Appendix 1

The alkaloid content of comfrey

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Introduction

Alkaloids are organic substances other than certain simple amines and amino-acids associated with proteins which are produced by certain plants and which have a basic nitrogen atom. Although alkaloids may be regarded as metabolic by-products, they frequently serve the plant as a nitrogen reserve. They are widely distributed throughout the plant kingdom, being present in about 5 per cent of all species, and occur in a wide variety of chemical forms. Many of the alkaloids are pharmacologically active being either poisonous or having medicinal properties. Thus green potatoes produce a poisonous solanine, certain poppies—morphine, nux vomica—strychnine, tobacco—nicotine, and foxgloves—digitalis, and alkaloids are frequently the active component in herbal medicines.

One group of alkaloids known as the pyrrolizidines has come into prominence more recently. This group is hepatoxic, causing in the liver either acute reactions with massive necrosis (that is, total destruction of tissues) or slower chronic symptoms of wasting with the development of extensive liver tumours according to the level and duration of ingestion. This type of alkaloid is responsible for wasting and fatalities which occur in cattle grazing in fields containing *senecio* species (Ragwort) of the compositae. Other examples of the pyrrolizidine alkaloids also occur in Crotalaria (Leguminosae), Heliotropium (Boraginaceae) and many other species of plants from Graminaceae, Orchidaceae, Sapotaceae, etc. The pyrrolizidine group of alkaloids is generally considered to be highly toxic.

Comfrey belongs to the plant family of the Boraginaceae which includes the Heliotropium species with their high alkaloid content. Furthermore comfrey belongs to the subdivision of this family (Tribe Anchusa) which contains *Anchusa officinalis* and *Borago officinalis* (L.) amongst others which are known to contain this alkaloid. It would be reasonable to expect therefore an alkaloid of this group to be present in comfrey and this has previously been found to be the case by others in *Symphytum perigrinum* (Ledeb) at a concentration of 0·03 per cent. Nevertheless experience gained over many years with feeding cattle and horses on comfrey in different parts of the world has failed to produce any evidence of an acute reaction. Equally well there is an absence of any direct evidence of liver tumours of the chronic reaction in comfrey-fed animals having been observed in slaughter houses. However, from this negative indirect evidence it cannot be decisively concluded that comfrey does not present the toxic hazard of a chronic reaction because cattle bred for meat are slaughtered early in life when at their prime and long before the chronic reaction develops later in life following the slow accumulation of alkaloid. Thus with the fairly recent isolation and identification of two separate pyrrolizidine alkaloids, symphytine and echimidine in *Symphytum officinale* (L.) and the possibility of producing the hepatic tumours of a chronic reaction existing, it was decided that it was vitally necessary that a further investigation should be made of the alkaloids in comfrey before continuing to recommend comfrey for human or animal consumption. There is every indication that the consumption of comfrey by animals could sharply increase as an alternative source of protein in this protein-deficient world and it is being increasingly consumed by mankind because of medicinal properties. It is used in the form of fresh leaves in salads, dried leaves for tea and the root may be ground to produce a comfrey flour.

Method of Approach

Studies on the alkaloid content and toxicity were co-ordinated between the Chemistry Department of the University of Exeter, the Toxicology Unit of the Medical Research Council at Carshalton and the Michaelis Nutritional Research Laboratory at Harpenden. The studies consisted of:

1. The extraction and purification of the alkaloid for direct injection into rats to determine immediate toxicity.
2. Direct feeding experiments with rats to determine long-term chronic effects.
3. The determination of alkaloid content in the green leaves of various comfrey clones.

Comfrey root is known to have the highest concentration of alkaloid and this was used both for the source of alkaloid for injection and for the long-term feeding experiment. The alkaloid was extracted and purified in the Department of Chemistry at Exeter before being sent to the M.R.C. Toxicology Unit where the experiments with rats were conducted. The method for extraction was basically similar to that used at Harpenden for the determination of alkaloid in various comfrey clones described below.

Laboratory Technique

In order to ensure full development of the alkaloid, samples of fully grown comfrey herbage were taken from the Henry Double-day Research Association trial ground at Bocking to the laboratory and dried at 70°C. and milled to pass through a 2mm screen. Simultaneously a determination of moisture content of the fresh material was also made.

Maximum weighed quantities of the milled leaf were extracted in a Soxhlet apparatus for 8 hours, using methanol as solvent. The methanol was then removed from the extract by carefully heating under reduced pressure on a thermomantle and the 'tarry' residue extracted with a known volume of 0·5N H_2SO_4. The acid extract was divided equally into two separate plastic bottles (a) and (b) and sufficient concentrated H_2SO_4. To this bottle was also added an excess of zinc powder and the contents were kept mixed with a magnetic stirrer for 3 to 4 hours, keeping the temperature below 45°C. Immediately after reduction with the zinc powder, both bottles were filtered into individual separatory funnels, rinsing the bottles and filter funnels with distilled H_2O. A few drops of phenolphthalein were added and enough concentrated NH_4OH added to turn the solution just pink—reddish brown colour. The solutions were then extracted with four small portions of $CHCl_3$ collecting the $CHCl_3$ extracts into two separate 'Quickfit' boiling tubes, any emulsions that formed being separated by passing the extract through a phase paper into the boiling tube. After the addition of enough 40 per cent NaOH to the separatory funnels to make them strongly alkaline, they were extracted with a further four small portions of $CHCl_3$ which were added to the previous $CHCl_3$ extracts in the boiling tubes.

