

Review: Colchicine, current advances and future prospects

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Abstract. Ade R, Rai MK. 2010. *Colchicine, current advances, and future prospects*. Nusantara Bioscience 2: 90-96. Colchicine is a toxic natural compound and secondary metabolite commonly produced by plants like *Colchicum autumnale* and *Gloriosa superba*. It is originally used to treat rheumatic complaints, especially gout, and still finds its uses for these purposes today despite dosing issues concerning its toxicity. It is also prescribed for its cathartic and emetic effects. Initially, oral colchicine has not been approved as a drug by U.S. Food and Drug Administration (FDA). But now FDA approved colchicine as a drug for some disorders. Colchicine's present medicinal use is in the treatment of gout and familial Mediterranean fever. It is also being investigated for its use as an anticancer drug. In neurons, axoplasmic transport is disrupted by colchicine. Due to all the pharmacological application of colchicine, there is an urgent need to enhance the properties and increase the production of colchicine with the help of *in vitro* technologies. The present review is mainly focused on the chemistry of colchicine, its medicinal uses, and toxicity.

Key words: colchicine, photoisomerization, colchicinamide, toxicity, polyploidy

Abstrak. Ade R, Rai MK. 2010. *Kolkisin, kelebihanannya pada saat ini dan prospeknya di masa depan*. Nusantara Bioscience 2: 90-96. Kolkisin adalah senyawa alami beracun dan metabolit sekunder yang umumnya dihasilkan oleh tanaman seperti *Colchicum autumnale* dan *Gloriosa superba*. Senyawa ini pada awalnya digunakan untuk mengobati keluhan rematik, terutama asam urat, dan tetap digunakan hingga kini, meskipun terdapat perdebatan mengenai dosis toksisitasnya. Senyawa ini juga diresepkan untuk efek katarsis dan emetik. Semula pemberian kolkisin secara oral sebagai obat belum disetujui oleh Badan Pengawas Obat dan Makanan Amerika Serikat (FDA), tetapi sekarang FDA menyetujui kolkisin sebagai obat untuk beberapa gangguan. Kolkisin digunakan dalam pengobatan asam urat dan demam mediterania familial. Senyawa ini juga sedang diselidiki kegunaannya sebagai obat antikanker, karena dalam neuron, transportasi akoplasma dapat dipengaruhi oleh kolkisin. Mengingat adanya aplikasi farmakologi kolkisin, maka perlu adanya upaya untuk memperbaiki sifat dan meningkatkan produksi kolkisin dengan bantuan teknologi *in vitro*. Telaah ini terutama difokuskan pada kimia kolkisin, serta penggunaannya sebagai obat dan toksisitasnya.

Key words: kolkisin, fotoisomerisasi, colchicinamide, toksisitas, poliploid.

INTRODUCTION

Colchicine is a traditional drug for gout (Wendelbo and Stuart 1985), and has been in use for treating acute gout dates back to 1810. It is obtained from corms of *Gloriosa superba* and also from *Colchicum autumnale* (Family Liliaceae). Since the approval of colchicine as drug for gout in 2009 by the Food and Drug Administration (FDA, USA) there has been revival of interest in colchicine research and applications (Schlesinger 2010). Colchicine is an extremely poisonous alkaloid, originally extracted from *Colchicum autumnale* (autumn crocus, meadow saffron) medicinal plants. It is used to treat rheumatic complaints.

Colchicine was first isolated in 1820 by the two French chemists Pelletier and Caventon and extract of *Colchicum* plant was first described as a treatment for gout in *De Materia Medica* of Padanius Dioscorides. It was later identified as a tri-cyclic alkaloid and its pain relieving and anti-inflammatory effects for gout were linked to its binding with the protein tubulin. The molecular formula of colchicine is $C_{22}H_{25}NO_6$ and its chemical name is N-[(7S)-

5, 6, 7, 9-tetrahydro-1, 2, 3, 10-tetramethoxy-9-oxobenzo[a]heptalen-7-yl) acetamide].

