colchicine.[34]
3. Central nervous system (CNS): There is progressive paralysis of the central nervous system and peripheral nervous system[35]
4. Peripheral nervous system: Ascending polyneuropathy, weakness, loss of deep tendon reflexes may be described.
5. Skeletal and smooth muscle: Colchicine could have a direct toxic effect on skeletal muscles causing muscular weakness. Rhabdomyolysis may occur with significant increase in muscle enzymes and myoglobinuria as a result of direct muscular damage. Muscle weakness that may persist for many weeks may contribute to respiratory deficiency.[36]
6. Gastrointestinal: Gastroenteritis including nausea, vomiting, diarrhoea with blood accompanied by colic and tenesmus. Loss of fluids and electrolytes leads to hypovolaemia. Intestinal ileus may develop within the first few days and may persist up to a week.[37]
7. Hepatic: Colchicine may exert direct hepatic toxicity with moderate cytolysis.
8. Renal: Any direct toxic effect of the toxin on kidney is not clear. Renal failure is probably secondary to excess fluid loss or hypovolaemia and is preceded by oliguria and haematuria. Proteinuria could also occur[38].
9. Endocrine and reproductive systems: Vaginal bleeding has been reported as a feature of intoxication. Tubers are used as an abortifacient in some countries.
10. Dermatological: Alopecia usually occurs one or two weeks after the ingestion of *G. superba*. A case of generalized depilation has also been reported Eye, ear, nose, and throat: Subconjunctival haemorrhages have been observed. Burning and rawness of the throat may be early symptoms of toxicity.
11. Fluid and electrolyte disturbances: There is an extensive fluid and electrolyte loss due to intense vomiting and diarrhoea or sometimes due to haemorrhages. Hypokalaemia, hypocalcaemia, hypophosphataemia and hyponatraemia may occur.
12. Haematological: Colchicine has a depressant action on the bone marrow which is characterized by a transient leucocytosis followed by leucopenia. It could also cause thrombocytopenia that may give rise to various coagulation disorders resulting in vaginal bleeding, conjunctival and gastrointestinal haemorrhages. Severe thrombocytopenia occurring within 6 hours of poisoning has been documented. Anaemia may occur, mostly secondary to haemorrhages.[39]

CASE REPORTS

Toxic encephalopathy due to colchicine-Gloriosa superba poisoning CASE REPORT -1

A 28-year-old woman, previously healthy, presented with abdominal pain, diarrhoea and profuse vomiting. Six hours before, she had eaten G superba tubers when attempting to end her life after a domes tic dispute. On admission, she was stable; we gave activated charcoal and treatedher symptomatically. After initially improving, on the 5th day she developed generalised tonic-clonic sei zures. She was hypocalcaemic, with serum ionised calcium of 0.9 mmol/L (1.1–1.4). We gave intravenous calcium and anticonvulsants and achieved seizure control.

On day 7, her level of consciousness declined to a Glasgow coma scale score of 6 out of 15. There were no focal neurological signs. Her full blood count, arterial blood gas tensions, urinalysis, erythro cyte sedimentation rate, serum C-reactive protein, urea, electrolytes, glucose, ammonia, calcium and magnesium were normal. Her serum transaminases were transiently mildly elevated: serum aspartate ami notransferase 278 U/L (1–31) and alanine aminotrans ferase 216 U/L (5–35). CT scan of head, lumbar puncture, ultrasound scan of pelvis and abdomen were normal. Serology for Epstein-Barr virus, cyto megalovirus, Japanese encephalitis (JE) virus, and herpes simplex virus were negative. EEG showed bilat eral diffuse slow waves, suggestive of encephalopathy. MR scan of her brain (figure 3) showed bilateral T2 hyperintensities in the basal ganglia. We managed her supportively in the intensive care unit. By day 15, her Glasgow coma scale score had improved to 11/15 and remained static up to the point of the current report. She also developed alopecia.

DISCUSSION

This case highlights a delayed toxic encephalopathy following Gloriosa poisoning. Based on the unequivo cal history of ingestion of Gloriosa tubers, the extra central nervous system clinical features supporting Gloriosa toxicity, the temporal profile of the onset of encephalopathy and the exclusion of other neoplastic, immune, infective and metabolic causes for encephal opathy, we conclude that her encephalopathy resulted from colchicine toxicity following Gloriosa tuber ingestion. However, we had no facilities to assess serum levels of colchicine. Patients with colchicine poisoning occasionally develop confusion and sei zures, but all such cases have concomitant renal or liver dysfunction. By contrast, our patient had no significant metabolic

derangement. The other unusual feature of her encephalopathy was the delayed onset, reminiscent of that following anoxic encephalopathy and carbon monoxide poisoning.MR brain scan findings of bilateral T2 hyper inten sities in the caudate and lentiform nuclei of the basal ganglia in this patient are consistent with a toxic encephalopathy, further strengthening the case for a Gloriosa- induced encephalopathy. The thalamic regions were unaffected. The putamen and globus pallidus of the basal ganglia have abundant mitochondria, neurotransmitter and chemical content. They are also extremely metabolically active and are exceedingly prone to metabolic and toxic damage.

