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CLINICAL RESEARCH

Activated charcoal significantly reduces the amount of colchicine released from *Gloriosa superba* in simulated gastric and intestinal media

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ABSTRACT

Background: Poisoning with *Gloriosa superba*, a plant containing colchicine, is common in Sri Lanka.
Objectives: This study was to estimate release of colchicine from 5 g of different parts of *Gloriosa superba* in simulated gastric and intestinal media, and examine the binding efficacy of activated charcoal (AC) to colchicine within this model.
Methods: A USP dissolution apparatus-II was used to prepare samples for analysis of colchicine using HPLC.
Results: Cumulative colchicine release from tuber in gastric media at 120 minutes was significantly higher (2883 μg/g) than in intestinal media (1015 μg/g) ($p < .001$). Mean ± SD cumulative colchicine concentration over 2 hours from tuber, leaves and trunk in gastric medium was 2883.15 ± 1295.63, 578.25 ± 366.26 and 345.60 ± 200.08 μg/g respectively and the release in intestinal media was 1014.75 ± 268.16, 347.40 ± 262.61 and 251.55 ± 285.72 μg/g respectively. Introduction of 50 g of AC into both media made colchicine undetectable (<0.1 μg/ml).
Conclusions: The tuber released the highest quantity of colchicine. The colchicine release and elapse time to achieve saturated, equilibrium dissolution mainly depends on physicochemical properties of plant part. Significant *in vitro* binding of colchicine to AC suggests that AC has a role in decontamination of patients presenting to hospital after ingestion of *Gloriosa superba*.

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1. Introduction

Self-poisoning is a major clinical problem in rural Asia with up to a 42% case fatality [1,2]. While agrochemicals cause the majority of deaths, plant poisoning contributes to significant morbidity and mortality [3–5]. Poisoning with *Thevetia peruviana* (yellow oleander) [6,7], *Cerbera manghas* (pink-eyed cerbera or sea mango) [8], *Gloriosa superba* (glory lily) [9–13] and *Cleistanthus collinis* (a species of teak) [14,15] causes significant numbers of deaths each year in South Asia. Yellow oleander is the commonest cause of plant poisoning in Sri Lanka although *Gloriosa superba* accounts for about 44% of plant poisoning in some parts of Sri Lanka [16].

Gloriosa superba poisoning has a case fatality rate of 15% [16]. The toxicity of *Gloriosa superba* relates to colchicine released from the plant parts after ingestion. Although colchicine is one of the oldest antimitotic drugs and is still used in the treatment of gout and Familial Mediterranean Fever (FMF) [17–19], its toxicity limits any extensive therapeutic application. Acute pharmaceutical colchicine overdose is also associated with a high mortality rate and the mortality is directly related to dose ingested [4,20,21]. Patients with early hemodynamic collapse due to colchicine overdose have particularly poor prognosis [21,22], and there has been no effective treatment for this complication of severe colchicine intoxication and management is limited to decontamination, supportive care and symptomatic management [23–25]. Colchicine binds reversibly to tubulin [26], and colchicine-specific antibodies have been shown to restore the activity of tubulin *in vitro* [27,28]. Polyclonal Fab fragments that have been developed against colchicine though effective [29], they are unfortunately prohibitively expensive and not commercially available for clinical use.

In the absence of specific antidotes, management of plant poisoning in developing Asia is limited to supportive care and management of complications. Gastric decontamination with activated charcoal (AC) is recommended in most guidelines. However, the dissolution characteristics of colchicine from plants and efficacy of AC in this situation have not been described. Understanding of both of these factors would inform the evidence base for rational treatment guidelines. The aim of this study was to investigate the binding affinity of colchicine released from different parts of *Gloriosa superba* to AC (in a 1:10 ratio of plant: charcoal) in simulated gastric and intestinal media.

