

Analysis of herbal teas made from the leaves of comfrey (*Symphytum officinale*): reduction of *N*-oxides results in order of magnitude increases in the measurable concentration of pyrrolizidine alkaloids

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Abstract

Objectives: To determine the relative quantities of two hepatotoxic pyrrolizidine alkaloids, symphytine and echimidine, in teas prepared from comfrey leaves (*Symphytum officinale*), and to determine the potential contribution of the *N*-oxide forms of these alkaloids to levels of the parent alkaloids.

Design: Comfrey leaves were purchased from three commercial sources and used to prepare tea in a manner consistent with the methods used by consumers. An extraction scheme was devised for extraction of the alkaloids, and a gas chromatographic method was developed to quantify the two major alkaloids, symphytine and echimidine. Recognising that the *N*-oxide derivatives of these alkaloids have also been identified in comfrey preparations, chemical reduction was applied to determine the total quantities of the alkaloids as free bases and as *N*-oxide derivatives.

Results: The concentration of symphytine and echimidine varied considerably between teas prepared from leaves purchased from the different vendors of plant material. Moreover, a much higher concentration of symphytine was found in the tea when steps were included to reduce *N*-oxides prior to analysis. The treatment of pure symphytine with hot water did not generate the *N*-oxide derivative *de novo*.

Conclusions: Since the pyrrolizidine alkaloids are known to be hepatotoxic, consumption of herbal teas made from comfrey leaves may be ill-advised. The concentration of pyrrolizidine alkaloids in such teas may be underestimated substantially unless the concentration of *N*-oxides is taken into consideration.

Keywords: Comfrey, Pyrrolizidine, *N*-oxide, *Symphytum officinale*, Symphytine, Echimidine

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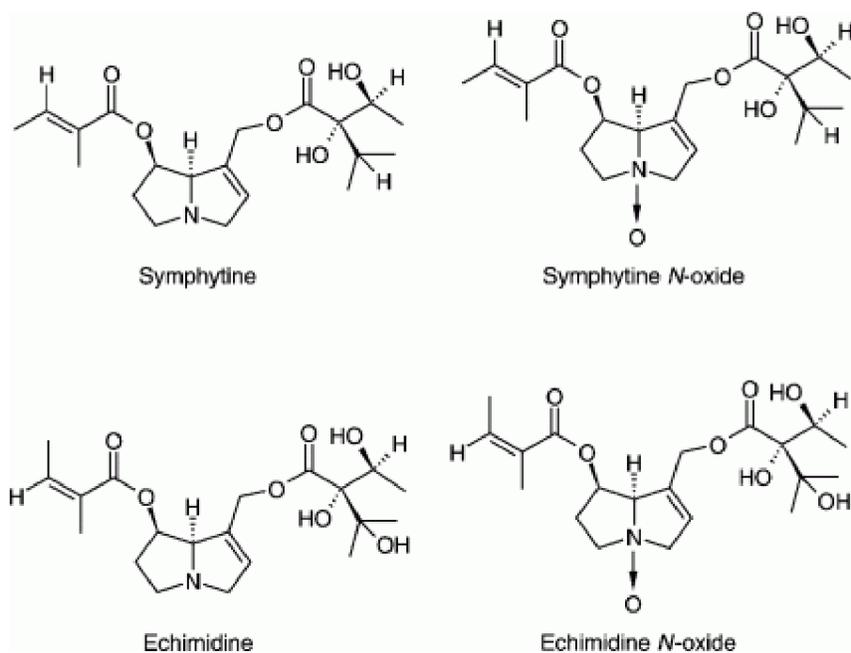
In folk medicine, comfrey has been used as an externally applied poultice to promote wound healing¹. This practice has been extended to the treatment of stomach ulcers and other diseases of the digestive tract via the consumption of teas and/or capsules made from comfrey¹⁻³. However, the presence of hepatotoxic pyrrolizidine alkaloids (PAs) in comfrey has raised concerns over the chronic consumption of teas or other products made from it^{4,5}.

