Formulation of colchicine ointment for the treatment of acute gout

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INTRODUCTION In spite of being the fastest acting drug available for the control of an acute gout attack, colchicine is generally considered a last alternative in gout therapy. This is mainly due to the severe adverse effects associated with its administration through the enteral and parenteral routes, as well as its high risk/benefit ratio. The preparation of dosage forms of colchicine that can be administered by alternative routes is therefore a beneficial exercise. Among the formulable substitute dosage forms of colchicine, its ointment seems to be the best option available due to its ability to deliver the drug transdermally as well as its ease of preparation and evaluation. In this study, we prepared and tested 0.2% and 0.5% colchicine ointments for their effectiveness in delivering colchicine transdermally.

METHODS Colchicine ointment was prepared using a self-formulated water-in-oil type of emulsion ointment base, with the colchicine dissolved in the water portion of the ointment base. *In vitro* drug release studies were carried out using the Franz diffusion test apparatus and an ultraviolet (UV)-visible spectrophotometer was used to quantify the drug in the samples. Rabbits were used as test animals for *in vivo* studies and the blood samples were analysed using the UV-visible spectrophotometer.

RESULTS Colchicine was found to be well-absorbed transdermally, although absorption was not 100%. No side effects were associated with its 0.2% formulation.

CONCLUSION Ointments containing colchicine in low concentrations may be a feasible and effective treatment option for the prevention and treatment of acute gout attacks.

Keywords: colchicine, Franz diffusion test apparatus, in vitro drug release studies, in vivo drug release studies
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INTRODUCTION

Acute gout is one of the most painful conditions experienced by humans.[1] An acute gout attack occurs due to the sudden inflammatory response triggered by a precipitation of sodium urate crystals in the joints and is often associated with hyperuricaemia. The various drugs that are currently used for the treatment of acute gout include non-steroidal anti-inflammatory drugs, corticosteroids, uricosurics and colchicine.[2]

Colchicine, an alkaloid extracted from the plant *Colchicum autumnale* is the fastest acting drug among the currently available drugs for the control of an acute attack of gout.[3] Colchicine is believed to reduce inflammation by a combination of effects produced by the inhibition of monosodium urate-induced migration of neutrophils and other leucocytes via a blockade of microtubule formation, along with the suppression of superoxide production by neutrophils.[4-6] However, a recent study showed that colchicine can also be used for the suppression of neutrophil superoxide production alone at doses 100 times lower than that necessary to prevent neutrophil infiltration.[4] This finding has provided a rationale for using low-dose colchicine for the treatment of acute gout attack.

Even so, and in spite of being the fastest and most effective drug against acute gout attacks, colchicine is considered a last alternative in gout therapy, mainly due to the severe adverse effects associated with its administration through the enteral and parenteral routes, and its high risk/benefit ratio. Upon oral administration, colchicine causes nausea, vomiting, diarrhoea and stomach upset.[7] Sometimes, diarrhoea can be bloody due to the accumulation of colchicine in the intestine and the inhibition of mitosis in its rapid turnover mucosa.[3] Perhaps another reason for the high incidence of side effects associated with colchicine use is the unavailability of reliable data regarding the well-defined optimal dose for oral colchicine, which makes dose determination very difficult for the prescribing physician. A popular dosage regimen recommends dosing until relief of pain, vomiting or diarrhoea occurs, up to a maximum dose of 6–8 mg.[8,9] However, in the only randomised controlled trial that assessed the efficacy of this dosing regimen, all participants treated with colchicine experienced significant gastrointestinal side effects, with only 66% of patients reporting a 50% reduction in pain and clinical symptoms.[10] Another limiting factor for the oral administration of colchicine is that it cannot be used continuously for more than a week, as it may lead to bone marrow suppression due to its accumulation in the body.[11]

Intravenous administration of colchicine is no better, as it may lead to potentially adverse side effects such as necrosis, cytopenias, disseminated intravascular coagulation and even death.[11] The side effects of intravenous colchicine administration are mainly

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Table I. Compositions of 0.2% and 0.5% colchicine ointments.