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2. Materials and methods

Gloriosa superba plants were collected from various geographical areas (Central Province, North Central Province, Southern Province, and Western Province) of Sri Lanka and taxonomically identified at the Faculty of Agriculture, University of Peradeniya, Sri Lanka. All the plant parts from different regions were collected within a period of two weeks (1 September to 15 September) and dissolutions were carried out during the same period. Different fresh plant parts (tuber, leaves and trunk) of *Gloriosa superba* each weighing 5 g (using a digital electronic scale) were crushed for two minutes using separate standard size mortars and pestles to mimic chewing.

The dissolution studies were conducted using the USP dissolution apparatus-II basket method [30]. As stated in official monograph of United State Pharmacopeia (USP) the dissolution test should be carried out for colchicine tablets using 500 ml water as the dissolution medium with controlled temperature (±37 °C), 100 rpm rotation and recommended dissolution time is 30 minutes [31]. Considering the greater weight of plant parts (5 g), to improve the feasibility to run the test, we used 900 ml (maximum volume of the vessel). We used 0.1 N hydrochloric acid (HCl) (pH 1.2) and 1.5% sodium bicarbonate ($NaHCO_3$) (pH 7.5) to simulate gastric and intestinal media respectively instead of water. The USP dissolution apparatus-II was calibrated with a disintegrating type (prednisolone) and a non-disintegrating type (salicylic acid) agent. The temperature was maintained at 37 °C ± 0.5 °C throughout the process by auto temperature control of the water bath. Weighed and crushed plant part (we studied individual plant parts from each of the geographical areas) was placed in a basket that was allowed to sink to the bottom of the vessel that contained 900 ml of fresh media. The unit was operated immediately at a rotation speed of 100 rpm. Ten milliliters of dissolution samples were withdrawn from the vessel at 10 min, 20 min, 30 min, 45 min, 60 min, 90 min and 120 min time intervals using clean and separate pipettes and each was filtered using separate filter paper. To maintain the total volume of the media constant in the vessel, 10 ml of fresh media pre heated at 37 °C ± 0.5 °C was added after each withdrawal of 10 ml sample. The dissolution samples were labelled and stored in light protected containers at −22 °C. The dissolution samples were analysed for colchicine using high performance liquid chromatography (HPLC): Dissolution samples (100 µl) and internal standard (500 ng/ml midazolam-d4, 100 µl) were diluted with 50% methanol (5 ml) prior to analysis. Five microliters of each sample was injected on to the HPLC (Alltech Alltima C18 5µ, 150 mm × 2.1) column held at 40 °C mobile phase with 65% methanol/35% de-ionised water containing 1 ml formic acid per litre pumped at 300 µl/min. Detection of colchicine was undertaken using API4000 MS/MS with anion spray interface running at 500 °C in positive mode.

The experiment was conducted in both freshly prepared simulated gastric and intestinal dissolution media separately using one 5 g sample each of fresh tubers, leaves and trunks collected from each of four different geographical regions of Sri Lanka.

The experiments were repeated separately in the dissolution media treated with 50 g AC using a single 5 g sample each of the fresh tubers, leaves and trunk collected from the same geographical areas. Fifty grams of AC was added to the fresh media prior to introducing the basket (containing 5 g of plant part) into the media on each occasion and the results compared to the studies undertaken without AC.

Statistical analysis was done using SPSS18 statistical software (SPSS Inc., Chicago, IL). Results were expressed as mean ± SD (standard deviation). Assessment of difference between colchicine release in different physical and geographical conditions was analysed by using independent *t* test and one-way ANOVA and whenever appropriate post hoc Bonferroni's multiple comparison test was also conducted. In all cases, *p* values lower than .05 were set as statistically significant.

3. Results

The average of four geographical regions on colchicine release from different parts of *Gloriosa superba* plant in acidic media as well as alkali media were calculated (Table 1) and the subsequent analyses were carried out with the pooled amount of colchicine for each plant part at each time point.