The basal ganglia lesions in toxic and metabolic encephalopathies are global and symmetrical, as in this lesions without concomitant thalamic involvement occur with only a few causes of toxic encephalopathy, suchas hyperammonaemia and hypoglycaemia:we excluded both these in this patient. Infectious disease can also cause basal ganglia abnormalities, but such lesions are usually more asymmetrical and patchy. It is also possible that she had ingested another neurotoxic agent along with the G superbaGloriosa's active toxic compound is the alkaloid col chicine, which binds to tubulin and inhibits its poly merisation into microtubules. Colchicine's toxic effects are mediated through microtubule disruption, which, in neural cells, interferes with axonal and den dritic transmission. In experimental and animal models, colchicine causes the neuronal cell death of certain neuronal populations, such as the granular cells of the dentate gyrus and pyramidal cells of the hippocampus. Colchicine may induce an autotoxic response, leading to neuronal death of certain populations, including the basal ganglia, due to the accumu lation of a toxic cellular product which is normally transported by a microtubule-dependent process.

This may be the explanation for the delayed onset ofthis patient's encephalopathy, as a time lag may be evident before a critical neuronal toxic concentration of these substances is reached. The concentration of colchicine in cerebral tissue is usually low following poisoning, perhaps explaining why, despite these experimental findings, clinical cases of colchicine poising are so rare.[40,41,42,43]

Colchicine cardiotoxicity following ingestion of Gloriosa superba tubers CASE REPORT-2

A 29 year old man who attempted suicide by ingesting tubers of Gloriosa superba was admitted 4 hours later to Teaching Hospital Peradeniya, complaining of burning in the mouth and throat, intense thirst, nausea, vomiting and abdominal colics. On examin ation he was restless, afebrile and dehydrated. Pulse rate was 90 beats/min. Blood pressure was 100/ 70 mmHg and respiratory rate was 20 per minute. Gastric lavage was performed and he was given intravenous fluids. Except for an elevated haematocrit (PCV = 0.541/1) his biochemical and haematological investigations were normal on admission. He was oliguric for 24 hours. By the second day the blood urea increased to 10.3 mmol/l and urinalysis showed proteinuria and mild haematuria. He developed a watery diarrhoea, complained of severe body ache and generalized chest pain. An electrocard iogram done at this stage was normal. Serum aspartate transaminase was 10IU/I (normal 0-12). Serum alanine transaminase was 20 IU/I (normal 0-11), serum bilirubin was 17 mmol/l, plasma potassium was 4.2 mmol/l and plasma sodium was 138 mmol/l. On the third day the patient complained of severe pain all over the chest associated with difficulty in breathing. His respiratory rate was 38/min, pulse was 100 beats/ min regular, and blood pressure 110/70 mmHg. He had a triple rhythm and bilateral basal crepitations. The electrocardiogram (Figure 1) showed ST eleva tion. Aspartate transaminase rose to over 60 IU/I (normal 0-12), creatine kinase was 40/IU/l (normal 0-12) and serum cholesterol was 5.0 mmol/l.

He was treated with analgesics and frusemide. He continued to complain of chest pain on the fourth day. The electrocardiogram showed further T elevation (Figure 2). By the fifth day he developed bleeding gums and subconjunctival haemorrhages and haematuria. At this stage haematological investi gations showed haemoglobin 9.5 g/dl, white cell count 2.8 x 99/1, platelet count 20 x 109/1, bleeding time 3 minutes, clotting time 11 minutes, prothrombin time 18 seconds (control 15 seconds). He was transfused three pints of fresh blood in the next 48 hours. Serum aspartate transaminase and creatine kinase returned to normal by the ninth day. The electrocardiogram showed only inverted T waves in V5. His condition gradually improved and 3 weeks afteradmission he was transferred to the Department of Psychiatry for further management.

Biochemical and haematological investigations and the electrocardio gram (resting and in response to exercise) were normal.