Ingredient	Composition	
	0.2% colchicine	0.5% colchicine
Colchicine (mg)	20	50
Wool fat (g)	2.9	2.9
White soft paraffin (g)	1.95	1.95
Sodium lauryl sulphate (mg)	100	100
Distilled water (g)	5	5

associated with the very narrow therapeutic range of colchicine. 0.015 mg/kg is the safe dose, but 0.1 mg/kg or slightly higher is defined as a toxic dose and 0.8 mg/kg is a lethal dose.[12] So far, no records of intra-articular injection of colchicine in humans are available, as such administration proved to be lethal in experiments conducted on rats, with intra-articular injection of colchicine leading to the degeneration of articular cartilage.[13]

As the only other viable route remaining for the safe and effective administration of colchicine is the transdermal route, researchers worldwide have developed various topical formulations for colchicine. However, until recently, successful development of topical formulations of colchicine was mostly limited to disorders such as psoriasis[14] and actinic keratosis,[15] where the transdermal delivery of colchicine is not necessary for the effective treatment of the disorder. Topical formulations for the treatment of both of these disorders were in the form of either ointments or gels.

Although the treatment of an acute gout attack requires efficient transdermal delivery of colchicine, such an approach is also associated with inflammation of the skin at the area of application due to intradermal retention of colchicine. For this reason, only two studies so far have attempted transdermal delivery of colchicine.[12,16] Both these attempts involved the use of elastic liposomes. In the first study by Singh et al, colchicine alone was enclosed in elastic liposomes, which showed good transdermal flux with a 10.2-fold higher delivery of colchicine across the skin than a normal drug solution.[16] In a second attempt, Singh et al complexed colchicine with cyclodextrin, following which the complexes were enclosed in elastic liposomes. These elastic liposomes, containing colchicine-cyclodextrin complexes, demonstrated a 12.4-fold higher colchicine delivery across the skin than the normal drug solution.[12] In both the attempts by Singh et al, there was no inflammation seen at the site of administration, as the liposomes containing colchicine were retained in the skin and colchicine was not in direct contact with the skin. However, a major limiting factor of the two formulations is the cost and complexity of preparation, the specialised storage conditions required and the need for tedious and complicated quality control tests.

Our aim therefore was to overcome these limitations by formulating the simplest possible dosage form for a successful transdermal delivery of colchicine. As the formulation and evaluation of any other dosage form could not be more simple than that of an ointment, it was decided that an ointment be prepared for the transdermal delivery of colchicine in our attempt. We formulated two ointments, with 0.2% and 0.5% colchicine, and carried out *in vitro* and *in vivo* drug release studies to determine the applicability of the two formulations for the transdermal delivery of colchicine.

Table II. Average absorbance values of colchicine at 350 nm.

Concentration (μg/mL)	Average absorbance*
10	0.650
20	1.254
30	2.359

* Average of three readings.

METHODS

Colchicine ointment was prepared using colchicine in its pure form (obtained as a gift sample from Inga Laboratories, Mumbai, India). Table I presents the compositions of the 0.2% and 0.5% colchicine ointments used in our study. The 0.2% ointment was prepared by dissolving 20 mg of colchicine and 100 mg of sodium lauryl sulphate in 5 g of water. This solution served as the aqueous phase of the mixture. Simultaneously, 1.95 g of white soft paraffin, followed by 2.9 g of wool fat, was melted in another beaker. This molten mixture served as the oily phase of the mixture. The aqueous phase was heated to about 60°C and the hot solution was slowly added to the oily phase with continuous stirring using a magnetic stirrer until the mixture cooled to form a 0.2% colchicine ointment. A similar method was followed for the preparation of the 0.5% colchicine ointment, with the only difference being that 50 mg of colchicine was dissolved in 5 g of water instead of 20 mg. To accurately measure 5 g of water, the density of water was first determined at room temperature (25°C) with the help of a pycnometer. The volume of water equivalent to 5 g at room temperature was then taken. In both these ointments, sodium lauryl sulphate acted as both a surfactant and permeation enhancer. The two ointments were then used in *in vitro* and *in vivo* drug release studies to ascertain the efficiency of these ointments in delivering colchicine transdermally.