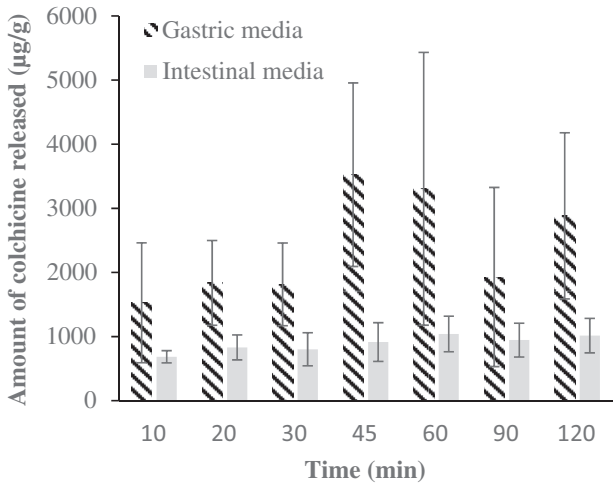
Figures 1–3 illustrate the mean amount of colchicine at different time intervals in simulated gastric and intestinal dissolution media. As shown in the figures, in the gastric dissolution media, the amount of colchicine released increased up to 45 minutes, but beyond which the amount fluctuated; the same trend was observed in intestinal media however, it was less prominent.

The mean ± SD of cumulative amount of colchicine released over two hours from 5 g of tuber, leaves and trunk in gastric medium were 2883.15 ± 1295.63 µg/g, 578.25 ± 366.26 µg/g and 345.60 ± 200.08 µg/g, respectively; these were higher than the release from 5 g of plant parts in intestinal media, i.e., 1014.75 ± 268.16 µg/g, 347.40 ± 262.61 µg/g and 251.55 ± 285.72 µg/g, respectively (Figure 4). The mean

Table 1. Cumulative amount of colchicine released over 120 minutes from 5 g of plant parts in gastric media pre and post activated charcoal treatment.

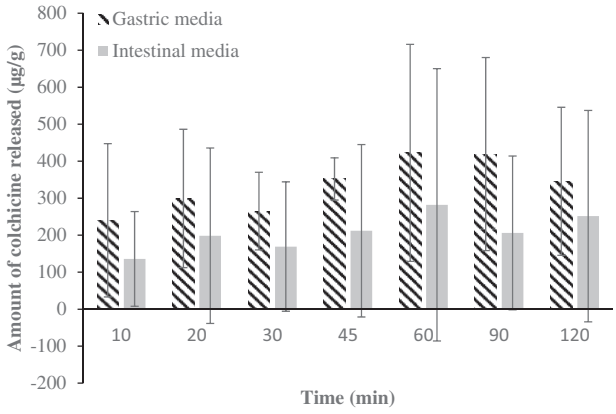
Plant parts	Colchicine release (Tuber) Pre AC treatment (µg/g)		Colchicine release (trunks) Pre AC treatment (µg/g)		Colchicine release (leaves) Pre AC treatment (µg/g)		Colchicine release post AC treatment (µg/g)
Province	SGM	SIM	SGM	SIM	SGM	SIM	All conditions and plant parts
Central Province	3272.4	853.2	579.6	88.2	165.6	486	<0.1
Western Province	1218.6	1301.4	441	212.4	1035	203.4	<0.1
North Central Province	2714.4	1175.4	212.4	666	662.4	639	<0.1
Southern Province	4327.2	729	149.4	39.6	450	61.2	<0.1

AC: activated charcoal; SGM: simulated gastric media; SIM: simulated intestinal media.



Values represent mean ± SD.

Figure 1. Pooled mean amount of colchicine released over two hours in gastric and intestinal media from 5 g of tuber collected from different geographical areas.



Values represent mean ± SD.

Figure 2. Pooled mean amount of colchicine released over two hours in gastric and intestinal media from 5 g of trunk collected from different geographical areas.

