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 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

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 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 proposed the early idea of action of colchicine in gout treatment. Gout and uric acid metabolism is same way...
division by inhibiting the development of spindles, from a pool of subunit during a distinct phase of cell cycle and then depolymerized during other phases.

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.

CHEMISTRY OF COLCHICINE

Colchicine is also known as methyl ether of colchicine. It is a major alkaloid of Gloriosa superba and Colchicum autumnale. N-formyl-N-de-acetyl colchicine and 3-demethyl colchicine designated as substances A, B, C respectively have occurred in lileaceae family (Figure 1).

The study of isolation started in 1820 and present method of Ziesel-methoxyl determination has its foundation in the determination of these functional groups in colchicine possessing only one asymmetric carbon atom at position C7. The alkaloid morphine and strychnine are now known.

Colchicine (C22H25NO4) is not an alkaloid, because the nitrogen atom is not basic, which is part of acetamide function, four oxygen atoms are present as 4-methoxy group, and remaining oxygen is unreactive towards reagent that affect acylation and affords no carbonyl derivative. Acid hydrolysis of colchicine in varying degrees of rigidity provides method used for the selective breaking cleavage of the functional groups. Dilute acid affords colchicine, an acidic substance that can be methylated to colchicine and iso-colchicine by diazo-methane. The assigned colchicine 9-methyl phenanthrene structure and the structural formula for ring. Proof of cycloheptane structure for ring-B was obtained by synthesis of dl-colchinol methyl ether, N-acetyl colchinol methyl anhydride, the degradation products of colchicine. Dewar (1945) reported troponol structure for ring-C and it was responsible for coining the term troponol for cycloheptatrienolon., It was proved that ring-C was 7 member by the synthesis of octa-hydro demethoxy des oxides acetamid colchicine, a degradation product of colchicine in which ring-C remain intact, so the correct structure of colchicine is assigned as methyl ether of colchicine. Colchicine is optically active by virtue of the single asymmetric carbon atom at position C-7. The absolute configuration at this center was established by oxidation of colchicine to N-acetyl-L-glutamic acid.

The synthesis of colchicine has attracted widespread attention as a synthetic object (Seganish et al. 2005). The starting material was 7-8-9 trimethoxy-benzo-suberone and end product was plus minus trimethylcolchicine acid which had been converted earlier to colchicine by resolution N-acetylating and O-methylamine, while in case of Alexander et al. (1994) the starting material was purpurogallin trimethyl ether and end product is similar to Van tamelan synthesis. In Nakamura synthesis, starting material is pyrogallolmethyl ether herring A and C formed first and then constructed to form end product.

Photoisomerization

When colchicine is irradiated by light, photo-isomerization occurs and structure of α-β-γ-lumicolchicine so formed has been elucidated. Now the process and methodology are currently at the cross road 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 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, 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 pharmacopoeia 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 are of enormous interest. A large number of synthetic and natural compounds with dissimilar structures have been shown to bind to 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 pharmacopoeia.

Raimond et al. (2004) reproted 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 complexe 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 provides 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 embryo regeneration 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 hour stimulated embryo regeneration 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 hour with low frequency of polyploid and chimeric plants. The experiment has shown that treatment duration of 30 hour 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 a 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. The effect of different NPK (Nitrogen, Phosphorous and Potassium) fertilizer levels on colchicine content of the two colchicum species at different growth stages were evaluated by High Performance Liquid Chromatography. Results indicates 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 antiabortive. It is also source of gloriosin and colchicoids, 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 also known as Malhar glory lily is a perennial tuberous climbing herb, widely scattered in the tropical and sub-tropical parts of India. It is called as
neurological manifestations. Electrocardiographic changes and haematological abnormalities were the main toxic manifestations. There was no hypotension and no suppression with resultant leukopenia, thrombocytopenia and possibly sepsis. 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 a potentially exposed person is being evaluated by health-care workers or public health officials for poisoning by a particular chemical agent, but no specific credible threat exists.

**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.
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 gastro-intestinal 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 on 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 constellation-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 treatmet. Side-effects include gastro-intestinal upset and neutropenia. High doses can also damage bone marrow and lead to anaemia. 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 contain 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, sensitiser, 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 cause 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 an 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 have 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

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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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