PAs may be metabolised to either pyrroles, possibly responsible for the hepatotoxicity, or *N*-oxides, possibly a detoxification process⁶⁻¹¹. Studies on the structure-activity relationships responsible for either of the above mechanisms have been inconclusive¹². However, Mattocks and Bird¹² suggested that these pathways are parallel but not competitive; the increased formation of *N*-oxides does not lead implicitly to a decreased formation of pyrroles, and *N*-oxides can be reduced to basic alkaloids in the gut¹³. As just one example, in a study on PAs from riddell groundsel (*Senecio riddellii*), Molyneux *et al.*¹⁴ showed that both the free base and the *N*-oxide derivatives of riddelliine are capable of inducing *Senecio* toxicosis in cattle. Nevertheless, some proponents of comfrey have maintained that consumption of tea made from comfrey leaves may not be a risk since the PAs are not particularly soluble in water, and thus the subsequent tea should contain only the more water-soluble *N*-oxides². Yet, case studies have reported severe liver disorder (veno-occlusive disease, VOD) and even fatalities from the chronic consumption of products that contain PAs^{1,15-21}. Prakash *et al.*²² recently published an

extensive analysis of the literature associated with the hepatotoxicity of PAs, and they confirm that PAs cause VOD via metabolic transformation to highly reactive pyrroles.

Despite its ban in several other countries, herbal products that contain comfrey are still available in the US marketplace. Earlier studies have reported the presence and concentration of PAs in several different comfrey consumer products^{2,23}. Huxtable *et al.*²⁴ noted the health risk associated with the consumption of comfrey—pepsin capsules and tablets because they found a high concentration of PAs in these digestive-aid products, both as the native PAs and as the *N*-oxide derivatives. However, comfrey leaves for use as herbal tea are readily available, and, to the best of our knowledge, no one has determined the concentration of PAs and their *N*-oxide derivatives in teas made from comfrey leaves.

Since comfrey-containing products continue to be sold in the burgeoning US herbal marketplace, it is important to evaluate the potential risks associated with their consumption in humans. This may be true particularly for products used in teas, as the consumer is unlikely to read label warnings or directions for making a drink by the universally familiar method of steeping leaves in hot water. In a recent publication, we described the isolation and characterisation of three PAs, symphytine, symlandine and echimidine (Fig. 1), from comfrey roots (*Symphytum officinale*) using countercurrent chromatography²⁵; the roots have been shown to have a much higher concentration of PAs relative to the leaves^{2,15,26}. Herein, we describe the use of symphytine and echimidine as reference standards to analyse herbal teas made from comfrey leaves. Moreover, by reducing *N*-oxides (Fig. 1) prior to analysis, we observed an order of magnitude increase in the measurable concentration of symphytine. This demonstrates the relatively high level of total PAs that may result from drinking comfrey herbal tea.



Structures of the pyrrolizidine alkaloids quantified in comfrey tea

Methods and materials

General experimental procedures

For gas chromatography (GC) analyses, a Hewlett-Packard 5890A instrument equipped with a J&W DB-1 (30 mx0.25 μm film thickness) column and a nitrogen/phosphorus detector (NPD) was utilised. The GC oven temperature was held at 55 °C for 1 min, and programmed to increase to 120 °C at 20 °C min^{-1} and then to 260 °C at 2 °C min^{-1} . The flow rate of the helium carrier gas was 0.65 ml min^{-1} at 250 °C, and the injector was split-less (1 min)/split at 190 °C. Data were managed using the Waters Millennium chromatography data system.

Plant material

Air-dried leaves purported to be *Symphytum officinale* L. (Boraginaceae) were purchased from Blessed Herbs (Oakham, MA), Richters (Goodwood, Ontario, Canada) and Frontier (Norway, IA) in 1998. Voucher specimens from each of these (NCU No. 566519, 566520 and 566521, respectively) have been deposited in the Herbarium of the University of North Carolina, Chapel Hill. To avoid any perceived conflict of interest, we coded the identity of these three samples as vendors A, B and C.