The $CHCl_3$ extracts (a) and (b) were then individually evaporated to dryness under reduced pressure on a thermomantle, care being taken not to burn the extract by wrapping the tubes in glass wool. The extracts were redissolved in 10 ml of AR $CHCl_3$ and a suitable aliquot was pipetted into a small conical flask for titrating with 0·01M p-toluene sulphonic acid adding a speck of methyl-yellow powder to act as an indicator. A similar volume of AR $CHCl_3$ was also titrated to serve as a blank. The results were then calculated according to the formula.

$$\left(\text{Vol. of titrate} - \text{vol. of blank}\right) \times \left(2 \times \cdot 0019 \times \frac{100}{\text{sample wt.}} \times \frac{\text{vol. extract}}{\text{vol. aliquot}}\right) = \text{grams \% alkaloid}$$

Where the titres of the unreduced solution (a) were indicative of total tertiary bases and those of the reduced solution (b) combined tertiary bases and N-oxides representing total alkaloid.

The crude alkaloid extracts were then examined further by thin layer chromatography. Activated plates were prepared with Kielselgel G (E. Merk AG) using $CHCl_3$—MeOH—NH_4OH (85:14:1) as eluent. The chromatographed alkaloids were detected with Dragendorff's—reagent (Thies, Reuther, modified by Vagujfalvi) and intensified by subsequent spraying with ·08N H_2SO_4. The alkaloid mono-crotaline was used for a standard reference for each chromatograph and the R_F values were determined.

Results and observations

For this study the different clones of comfrey have been identified by the Bocking No. given by the Henry Doubleday Research Association at their trial ground. Description of the clones as to their habit of growth, i.e. prostrate or erect, type of leaf, colour of flow, etc., are given in Comfrey Report No. 2 (Henry Doubleday Research Association). The results are given in the following table.

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TERTIARY AND TOTAL PYRROLIZIDINE ALKALOID CONTENT OF DRIED HERBAGE OF THREE SPECIES OF COMFREY, COMFREY MIXTURES AND SEPARATED BOCKING CLONES

Plant Material	*Tertiary alkaloid per cent*	*Total alkaloid per cent*	*R_F Group*
Bocking Clone			
No. 1	·024	·033	2
2	·024	·029	3
4	·015	·029	3
7	·026	·053	2
14	·022	·024	2
16	·025	·033	—
17	·011	·017	3
19	·006	·013	2
20	·014	·014	3
21	·017	·023	2
Mixed	·013	·025	—
USSR	·026	·033	—
S. officinale	·020	·034	—
S. asperum	·035	·062	1
S. Caucasicum	·024	·043	—
Tea—1	·004	·009	3
2	·010	·030	—
Monocrotaline	—	—	1
R_F Group 1	·70–·75		
„ 2	·76–·81		
„ 3	·87–·96		

Solvent: $CHCl_3$: MeOH : NH_4OH (85:14:1)

Although differences in alkaloid content were observed between the different clones the number of observations were too small to establish such differences statistically. Differences were observed between different sets of samples of the same clone and were no doubt at least in part due to slight differences in experimental technique involving in particular the time and temperature involved in leaf drying and extraction. Naturally owing to the lengthy procedure involved it was impossible to

carry out the ideal programme of processing all the samples simultaneously. This is possibly of even greater significance than first realized as the alkaloid proved to be unstable and was very easily oxidized and destroyed during extraction. However it can be seen that with one sample of comfrey tea a Tertiary alkaloid content of ·010 per cent and a total alkaloid content of ·030 per cent was obtained which agreed with that obtained at Exeter University and the Toxicology Unit, whereas with another sample of comfrey tea the values were ·004 per cent and ·009 per cent respectively. At least part of this difference may be due to differences in the plant material used for the different tea samples and the conditions for drying the leaves. Comfrey tea naturally is of considerable interest in this study due to the quantities consumed for medicinal purposes by mankind and further observations will be made on this later in the report. It should be noted that mean values are given for two or more different sets of samples taken at different times in the period Sept.-Nov. Although variations were observed between samples, no seasonal trend could be detected.

For tertiary alkaloids it is interesting to note that the lowest concentration was observed in Bocking Clone No. 19, whereas about six times this concentration was observed in the wild stock *S. asperum* and this was also reflected in the total pyrrolizidine alkaloid content. In general the tertiary alkaloids constituted about 50 per cent of the total value.