Prior to carrying out the various drug release studies, a calibration curve was plotted for colchicine using colchicine solutions of different concentrations. For plotting the calibration curve, 10 mg of colchicine was first dissolved in 10 mL of phosphate buffer (pH 7.4) to obtain a solution containing 1,000 μg/mL of colchicine. 1 mL was withdrawn from this solution and mixed with 9 mL of phosphate buffer to obtain a solution containing 100 μg/mL of colchicine. From this second solution, 1 mL, 2 mL and 3 mL of solution were withdrawn separately and mixed with 9 mL, 8 mL and 7 mL of phosphate buffer, respectively, to obtain solutions containing 10 μg/mL, 20 μg/mL and 30 μg/mL of colchicine. The absorbance values of these three colchicine solutions (10 μg/mL, 20 μg/mL and 30 μg/mL) were determined using a double beam ultraviolet (UV)-visible spectrophotometer at a λ_{max} of 350 nm,[17] using phosphate buffer (pH 7.4) as the reference solvent (Table II).

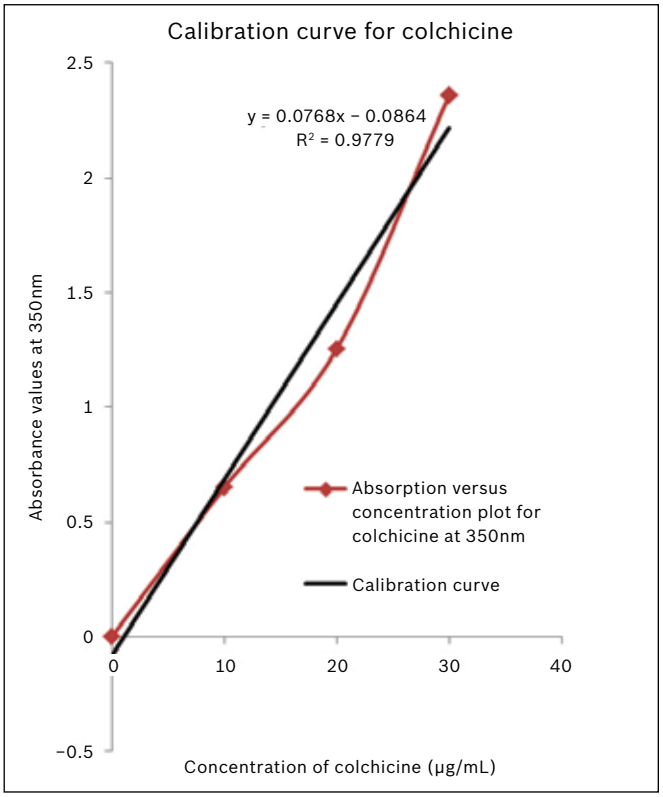

Fig. 1 Graph shows the calibration curve for colchicine.

The absorbance values obtained were plotted against the concentration of colchicine to obtain a curve to which linear regression was applied to arrive at the calibration curve for colchicine (Fig. 1). This calibration curve was then used as a reference to determine the quantity of colchicine delivered transdermally during our *in vitro* and *in vivo* drug release studies.