The term 'colchicine' is originated from area known as "Colchis" near black sea. *C. autumnale* grows wild in Europe and Africa while *Gloriosa* is distributed in Africa and Asia including foothills of Himalayas, Burma, Indonesia, Malaya, etc. Thomson was the first who proposed the early idea of action of colchicine in gout treatment. Gout and uric acid metabolism is same way linked and colchicine might act on this. Gout is caused by deposition of micro-crystals of uric acid in joints and may be due to defective regulatory mechanism for endogenous purine synthesis but conflicting results for the action of colchicine on synthesis and extraction of urates have been recorded. The colchicine interrupts the cycle of new deposition, which appears to be essential for the maintenance of acute gout. The frequent side effect has been recorded, but colchicine remains the ideal drug for acute gout. Modification of the side chain of rings does not eliminate anti-gout activity as long as the configuration of C-ring confirms to that of colchicine. It suppresses cell

Colchicine can solve an important problem of fuchsia breeding. The maximum fuchsia species are diploid or tetraploid. The crossing between diploid and tetraploid results often in a triploid, which is mostly sterile because the process of meiosis requires the pairing of similar chromosomes and also due to lack of mechanism so as to allow the alignment of three similar chromosomes. Triploid plants are not able to produce prolific reproductive cells, therefore, they remain sterile and unusable like parents. The advantage of polyploidy plants that all plant parts are bigger (flowers, leaves) a lot of big double fuchsias are polyploidy. A special problem of colchicine which induced ploidy, particularly in vegetatively propagated crops, is the chimerism caused by the simultaneous occurrence of tissue of different ploidy levels in one plant or plant part.

colchicine



The chemical structure of colchicine is shown. It features a complex polycyclic core consisting of a tropane ring system fused to a benzene ring, which is further fused to a cyclohexadiene ring. The tropane ring has a carbonyl group (C=O) and a methoxy group (OCH₃) attached. The benzene ring has two methoxy groups (OCH₃) attached. The cyclohexadiene ring has a methoxy group (OCH₃) attached. The overall structure is a complex polycyclic molecule with multiple methoxy groups.

When colchicine is irradiated by light, photoisomerization occurs and structure of α - β - γ -lumicolchicine so formed has been elucidated. Now the process and methodology are currently at the crossroad between the effectiveness of synthetic and natural compound in the improvement of human ailments. In comparison with allopathic or chemotherapy or antibiotic therapy, there are tremendous difficulties, allopathy has taken strong roots in most urban areas, the rural population of India has much faith in the usefulness and healing powers of age-old system of Ayurveda that is original system of medicines. Concentrated research in identifying and characterizing newer medicinal and aromatic plants can place us in a position of growth of the national economy. Also, we can help by fortifying the very grass root of Ayurveda by scientific interpretation to the pharmacodynamics of the many medicinal plant bases used in traditional treatments of the past. During the last three and half decades, various workers engaged in the field of Medicinal and Aromatic Plants in India, have increased manifold and so the output of research data on the subject. There is similar stepping up in research and development work in the growing and processing of medicinal and aromatic plants in many other developing countries like Asia, Africa and Latin America.

(Sudipto and Sastry 2000). This fact is powerfully reflected in the reports of many United Nation agencies, which has been advocating greater attention to those crops as a means of socio-economic uplift. However, in fact, revitalization of interest in natural plant products as these are biologically more well-matched with human system and relatively less toxic than the synthesis. Thus, the growing of medicinal and aromatic plants has got a great boost during the last two decades. Evidently, the need was felt for scientific literature on the growing and processing of these plants. Under such a situation retrieval of the information becomes a very painstaking process for the research and development.

Nguyen et al. (2005) studied the common pharmacophore for a diverse set of colchicine site inhibitor using a structure-based approach. In which the modulation of structure and function of tubulin and microtubule is most important route to anticancer therapeutics therefore small molecule bind to tubulin and cause mitotic arrest is of enormous interest. A large number of synthetic and natural compounds with dissimilar structures have been shown to bind at the colchicine site, one of the major binding sites on tubulin, and inhibit tubulin assembly. Using the recently determined X-ray structure of the tubulin colchicinoid complex as the template, and also employed docking studies to determine the binding modes of a set of structurally diverse colchicine site inhibitors. These binding models were subsequently used to construct a comprehensive, structure-based pharmacopeia.