DISCUSSION

There does not appear to be a clear separation of non-toxic, toxic and lethal dosages of colchicine.2-5 This patient had ingested about 100 grams of tuber. Dunuwille and others' have estimated that 10 g of fresh tuber contains approximately 6.0 mg of col chicine. Therefore the amount of colchicine ingested by this patient is in the region of 60 mg. Fatalities have been reported with doses as small as 7 mg.[44,45,46]

Massive generalized alopecia after poisoning by Gloriosa superba CASE REPORT-3

The patient, a married woman aged 21 years, was admitted on 17 September 1964 at about 2 p.m. to the University Medical Unit, General Hospital, Kandy. She had fallen ill after an afternoon meal the day before which consisted of a variety of boiled yams. The plant and the ingested tubers were identified as Gloriosa superba. The colchicine content of the tubers is 0.3%. Therefore, as the patient had eaten about 125 g. of tubers,

the total amount of colchinine taken was in the region of 350 mg. About two hours after this meal she had started vomiting, and about eight hours later had profuse watery diarrhoea, which continued throughout the night. She had vomited 25 times that night and had 20 watery stools. The patient had no children. Her past history revealed nothing of note. She attained puberty at 13 and her periods were regular.

She had no physical abnormalities. Her hair had never been cut since childhood, as is the custom among Ceylonese girls, and it came down to her knees. On admission she was unconscious, restless, dehydrated, and collapsed. Her pulse rate was 122/min., of moderate volume and in sinus rhythm; blood-pressure was 95/70 mm. Hg, and respiratory rate was 18 per minute. Apart from these findings, nothing abnormal was noted. She had no cyanosis or dyspnoea. Her white-cell count was 8,800/c.mm., with a differential count of polymorphs 76%, lymphocytes 21%, eosinophils 3%. Her blood urea was 37 mg./100 ml.; serum potassium 3.7 mEq/litre; serum sodium 142 mEq/litre. She did not pass any urine on the day of admission. On the day of admission she was treated with a slow intravenous drip-infusion of 3 pints (1.7 litres) normal saline and 1 pint (0.6 litres) 5% dextrose with added vitamins. Her condition improved on this regimen. Blood-pressure rose to 100/70 mm. Hg, and the pulse rate fell to 1 10/min. The following morning she collapsed again, the blood-pressure could not be recorded, and the pulse was imperceptible.

Hydrocortisone hemisuccinate, methoxamine, and noradrenaline were then added to the drip. Two days after admission, the day after her collapse in the ward, her general condition- improved, blood-pressure was 90/70 mm. Hg, pulse rate 104/min., of moderate volume, and in sinus rhythm. No other abnormalities were detected clinically. Examination of her urine showed " pus cells-field full," probably because she was catheterized previously. She was given sulphadimidine and the urine report came back to normal in four days. Blood urea was 39 mg./100 ml. Five days after admission the patient was found to have a subconjunctival haemorrhage in her left eye. Her menstrual period, which was ending the day she ate the tubers, continued for a further 20 days. A platelet count carried out at this stage was 475,000/ c.mm.; white-cell count was 5,000/c.mm., with polymorphs 50%, lymphocytes 49%, eosinophils 1%, and packed cell volume 35%. Haemoglobin was 11.3 g./100 ml., and mean corpuscular haemo globin concentration 30 mg./100 ml. Twelve days after admission marked alopecia was noticed, especially affecting the scalp hair (Fig. J), and within two days most of the hair on her scalp had dropped out, as had her axillarhair and part of the pubic hair. She was seen in the clinic subse quently, and within a week after her discharge from hospital, 23 days after admission, she was completely bald. Two months - later her scalp hair had regrown to half an inch (12.7 mm.) (Fig.2). Pubic hair showed regrowth. Her axillary hair remained very scanty. After five months her scalp hair was 2-3 in. (5.1-7.6 cm.) long.[47,48,49,50]

CONCLUSION

Application indigenous natural products has been alternative way to replace synthetic medicine. *Gloriosa superba* is a well known ethnomedicinal plant which is used in Ayurveda. Photochemical studies of *G. superba* showpresence of a highly active alkaloid, Colchicine. FDA- approved use of colchicine is to treat gout (it is one of the active ingredients of anti-gout tablets marketed by Merck & Co.), though it is also occasionally used in veterinary medicine totreat cancers in some animals. It is also used as an antimicrobial, antifungal, anticoagulant, antilipoxygenase agent and antidote in snake bite. However, ingestion of all parts of the plant is extremely poisonous and can be fatal. The commonest clinical presentation of poisoning is severe gastroenteritis with nausea, vomiting, diarrhoea with bleading to dehydration, hypovolaemic shock and acute renal failure. *Gloriosa superba* usually multiply by corm and seeds but due to low germination capability it restricts for the regeneration. Therefore, in order to safeguard and preserve this important plant biotechnological approachs would be very useful. Micropropagation of *Gloriosa superba* meets ever increasing demands for colchicine. The availability from both wild and cultivated sources make the plant of *Gloriosa superba* a potential source of Colchicine in India.

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