Preparation of comfrey tea and generation of the simple alkaloid extract

Ground comfrey leaves were used to make teas according to the procedure described by Betz *et al.*². Briefly, comfrey leaves (10 g) were added to 1 l of hot (90 °C) water, and the mixture was allowed to steep for 5 min. The resulting solution was decanted and passed through cheesecloth, allowed to cool to room temperature, and extracted three times with 1 l of chloroform–ammonium hydroxide (99:1). The chloroform extract was concentrated *in vacuo*; the PAs were visualised in this fraction via thin-layer chromatographic analysis using Dragendorff's reagent²³.

Preparation of comfrey tea followed by rigorous alkaloid extraction with and without an N-oxide reduction step

Comfrey leaves from one vendor (vendor A, 10 g) were added to 1 l of hot (90 °C) water, and the mixture was allowed to steep for 5 min. The resulting solution was decanted, passed through cheesecloth and allowed to cool to room temperature. Next, the eluent was brought to pH 2 with concentrated sulfuric acid²⁵ and zinc dust (5 g, Aldrich) was added. The resulting mixture was stirred for 3 h, then the zinc dust was removed by filtration and the acidic eluent shaken with chloroform. The aqueous layer was then made basic with ammonium hydroxide (pH 11) and partitioned between chloroform and water^{25,27}. The PAs partitioned into the organic fraction as free bases (visualised using Dragendorff's reagent). For the purpose of a direct comparison with a non-reduced sample, a separate 10-g aliquot of leaves from vendor A was treated in an identical manner, except zinc dust was not added. Furthermore, to determine if the hot water treatment induced the formation of N-oxides, a sample of pure symphytine (19 mg) was carried through analogous procedures (with and without the addition of 50 mg of zinc dust).

Calibration curves for symphytine and echimidine

The purification of symphytine and echimidine from the roots of *S. officinale* has been described previously²⁵; methanol solutions of these samples were used as standards to create calibration curves over the concentration range 0.1–20.0 ng μl^{-1} . Aliquots (1 μl) of each solution were injected into the GC–NPD system. Quadratic unweighted calibration curves were generated from measurements of the concentration of the reference standards versus peak area; r^2 for both equations was 0.99. The estimated limit of detection (ELOD) was calculated as 3 \times SD (standard deviation) of the peak area, expressed as concentration, for triplicate injections of standards at 0.4 ng μl^{-1} for symphytine and 0.4 and 1.0 ng μl^{-1} for echimidine. For confirmation, small amounts of each compound were analysed to observe experimentally the detection limit. From this procedure, the ELOD for symphytine was approximately 0.1 ng μl^{-1} , and the ELOD for echimidine was between 0.1 and 0.4 ng μl^{-1} .

Analysis of comfrey teas by GC–NPD

Aliquots of the residues from the teas were dissolved in 100 μl of methanol, and the solutions were centrifuged to remove a precipitate; alkaloids were not detected in the precipitate via GC–NPD analysis (data not shown). The methanol-soluble portions were analysed by GC–NPD (1 μl aliquots). Peaks for symphytine and echimidine in these extracts were determined by comparisons of the retention times with those of the reference standards. For confirmation, the tea samples were spiked with 25 μl of a 20 ng μl^{-1} methanol solution of pure symphytine or a 20 ng μl^{-1} methanol solution of pure echimidine, and these spiked samples were also analysed by GC–NPD.