In vitro drug release studies of colchicine ointments were carried out using the Franz diffusion test apparatus,[18] with a lower compartment of 300 mL capacity. The apparatus had a provision for a skin/membrane of 6 cm in diameter. We used a 1-mm thick epidermal layer from fresh goatskin for the study, which was fixed in the Franz diffusion apparatus. Phosphate buffer (pH 7.4) was taken in the lower compartment to simulate the pH of blood,[17] and water heated to a temperature of 37°C was circulated through the outer jacket of the lower compartment to maintain the temperature of the buffer solution at 37°C. 0.5 g of the 0.2% colchicine ointment was applied on the goatskin, and a 1-mL sample was withdrawn from the lower compartment at time intervals of 30 minutes. The sample was analysed for drug content using the UV-visible spectrophotometer at a wavelength of 350 nm,[17] with phosphate buffer (pH 7.4) as the reference solvent. The quantity of colchicine released was determined from the absorbance values obtained from the samples using the calibration curve plotted earlier, following which the quantity of colchicine was plotted against time to obtain a graph representing the drug release pattern (Fig. 2). A similar procedure was followed for the *in vitro* drug release study of 0.5% colchicine ointment. The *in vitro* drug release studies were followed by *in vivo* drug release studies using rabbit as the animal model.

In vivo drug release studies were conducted in accordance with the animal ethics guidelines of the institutional animal ethical committee for the purpose of control and supervision of experiments on animals. All rabbits were male, with weights in the range of 3.8–4.1 kg (average weight 3.95 kg). The animals were housed with free access to food and water, except for the final two hours prior to the experiment. A total of 20 rabbits were used (0.2% ointment n = 10; 0.5% ointment n = 10). Of the 10 rabbits assigned for each ointment, five in each group were used as control animals while the remaining five made up the test group. Prior to beginning the study, hair in the abdominal region of all the rabbits was shaved neatly to form a hairless circular area of 6 cm in diameter. An ointment base without colchicine was applied to all 10 control rabbits while, in the test groups, 0.2% and 0.5% colchicine ointments were applied to five test rabbits each. Following the application of the ointments, 2.5 mL blood samples were collected from the marginal ear vein of the rabbits at time intervals of 30 minutes into tubes each containing 0.1 mL of 5% disodium ethylenediaminetetraacetic acid (purchased from Keerthi Agencies, Andhra Pradesh, India). The tubes were then centrifuged at 4,000 rpm for 10 minutes. 1 mL of the resultant plasma was collected and analysed in the double beam UV-visible spectrophotometer at a wavelength of 350 nm,[17] using plasma from the control rabbits as a reference solvent. The quantity of drug released was determined from the absorbance values obtained from these samples using the calibration curve, as mentioned earlier. The quantity of drug released was then plotted against time to obtain the graph representing the drug release pattern (Fig. 3). The total amount of drug released into the plasma was calculated based on the assumption that plasma content would make up 7.5% of the body weight in the test animals.

Due to the long plasma half-life of colchicine, *in vivo* drug release studies were carried out only until peak plasma concentrations were achieved. Once achieved, the peak plasma concentration is likely to remain in a steady state for several hours. After the completion of the study, the rabbits were kept under observation for three days.

RESULTS

The results of our *in vitro* and *in vivo* studies showed that colchicine diffuses very slowly across the skin, and because of which, its concentration in the plasma only rises at a very slow rate. Table III and Fig. 2 present the results of the *in vitro* drug release studies of 0.2% and 0.5% colchicine ointments. The total drug release achieved in our attempt from the 0.2% ointment was 93.9%, while that from the 0.5% ointment was 97.5%. The results of the *in vivo* drug release studies of 0.2% and 0.5% colchicine ointments are given in Table IV and Fig. 3. The total drug release achieved from the 0.2% ointment was 87.8% while that from the 0.5% ointment was 93.9%.

Rabbits treated with the 0.2% colchicine ointment did not show any signs of inflammation or irritation at the site of ointment application during the three-day observation period. Side effects

Table III. *In vitro* drug release patterns of 0.2% and 0.5% colchicine ointments.