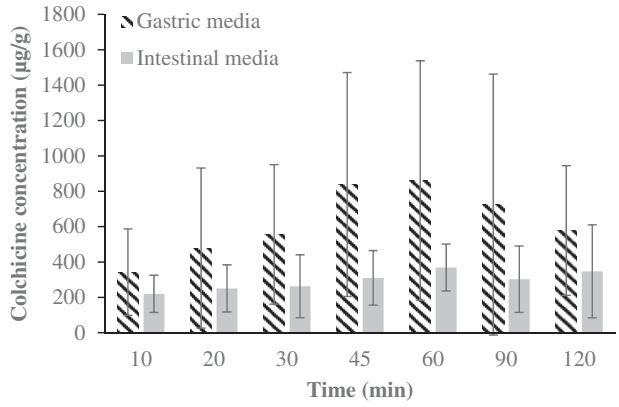
colchicine released from 5 g of tuber was significantly higher in the gastric media than intestinal media ($p = .01$) (Figure 4). Tubers released significantly more colchicine than leaves and trunks in both media (Figure 4).

Binding affinity of colchicine to AC: Free colchicine was not detectable (<0.1 µg/g) when both gastric and intestinal fresh media were pre-treated with 50 g of AC.

4. Discussion

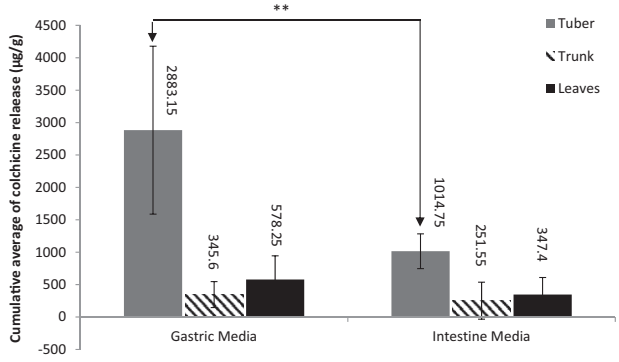
This study shows that there was no geographical variation in colchicine content of *Gloriosa superba* within the areas studied. The tuber contained the highest concentration of colchicine and all parts release more colchicine in gastric media than in intestinal media. Activated charcoal binds colchicine released from different parts of *Gloriosa superba* efficiently in both gastric and intestinal media.

Our study supports the administration of AC to patients with *Gloriosa superba* poisoning. This observation is



Values represent mean ± SD

Figure 3. Pooled mean amount of colchicine released over two hours in gastric and intestinal media from 5 g of leaves collected from different geographical areas.



Values represent mean ± SD. ** represent significance at p<0.01, independent T-test.

Figure 4. Pooled average amount of colchicine released from 5 g of plant parts at 120 min.

consistent with a study that showed that AC is effective in pharmaceutical colchicine self-poisoning. In this study 0.5 mg strength 1, 7 and 20 colchicine tablets were separately dissolved in simulated gastric juice and treated with 5 g of AC, it was found that more than 90% of the colchicine was adsorbed after 20 minutes for each of the three colchicine doses [32].

Tuber is the most commonly ingested part of *Gloriosa superba* for deliberate suicidal attempts and the estimated amount ingested (from patient or accompanying person's history on admission) varies from 5 g to 200 g [33], We only used a single dose of AC in our study and so were not able to study relative binding. However, Decker et al. demonstrated that 5 g of AC can effectively bind up to 90% of 20 colchicine tablets (approximately 10 mg of colchicine) [32]. Our study demonstrates that at AC:plant ratio of 10:1, AC is able to bind all the colchicine released. This could be further studied with different ratios of AC:plant parts to investigate if lower doses of AC could be used.

The demonstrated release of colchicine from tuber and other plant parts would support the administration of AC for at least two hours after ingestion as primary decontamination. In therapeutic oral colchicine administration, the Tmax is 1.07 ± 0.55 hours [34], which further supports the use of AC

up to two hours post ingestion. As we did not study colchicine release past two hours and colchicine and its metabolites undergo enterohepatic circulation [25,35–37], multiple doses of AC may be considered in patients with self-poisoning with *Gloriosa superba*.