Results and discussion

Currently, the US government does not have restrictions in place on the PA content of herbal drugs. However, in Germany, consumption of total PAs with 1,2-unsaturated necine moieties, such as seen in the structures of

both symphytine and echimidine, is limited to 1 $\mu\text{g dai}^{-1}$, although special consideration is given for comfrey tea, which is limited to a maximum dose of 10 $\mu\text{g daily}$ ⁹. At the onset of our studies, there was some question as to whether the PAs would be extracted into comfrey tea at all, since they are not particularly water-soluble. Using a procedure that approximates the methods of consumers², herbal teas were prepared by steeping comfrey leaves in hot water. To obtain the simple alkaloid extract, the decanted, aqueous decoction was shaken with a solution of chloroform–ammonium hydroxide (99:1), and the concentrations of symphytine and echimidine in this extract were determined using gas chromatography with nitrogen/phosphorus detection (GC–N PD) (see Methods and materials section).

As shown in Table 1, the weight of the simple alkaloid extract was nearly equivalent for all three commercial samples (-20 mg), and therefore this mild extraction technique treated them in a consistent manner. However, the concentrations of symphytine and echimidine varied considerably within these extracts. In the tea made from the comfrey leaves purchased from vendor A, the concentrations of symphytine and echimidine were approximately an order of magnitude greater than their respective concentrations in the tea made from the vendor C material. Similarly, the tea prepared from the vendor B comfrey leaves had a high concentration of echimidine. Huizing *et al.*³⁰ have shown that a genetically pure sample of *S. officinale* does not produce echimidine. Therefore, the observation of echimidine in these teas could be indicative of varying levels of contamination of the common comfrey leaves with other species of *Symphytum*, such as the hybrid *S. \times uplandicum* (Russian comfrey), which is known to contain echimidine. Also, it is possible that samples that have a higher relative concentration of the PAs may be contaminated with some root material, as the root is known to have a much higher concentration of PAs than the leaf^{2,15,26}. Alternatively, the observed variability in the concentration of symphytine and echimidine may be due to natural deviations among plant material grown and harvested under differing conditions^{31–36}. In fact, by studying the leaves of *S. \times uplandicum*, Mattocks³⁷ found a 16-fold higher concentration of total PAs in small leaves than in large ones, and in an investigation of *S. riddellii*, another PA-producing plant, Molyneux and Johnson³⁸ reported that samples from one specific collection site had a particularly high level of total PAs as measured against specimens collected elsewhere. Thus, our observation of variability in the concentration of PAs in these three tea samples reinforces that the profile of secondary metabolites in herbal samples is, by nature, variable. The consumption of teas made from comfrey leaves purchased from any of the three vendors exposes consumers to hepatotoxic PAs. Depending on the source of the plant material, this exposure could be quite substantial, especially for those who drink such teas chronically.

Vendor	Weight of chloroform fraction (mg)	Concentration of symphytine ($\mu\text{g l}^{-1}$ tea)	Concentration of echimidine ($\mu\text{g l}^{-1}$ tea)
A	18.8	13.7	14.5
B	21.7	5.3	13.9
C	22.8	1.1	1.5

The *N*-oxide derivatives of the PAs are more hydrophilic than the native free bases, yet, *in vivo*, these *N*-oxides can be reduced to the native PAs in the gut¹³. Since the aforementioned simple alkaloid extraction procedure did not include a step to reduce *N*-oxides, we hypothesised that the concentration of total PAs in the tea could be underestimated substantially. Using comfrey leaves from vendor A, which had the highest concentrations of the PAs symphytine and echimidine (Table 1), the effect of including an *N*-oxide reduction step prior to PA analysis of comfrey tea was examined. The leaves were steeped in hot water and decanted exactly as in the previous experiment, but this aqueous decoction was carried through a rigorous acid/base partition scheme that included treatment with zinc dust to reduce any *N*-oxides to the native PAs. For comparative purposes, a separate aliquot was carried through the identical procedure except that the zinc dust reduction step was omitted.