Duration (hr)	Drug concentration in buffer* (μg/mL)	
	0.2% colchicine	0.5% colchicine
0.5	0.9 ± 0.1	1.0 ± 0.1
1	1.7 ± 0.1	1.8 ± 0.1
1.5	2.4 ± 0.1	2.6 ± 0.2
2	3.1 ± 0.1	3.3 ± 0.1
2.5	3.1 ± 0.1	4.0 ± 0.1
3	3.1 ± 0.1	4.7 ± 0.1
3.5	-	5.6 ± 0.2
4	-	6.2 ± 0.2
4.5	-	6.9 ± 0.3
5	-	7.5 ± 0.3
5.5	-	8.1 ± 0.1
6	-	8.1 ± 0.1
6.5	-	8.1 ± 0.1

Data is presented as mean ± standard deviation. *Average of three readings.

Table IV. *In vivo* drug release patterns of 0.2% and 0.5% colchicine ointments.

Duration (hr)	Drug concentration in plasma* (μg/mL)	
	0.2% colchicine	0.5% colchicine
0.5	0.7 ± 0.1	0.9 ± 0.1
1	1.3 ± 0.1	1.6 ± 0.2
1.5	1.8 ± 0.3	2.3 ± 0.3
2	2.3 ± 0.2	2.9 ± 0.3
2.5	2.9 ± 0.1	3.6 ± 0.3
3	2.9 ± 0.1	4.2 ± 0.3
3.5	2.9 ± 0.1	4.9 ± 0.1
4	-	5.7 ± 0.3
4.5	-	6.4 ± 0.1
5	-	7.0 ± 0.2
5.5	-	7.9 ± 0.2
6	-	7.9 ± 0.2
6.5	-	7.9 ± 0.2

Data is presented as mean ± standard deviation. *Average of three readings.

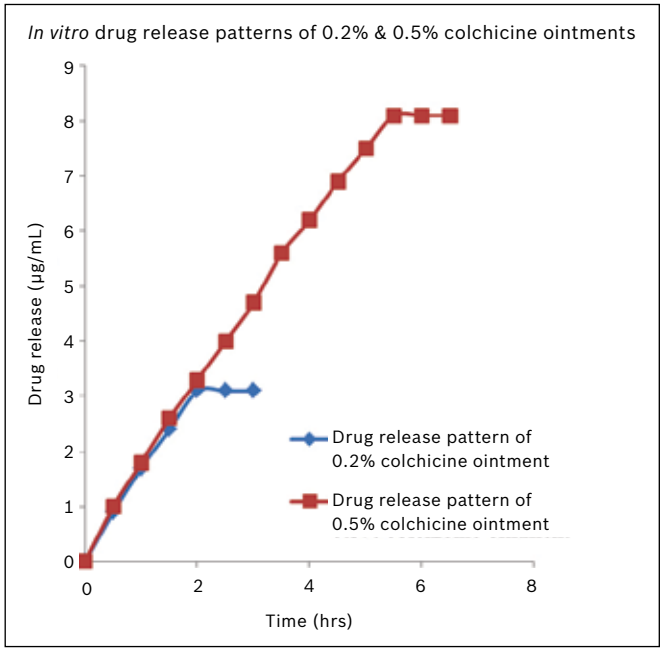

Fig. 2 Graph shows the *in vitro* drug release patterns of 0.2% and 0.5% colchicine ointments. The time taken for complete drug release from the 0.5% ointment is more than double that of the 0.2% ointment.