The amount of colchicine released increased over time up to 60 minutes and seemed to be reduced at later time points. The possible reason for this observation is dilution of colchicine in dissolution medium with the introduction of fresh media (total of 50 ml was added, representing about 7% dilution). Another reason may be hydrolysis of colchicine to dilute acid yields (methyl alcohol and colchicine) [38] and the yield products were not measured during HPLC test.

4.1. Limitations

We did not simulate gastric media with enzymes released in the stomach and the intestines, therefore the effect of digestive enzymes in the gastric and intestinal mucosa on the release of colchicine from the plant parts remains unknown.

The study did not investigate the influence of chewing on the release of colchicine in gastric and intestinal media. However, crushing was an attempt to simulate chewing. We only studied one dose of charcoal and so were not able to investigate relative binding of colchicine released from *Gloriosa superba* to AC.

5. Conclusions

The dissolution of colchicine from all plant parts for at least two hours occurs most extensively in simulated gastric media. The tuber has the highest quantity of colchicine. Activated charcoal was extremely effective in binding colchicine at these amounts in this *in vitro* study and should have a role in the decontamination of patients presenting to hospital with self-ingestion of *Gloriosa superba*.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Eddleston M, Phillips MR. Self poisoning with pesticides. BMJ. 2004;328:42–44.
[2] Eddleston M. Patterns and problems of deliberate self-poisoning in the developing world. QJM. 2000;93:715–731.
[3] Fuchs J, Rauber-Lüthy C, Kupferschmidt H, et al. Acute plant poisoning: analysis of clinical features and circumstances of exposure. Clin Toxicol. 2011;49:671–680.
[4] Gaultier M, Bismuth C. Acute colchicine poisoning. Rev Prat. 1978;28:4545–4554.
[5] Rochdi M, Sabouraud A, Baud FJ, et al. Toxicokinetics of colchicine in humans: analysis of tissue, plasma and urine data in ten cases. Hum Exp Toxicol. 1992;11:510–516.
[6] Eddleston M, Ariaratnam CA, Meyer WP, et al. Epidemic of self-poisoning with seeds of the yellow oleander tree (*Thevetia peruviana*) in northern Sri Lanka. Trop Med Int Health. 1999;4:266–273.
[7] Saravanapavananthan N, Ganeshamoorthy J. Yellow oleander poisoning-a study of 170 cases. Forensic Sci Int. 1988;36:247–250.
[8] Narendranathan M, Das KV, Vijayaraghavan G. Prognostic factors in Cerbera Odollum poisoning. Indian Heart J. 1975;27:283–286.
[9] Angunawela RM, Fernando HA. Acute ascending polyneuropathy and dermatitis following poisoning by tubers of *Gloriosa superba*. Ceylon Med J. 1971;16:233–235.
[10] Gooneratne BW. Massive generalized alopecia after poisoning by *Gloriosa superba*. Br Med J. 1966;1:1023–1024.
[11] Aleem HM. *Gloriosa superba* poisoning. J Assoc Phys India. 1992;40:541–542.
[12] Mendis S. Colchicine cardiotoxicity following ingestion of *Gloriosa superba* tubers. Postgrad Med J. 1989;65:752–755.
[13] Nagaratnam N, De Silva DP, De Silva N. Colchicine poisoning following ingestion of *Gloriosa superba* tubers. Trop Geogr Med. 1973;25:15–17.
[14] Thomas K, Dayal AK, Gijsbers A, et al. Oduvanthalai leaf poisoning. J Assoc Phys India. 1987;35:769–771.
[15] Thomas K, Dayal AK, Narasimhan, et al. Metabolic and cardiac effects of *Clistanthus collinus* poisoning. J Assoc Phys India. 1991;39:312–314.
[16] Fernando R, Fernando DN. Poisoning with plants and mushrooms in Sri Lanka: a retrospective hospital based study. Vet Hum Toxicol. 1990;32:579–581.
[17] Garcia-Gonzalez A, Weisman MH. The arthritis of familial Mediterranean fever. Semin Arthritis Rheum. 1992;22:139–150.
[18] Moreland LW, Ball GV. Colchicine and gout. Arthritis Rheum. 1991;34:782–786.
[19] Zemer D, Livneh A, Pras M, et al. Familial Mediterranean fever in the colchicine era: the fate of one family. Am J Med Genet. 1993;45:340–344.
[20] Stapczynski JS, Rothstein RJ, Gaye WA, et al. Colchicine overdose: report of two cases and review of the literature. Ann Emerg Med. 1981;10:364–369.
[21] Bismuth C, Gaultier M, Conso F. Medullary aplasia after acute colchicine poisoning. 20 cases. Nouv Presse Med. 1977;6:1625–1629.
[22] Roberts WN, Liang MH, Stern SH. Colchicine in acute gout. Reassessment of risks and benefits. JAMA. 1987;257:1920–1922.
[23] Ellenhorn M, Barceloux D. Colchicine overdose and colchicine-containing plant species. In: Medical toxicology, diagnosis and treatment of human poisoning. Amsterdam: Elsevier; 1988. p. 1234–1237.
[24] Kande Vidanalage CJ, Ekanayeka R, Wijewardane DK. Case report: a rare case of attempted homicide with *Gloriosa superba* seeds. BMC Pharmacol Toxicol. 2016;17:26.
[25] Finkelstein Y, Aks SE, Hutson JR, et al. Colchicine poisoning: the dark side of an ancient drug. Clin Toxicol. 2010;48:407–414.
[26] Hastie SB. Interactions of colchicine with tubulin. Pharmacol Ther. 1991;51:377–401.
[27] Wolff J, Capraro HG, Brossi A, et al. Colchicine binding to antibodies. J Biol Chem. 1980;255:7144–7148.