Raimond et al. (2004) reported the tubulin regulation from a complex with colchicine and stathmin-like domain. The microtubules are cytoskeletal polymers of tubulin involved in many cellular functions. Their dynamic instability is controlled by numerous compounds and proteins including colchicine and stathmin family proteins. The way in which microtubule instability is regulated at the molecular level has remained controlled, mainly due to lack of appropriate structural data. The structure at 3.5 Å resolution of tubulin in complex with colchicine and with the stathmin-like domain (SLD) of RB3 is the interaction of RB3-SLD with two tubulin heterodimers in a curved complex capped by the SLD amino-terminal domain, which prevents the incorporation of the complex tubulin into microtubules. A comparison with the structure of tubulin in protofilaments shows change in the subunits of tubulin as it switches from its straight conformation to a curved one. These changes correlate with the loss of lateral contacts and provide a validation for the rapid microtubule depolymerization characteristic of dynamic instability. Moreover, the tubulin-colchicine complex sheds light on the mechanism of colchicine activity. Colchicine binds at a location where it prevents curved tubulin from adopting a straight structure, which inhibits assembly.

Zhou et al. (2000, 2002) reported increasing embryogenesis and doubling efficiency by immediate colchicine treatment of isolated microspores in spring *Brassica napus* in which immediate colchicine treatment of isolated microspores with the concentrations 50 and 500 mg/L for 15 hours stimulated embryogenesis and produced large amounts of healthy-looking embryos. These normal embryos

germinated well at 24°C after being transferred to solid regeneration medium and an initial period of low temperature (2°C) for 10 days, and could directly and rapidly regenerate vigorous plants. A high doubling efficiency of 83-91% was obtained from 500-mg/L colchicine treatments for 15 hours with low frequency of polyploid and chimeric plants. The experiment has shown that treatment duration of 30 hours revealed less positive effects on embryogenesis and doubling efficiency, especially at higher colchicine concentration (1000 mg/L). Poor embryogenesis and embryo germination were observed from ordinary microspore culture without change of induction medium and colchicine treatment, and several sub-cultures were required for induction of secondary embryogenesis and plant regeneration (Bourgaud et al. 2001; Hadacek et al. 2002).

PLANT SOURCE OF COLCHICINE

Gigantic important flora has been a major source of secondary metabolites, which is now the main source of pharmaceuticals, food additives, fragrances and pesticides (Figure 2).

Colchicum spp.

Al-fayyad et al. (2002) studied determination of colchicine from *Colchicum autumnale*, and several others species, for example, in corms of *Colchicum hierosolymitanum* and *Colchicum tunicatum* colchicine was reported in an appreciable amount. High-Performance Liquid Chromatography evaluated the effect of different NPK (Nitrogen, Phosphorous, and Potassium) fertilizer levels on colchicine content of the two colchicum species at different growth stages. Results indicate that increasing NPK fertilizer levels significantly improve colchicine content in different plant parts and stages. The highest colchicine content observed in corms was at maturity stage 0.766 mg/g and 0.688 mg/g dry weight with *C. hierosolimitanum* and *C. tunicatum* respectively.

Gloriosa superba

Gloriosa superba is one of the important species in the world particularly, Asia and Africa produces two important alkaloids colchicine and gloriosin which is present in seeds and tubers (0.7% to 0.9%) and other is lumicolchicine, 3-demethyl-N-deformyl-N-deacetylcolchicine, 3-demethylcolchicine, N-formyldeacetylcolchicine have been isolated from the plant (Chulabhorn et al. 1998). It is used in almost all diseases, like cancer, gout, scrofula, and act as antipyretic, antihelmintic, purgative and anti-abortion. It is also source of gloriosin and colchicocides, which are very costly, being highly demanded by pharma industries. (Finnie and van Staden 1989; 1991). Due to excessive use of the plant for diverse medicinal purposes the species is on the verge of extinction and included in Red data book (Sivakumar et al. 2003a; 2003b; 2004; 2006).

Gloriosa superba was also known as Malabar glory lily is a perennial tuberous climbing herb, widely scattered in the tropical and sub-tropical parts of India. It is called as

'Mauve beauty', 'Purple prince', 'Modest', 'Orange gem', 'Salman glow' and 'Orange glow' (Bose and Yadav 1989). It is adapted to different soil textures and climatic variations. The leaf juice is used to kill-lice in hair, the tuber contains the bitter principles, superbine and gloriosin, which in large doses are fatal ; however, in small doses they are used as tonic, antiabortive, and purgatives (Bellet and Gaignant 1985; Somani et al. 1989, Finnie and van Staden 1991; Samarajeewa 1993). The white flour prepared from the tubers is bitter and used as a stimulant.