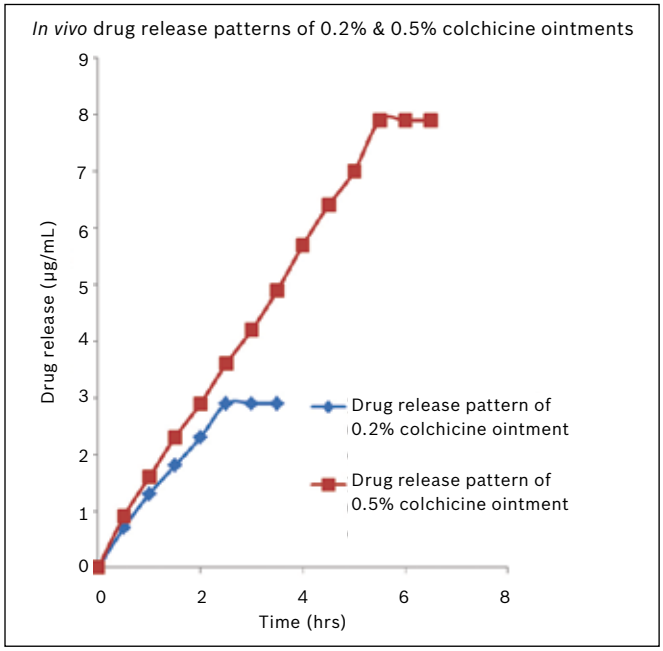

Fig. 3 Graph shows the *in vivo* drug release patterns of 0.2% and 0.5% colchicine ointments. The time taken for complete drug release from the 0.5% ointment is more than double that for the 0.2% ointment.

such as vomiting and diarrhoea were also not noticed in this animal group during this period. On the other hand, rabbits that were treated with the 0.5% ointment showed slight redness at the site of application. Swelling of any kind was not associated with the redness. This redness started disappearing from the site of application from the third day after the application of ointment. Other side effects such as diarrhoea or vomiting were not noticed during the observation period in the 0.5% ointment group.

DISCUSSION

From the results of our *in vitro* and *in vivo* drug release studies, it is clear that 100% of the drug administered as a transdermal colchicine ointment did not enter the systemic circulation. Since the plasma half-life of colchicine is very long and its hepatic metabolism negligible, the only reason for its absorption into the plasma not being 100% would be that colchicine was getting trapped in the skin. Also, interestingly, although the total drug release achieved into the plasma was higher from the formulation containing a higher concentration of colchicine in our *in vivo* drug release studies, the final plasma concentration of colchicine in the rabbits administered with 0.2% and 0.5% ointments differed only by 0.4 μg from what the plasma concentration would actually have been, had 100% of the colchicine administered been absorbed into the systemic circulation. Even in the case of

in vitro drug release, the difference is the same in both ointments and is 0.2 µg less than what it would have been if 100% of the colchicine was absorbed into the lower compartment of the Franz diffusion test apparatus. These results show that when the same quantity of colchicine ointments of different concentrations is applied to a skin area of the same size, the amount of the drug trapped in the skin layers is the same irrespective of the concentration of the formulation. As the total amounts of drug present in the two ointments were different, the percentage decrease in the plasma drug content due to trapping of the drug in the skin was also correspondingly different.

As the amount of ointment that got trapped in the skin was the same for both formulations of colchicine, we were able to arrive at the deduction that the redness of the skin noticed in the animals administered with the 0.5% colchicine ointment was not caused by the trapping of the ointment in the skin, but rather was a result of prolonged exposure of the skin to incoming colchicine from the ointment. Our results indicate that the ability of the skin to bind colchicine is limited, and therefore, the amount of colchicine bound to the skin would not increase with the increasing concentration of colchicine in the ointment.

It should be noted that although the results of our study support the use of transdermal colchicine ointments for the prevention and treatment of acute gout attacks (prior to assuming the applicability of such ointments in humans), physicians should keep in mind the fact that the results of such *in vivo* drug release studies may not be replicated in human subjects. This is especially so because of the difference in the absorption of colchicine by the skin of humans and rabbits – hair follicles promote the absorption of drugs across the skin and, when compared to human skin, rabbit skin has a much larger number of hair follicles per square inch.

In conclusion, our results indicate that the 0.2% colchicine ointment is safer than its 0.5% formulation. The likelihood of adverse effects arising from such transdermal application would be less for the 0.2% ointment, even if applied in excessive quantities by patients, indicating a very high margin of safety associated with the formulation. Another advantage found to be associated with the 0.2% colchicine ointment was that the duration of exposure of the skin to the incoming drug was shorter, which therefore did not result in any skin inflammation.

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