As shown in Table 2, the weights of the alkaloid extracts formed either with or without the zinc dust reduction step were nearly identical (~7 mg). The concentration of symphytine was measured in each of these using GC–NPD (see Methods and materials section). By comparing Tables 1 and 2, it can be seen that the reduction procedure produced a larger amount of PAs from the aqueous tea as evidenced by a higher concentration of symphytine measured in the vendor A material (14.5 vs. 110 $\mu\text{g l}^{-1}$). In fact, the zinc dust reduction step resulted in a 10-fold increase in the amount of symphytine measured in the tea over the non-reduced samples (Table 2). This suggests that there are high concentrations of *N*-oxide derivatives of the PAs present in the tea, and thus the total PA content of the tea may be underestimated substantially unless procedures to reduce the *N*-oxides are followed. These *N*-oxide derivatives can be reduced to the native alkaloid in the gut with subsequent metabolism to the hepatotoxic pyrrole. Given that the German government limits the maximum dose of PAs in comfrey tea to 10 μg daily²⁹, consumption of 1 l of vendor A tea (approximately three cups) would exceed that limit by two orders of magnitude solely on the concentration of symphytine.

Procedure	Weight of chloroform fraction (mg)	Concentration of symphytine ($\mu\text{g l}^{-1}$ tea)
Before <i>N</i> -oxide reduction	8.4	110
After <i>N</i> -oxide reduction	5.9	1115

Finally, since the *N*-oxides of symphytine are in such a high concentration in tea made from vendor A leaves, it was questioned whether these are being formed *de novo* during the tea-making process. To test this, a pure sample of symphytine was steeped in hot water, and the decanted aqueous decoction was carried through the aforementioned rigorous acid/base alkaloid extraction procedure. In parallel, one sample of symphytine was treated with zinc dust while the other was not reduced. These samples were analysed for the concentration of symphytine using GC–N PD; there was no evidence for the formation of the *N*-oxide derivative of symphytine during the tea-making process.

Conclusions

PAs symphytine and echimidine were extracted into herbal teas made from comfrey leaves; the concentration of both compounds varied based on the source of the plant material. In similar studies, both Betz *et al.*² and Awang *et al.*²³ reported significant variations in the concentration of PAs in several different commercial comfrey products. Over 20 years ago, Roitman³⁹ noted that significant concentrations of PAs could be measured in tea made from comfrey root material – as much as 26 mg per cup. The root is known to produce a higher concentration of PAs than the leaf^{2,15,26}, but he was not able to detect PAs in tea made from comfrey leaves, possibly due to the insensitivity of the methods or instrumentation used at the time. By using a rigorous extraction procedure that compared the inclusion of zinc dust as a reducing agent, a high concentration of symphytine was present in herbal tea made from comfrey leaves as the *N*-oxide. This is not unexpected based on the increased water solubility of the *N*-oxides, but the literature suggests that this does not represent a detoxification process, as such derivatives could be reduced *in vivo* to the native PA, which is then metabolised subsequently to the hepatotoxic pyrrole^{12,22,40}. In a case report of VOD that stemmed from the consumption of numerous herbal products, including a powder purported to be from comfrey root, Ridker *et al.*¹⁸ found the *N*-oxide levels in a tea made from this herbal preparation to be seven times greater than levels of the free base of the PAs. Thus, to most accurately determine the total concentration of PAs in teas made from comfrey leaves, procedures that account for the *N*-oxides, such as zinc dust reduction as shown herein, should be utilised.

Furthermore, since treatment of symphytine with boiling water did not generate the *N*-oxide derivative *de novo*, these *N*-oxide derivatives of PA are apparently not produced during the tea-making process; therefore, they may be present to a varying extent in comfrey leaves based on natural differences between plants or in the storage and/or harvest conditions. As has been suggested by other authors^{1,2,22,23,41}, consumption of tea made from comfrey leaves is ill-advised because of the presence of hepatotoxic PAs, both in the native form and, possibly to a much larger extent, in the form of the more water-soluble *N*-oxides.

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