Ghosh et al. (2002; 2005) reported colchicine production by using aluminum chloride as an elicitor. Root cultures of *Gloriosa superba* were treated with 5 mM methyl jasmonate and 125 M $AlCl_3$ which enhanced the intracellular colchicine content of the roots by 50-folds and 63-folds respectively. 10 mM of $CaCl_2$ and 1 mM $CdCl_2$ enhanced biomass significantly (7 to 8.6-fold respectively), while the maximum release of colchicine into the medium was obtained with 10 mM $CdCl_2$. Casein hydrolysate, yeast extract, and silver nitrate had no significant effect on growth and colchicine accumulation in root cultures.

Muzaffar and Brossi (1991) investigated the chemical structures of colchicine and related analogs, including allo-compounds with a six-membered ring. It was reported that colchicine cardiotoxicity by ingestion of *Gloriosa superba*, in which the clinical features of colchicine toxicity in a patient following ingestion of *G. superba* tubers were studied. Gastroenteritis, acute renal failure, cardiotoxicity, and hematological abnormalities were the main toxic manifestations. There was no hypotension and no neurological manifestations. Electrocardiographic changes were noteworthy and have not been reported previously.

Sivakumar et al. (2004) reported colchicine production in *Gloriosa superba* calli by feeding precursors, phenylalanine and tyrosine. The lack of biosynthetic precursors and signal inducing enzyme activity are responsible for the lower production of colchicine *in vitro*. B_5 medium nutrient grown calli have a low content of colchicine indicating that an optimal precursor level is required to increase PAL and TAL activity for colchicine accumulation. These results suggest that precursors are an important regulatory factor in colchicine accumulation in *in vitro*.

Other plants

Jha et al. (2005) reported production of forskolin, withanolides, colchicine, and tylophorine from plant source by using biotechnological approaches in which three alkaloids such as forskolin from *Coleus forskohlii* Briq, withanoloid from *Withania somifera* (L.) Dunal and colchicine from *Gloriosa superba* are discussed (Mukherjee et al. 2000; Furmanowa et al. 2001). The *Coleus forskohlii* Briq., a member of the family Lamiaceae is a common and ancient medicinal plant of India, and is used traditionally in Ayurvedic medicine (Bhattacharyya and Bhattacharya 2001; Engprasert et al. 2004). Large-Scale screening of medicinal plants by the Central Drug Research Institute, Lucknow, India, in 1974 revealed the presence of a hypotensive and spasmolytic component of *C. forskohlii* that was named coleonol. Further

investigation (Saksena et al. 1985) determined micropropagation and *in vitro* culture for production of forskolin. Forskolin synthesis in transformed cultures transformed cell and organ cultures have proved valuable transformed cell suspension culture withanolides from *W. somnifera*. Cell and tissue culture of *G. superba* for production of colchicine. There are few reports on micropropagation of *Gloriosa* sp. Since the active principle is mainly concentrated in the tubers, multiplication of tubers *in vitro* is essential. *In vitro* tubers have several advantages. They are harder, easier to handle, can be transported dry, there is no dormancy period thereby year-round cultivation is possible. These *in vitro* generated plantlets could serve as a source of cultures for studying the relationship between secondary metabolite accumulation and tissue differentiation. The productivity of the culture systems (transformed/ untransformed) needs to be improved significantly and to be shown to be competitive with field plants for production of target secondary metabolites on an industrial scale (Brodellus and Pedersen 1994). The lack of understanding of the molecular mechanism of regulation of secondary metabolism is the main bottleneck in attempts for further study.

POISONING OF COLCHICINE

Colchicine is often used to treat gout and acute rheumatoid arthritis and is known to relieve pain effectively (Neuwinger 1994). The mode of action of colchicine in gout is unknown; however, it is believed to decrease lactic acid production by the leukocytes and consequently, decrease urate crystal deposition and the subsequent reduction in phagocytosis with the inflammatory response. It also alters neuromuscular functions, intensifies gastrointestinal activity by neurogenic stimulation, increases sensitivity to central depressants, and depresses the respiration.