[28] Rouan SK, Otterness IG, Cunningham AC, et al. Reversal of colchicine-induced mitotic arrest in Chinese hamster cells with a colchicine-specific monoclonal antibody. Am J Pathol. 1990;137:779–787.
[29] Eddleston M, Persson H. Acute plant poisoning and antitoxin antibodies. J Toxicol Clin Toxicol. 2003;41:309–315.
[30] USP., The United States Pharmacopeia, the National Formulary: USP XXII, NF XVII. Supplement 5. 17th ed. Rockville (MD): US Pharmacoperial Convention; 1991.
[31] USP., United States Pharmacopeia-National formulary: The official Compedia of Standards Vol. 28. Rockville (MD): United States Pharmacopeial Convention; 2005.
[32] Decker WJ, Combs HF, Corby DG. Adsorption of drugs and poisons by activated charcoal. Toxicol Appl Pharmacol. 1968;13:454–460.
[33] SACTRC. Prospective cohort study on poisoning patients in Sri Lankan Hospitals from 2004–2009. Kandy, Sri Lanka: Faculty of Medicine, University of Peradeniya; 2009.
[34] Ferron GM, Rochdi M, Jusko WJ, et al. Oral absorption characteristics and pharmacokinetics of colchicine in healthy volunteers after single and multiple doses. J Clin Pharmacol. 1996;36:874–883.
[35] U.S. Food and Drug Administration. Information for healthcare professionals: new safety information for colchicine (marketed as Colcrys). Silver Spring (MD): U.S. Department of Health and Human Services: 20993; 2013.
[36] Rudi J, Raedsch R, Gerteis C, et al. Plasma kinetics and biliary excretion of colchicine in patients with chronic liver disease after oral administration of a single dose and after long-term treatment. Scand J Gastroenterol. 1994;29:346–351.
[37] Hunter AL, Klaassen CD. Biliary excretion of colchicine. J Pharmacol Exp Ther. 1975;192:605–617.
[38] Bhat SV, Nagasampagi BA, Sivakumar M. Chemistry of natural products. Berlin Heidelberg: Springer-Verlag; 2005.