Ingestion of colchicine typically leads to profuse vomiting and diarrhea, which can be bloody, followed by hypovolemic shock and multisystem organ failure within 24-72 hours. Coma, convulsions, and sudden death might also occur. Subsequent complications include bone marrow suppression with resultant leukopenia, thrombocytopenia and possibly sepsis.

Laboratory diagnosis

There are two methods of detection of colchicine, (i) Biological- in which colchicine is detected in urine, serum, or plasma as determined by a commercial laboratory, and (ii) Environmental - colchicine in environmental samples can be determined as per rules of Food and Drug Administration.

Case classification

Suspected: A case in which health-care workers or public health officials are evaluating a potentially exposed person for poisoning by a particular chemical agent, but no specific credible threat exists.



Figure 1. Several main plant source of colchicines. A. *Colchicum autumnale*, B. *Gloriosa superba*, C. *Coleus forskohlii*. (photos from several sources)

Probable: A clinically compatible case in which a high index of suspicion (credible threat or patient history regarding location and time) exists for colchicine exposure or an epidemiologic link exists between this case and a laboratory-confirmed case.

Confirmed: A clinically compatible case in which laboratory tests have confirmed exposure.

Colchicine is FDA-approved drug in USA recently for the treatment of gout and also for familial Mediterranean fever, amyloidosis, and scleroderma (Kallinich et al. 2007). Side effects include gastrointestinal upset and neutropenia. Starting the drug early during an attack of gout can exacerbate the symptoms. High doses can also damage bone marrow and lead to anemia. It's not used in the treatment of cancer, as the dose required would lead to intolerable side effects.

Toxicity

Poisoning resembles intoxication with arsenic: symptoms start 2 to 5 hours after the toxic dose has been ingested and include burning in the mouth and throat, fever, vomiting, abdominal pain, and kidney failure. Death from respiratory failure can follow (Goldbart et al. 2000). There is no remedy. It was later identified as a tricyclic alkaloid and its pain relieving and anti-inflammatory

effects for gout were linked to it binding with the protein tubulin. It inhibits the cytoskeleton by binding to tubulin, one of the main constituents of microtubules. Apart from inhibiting mitosis, a process heavily dependent on cytoskeletal changes, it also inhibits neutrophil motility and activity, leading to a net anti-inflammatory effect.

COLCHICINE IN CELL DEVELOPMENT

Pharmacology

Colchicine inhibits microtubule polymerization by binding to tubulin, one of the main constituents of microtubules. Availability of tubulin is essential to mitosis, and therefore colchicine effectively functions as a "mitotic poison" or spindle poison. Since one of the defining characteristics of cancer cells is a significantly increased rate of mitosis, this means that cancer cells are significantly more vulnerable to colchicine poisoning than normal cells. However, the therapeutic value of colchicine against cancer is limited by its toxicity against normal cells.

Apart from inhibiting mitosis, a process heavily dependent on cytoskeletal changes, colchicine also inhibits neutrophil motility and activity, leading to a net anti-inflammatory effect. Colchicine also inhibits urate crystal

deposition, which is enhanced by a low pH in the tissues, probably by inhibiting oxidation of glucose and subsequent lactic acid production in leukocytes. The inhibition of uric acid crystals is a vital aspect of the mechanism of gout treatment. It is also used as an anti-inflammatory agent for long-term treatment of Behçet's disease. The Australian biotechnology company "Giaconda" has developed a combination therapy to treat constipation-predominant irritable bowel syndrome which combines colchicine with the anti-inflammatory drug olsalazine.

The British drug development company "Angiogene" is developing a prodrug of colchicine, ZD6126 (also known as ANG453) as a treatment for cancer. Colchicine has a relatively low therapeutic index. Colchicine is "used widely" off-label by naturopaths for a number of treatments, including the treatment. Side-effects include gastrointestinal upset and neutropenia. High doses can also damage bone marrow and lead to anemia. Note that all of these side effects can result from hyper-inhibition of mitosis.

Induction of polyploidy

Since chromosome segregation is driven by microtubules, colchicine is also used for inducing polyploidy in plant cells during cellular division by inhibiting chromosome segregation during meiosis, half the resulting gametes, therefore, contain no chromosomes, while the other half contains double the usual number of chromosomes (*i.e.*, diploid instead of haploid as gametes usually are) and lead to embryos with double the usual number of chromosomes (*i.e.*, tetraploid instead of diploid). While this would be fatal in animal cells, in plant cells it is not only usually well tolerated, but in fact frequently results in plants which are larger, faster growing, and in general more desirable than the normally diploid parents for this reason, this type of genetic manipulation is frequently used in commercial plant breeding. In addition, when such a tetraploid plant is crossed with a diploid plant, the triploid offspring will be sterile, which may be commercially useful in itself by requiring growers to buy seed from the supplier, but also can often be induced to create a "seedless" fruit if pollinated (usually the triploid will also not produce pollen, therefore a diploid parent is needed to provide the pollen). This is the method used to create seedless watermelons, for instance. On the other hand, colchicine's ability to induce polyploidy can be exploited to render infertile hybrids fertile, as is done when breeding triticale from wheat and rye. Wheat is typically tetraploid and rye diploid, with the triploid hybrid infertile. Treatment with colchicine of triploid triticale gives fertile hexaploid triticale.

When used to induce polyploidy in plants, colchicine is usually applied to the plant as a cream. It has to be applied to a growth point of the plant, such as an apical tip, shoot or sucker. Seeds can be presoaked in a colchicine solution before planting. As colchicine is so dangerous, it is worth noting that doubling of chromosome numbers can occur spontaneously in nature, and not infrequently. The best place to look is in regenerating tissue. One way to induce it is to chop off the tops of plants and carefully examine the lateral shoots and suckers to see if any look different.

COLCHICINE IN MEDICINES

Colchicine poisoning and potential acute health effects

It is extremely hazardous in case of skin contact (corrosive, irritant, sensitizer, permeator), of eye contact (irritant), of ingestion, of inhalation. The amount of tissue damage depends on length of contact. Eye contact can result in corneal damage or blindness. Skin contact can produce inflammation and blistering. Inhalation of dust will produce irritation to gastrointestinal or respiratory tract, characterized by burning, sneezing and coughing. Severe over-exposure can produce lung damage, choking, unconsciousness or death. Inflammation of the eye is characterized by redness, watering, and itching. Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering, ingestion, of inhalation. The substance is toxic to blood, kidneys, lungs, the nervous system, the reproductive system, liver, mucous membranes. Repeated or prolonged exposure to the substance can produce target organs damage. Repeated exposure of the eyes to a low level of dust can produce eye irritation. Repeated skin exposure can produce local skin destruction, or dermatitis. Repeated inhalation of dust can produce varying degree of respiratory irritation or lung damage. Repeated exposure to a highly toxic material may produce general deterioration of health by an accumulation in one or many human organs. Repeated or prolonged inhalation of dust may lead to chronic respiratory irritation. The substance is toxic to blood, kidneys, lungs, the nervous system, the reproductive system, liver, mucous membranes.

Action and clinical pharmacology

Although its exact mode of action in the relief of gout is not completely understood, colchicine is known to decrease the inflammatory response to urate crystal deposition by inhibiting migration of leukocytes, to interfere with urate deposition by decreasing lactic acid production by leukocytes, to interfere with kinin formation and to diminish phagocytosis and the subsequent anti-inflammatory response. The anti-inflammatory effect of colchicine is relatively selective for acute gouty arthritis. However, other types of arthritis occasionally respond. It is neither an analgesic nor a uricosuric and will not prevent progression to chronic gouty arthritis. It does have a prophylactic, suppressive effect that helps to reduce the incidence of acute attacks and to relieve the residual pain and mild discomfort that patients with gout occasionally experience.

CONCLUSION

Colchicine has been approved as the drug for gout by Food and Drug Administration, USA in 2009. Thereafter, the interest of the scientist has revived. Since colchicine has wide array of properties and applications from ancient periods to modern era of medicine, it is necessary to understand its pharmacology. It is a pressing need to enhance the properties and percentage of colchicine by

application of *in vitro* technologies. In addition to that, besides chemical synthesis, *in vitro* biological synthesis by using precursors would be a novel method for the production of colchicine.

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