The variations observed in the analytical values indicated that much larger numbers of samples and uniform conditions were required for a comparison of the results to be statistically significant but unfortunately this could not be financially justified at this stage. A larger number of chromatographs was also needed for the determination of the R_F values using T.L.C. The method used is very effective in separating out closely related alkaloid compounds but unfortunately is less reliable in giving repetitively reproduceable results. In Table 1 values are given for the main spot on the plate with a second fainter spot following fairly closely behind it. Much more work needs to be done to sort out and identify these chromatographic spots but this was considered to be beyond the scope of this study. However it can be seen that with the possible exception of *S. asperum*, the alkaloids behaved quite differently from the monocrotaline standard alkaloid having higher R_F values. In their paper (1) on *S. officinale* Furuya and Araki detected symphytine and echimidine both of which they showed to be diesters having the typical nitrogenous pyrrolizidine nucleus and they found that echimidine had a lower R_F value. Thus the spots observed by us are most probably those of symphytine and echimidine or their closely related compounds. For their research the Japanese workers confined themselves to *S. asperum* only and used 5Kg (11 lb.) of root material which contained 0·226 per cent of the crude alkaloid, a massive quantity compared with the 50 gm (1·8 oz.) of dried leaf containing ·030 per cent used by us. However, our results suggest that minor differences in the alkaloid structure may well exist between the clones. Such differences could be important in terms of the stability of the alkaloids and also their toxicity.

In their paper, Furuya and Araki (1) also report on a pharmacological test with rats showing symphytine to have an LD_{50} of about 300 mg/Kg; that is an intravenous injection of 300 mg of the purified alkaloid per Kg of rat tissue caused death in approximately 50 per cent of the experimental animals. Similar experiments with alkaloid extracted from the roots of the Bocking clone No. 14 failed to demonstrate a similar toxicity at this level which may reflect slight differences in structure to which reference was previously made. For many purposes toxicity in rats is equivalent to that in mankind so that these results afford definite indication of the level of possible danger comfrey may be in human consumption. Thus in the case of comfrey tea if it be assumed that normal methods of infusion could extract just over half the alkaloid that was extracted by 8 hours in a Soxhlet apparatus in the laboratory, each cup of tea could contain 100 micrograms of alkaloid. At this level the consumer could never attain the lethal dose of 300 milligms/Kg tissue found necessary to produce the acute reaction in rats. Even to consume this quantity it would take a 150 lb. man drinking 4 cups of tea per day a total of 140 years. Furthermore it is known that to produce chronic reactions sub-lethal doses over a prolonged period are necessary. Normally such sub-lethal doses would need to be of a much higher order particularly as sensitivity to the alkaloid decreases with age.

Rats fed on a diet containing a high proportion of comfrey flour over a long period were examined after death anatomically, biochemically and histologically for symptoms of a chronic reaction of the liver to the alkaloid. Typically there is a colour reaction in the liver tissue itself, followed by the appearance of tumour cells and ultimately large visible protruding tumours. None of these symptoms were found in the experimental rats. As has been stated the alkaloid proved to be an unstable compound and was easily oxidized and it would appear conceivable that apart from its low toxivity in the purified state a considerable portion of the alkaloid would be destroyed in preparation and cooking of the comfrey flour. With fresh herbage such as leaves in salads it is possible to eat only a relatively small quantity and with its high water content the amount of alkaloid thus actually eaten by man would be very small and there would naturally be present in such herbage catalytic enzymes which would hasten its destruction. Livestock which may consume larger amounts of herbage frequently only eat it when wilted, and thus at a time when enzymatic breakdown could well have begun. Certainly prolonged and extensive use of comfrey herbage as a feeding stuff for animals has failed to reveal any deleterious effects, but rather that of considerable benefit to the health of the livestock. Furthermore it must also be remembered that many other species of plant considered

safe for foodstuffs actually contain toxic alkaloids but in amounts too small to be harmful.

Conclusion

Thus from these experiments and other considerations it may be concluded that the use of comfrey as a food for mankind or animals does not present a toxic hazard from alkaloids, there being no evidence of acute or chronic hepatic reactions either to the direct injection of purified alkaloid or to prolonged consumption of comfrey root flour, which has the highest alkaloid content, by rats. From this and the much lower content of alkaloid in herbage and tea, together with the lack of any toxic evidence in livestock and the very low level of toxicity of the alkaloid itself, it may be concluded there is no toxic hazard from the use of comfrey herbage and tea.

Acknowledgements

I would like to thank Mrs. P. Walshaw of the Michaelis Nutritional Research Laboratory for her considerable help with the delicate and time-consuming processes of extraction, purification, and determination of the alkaloids and also to Dr. D. Crout of Exeter University for his personal interest and advice and also for his help in arranging the pharmacological tests with Dr. Mattocks at the M.R.C. Unit of Toxicology, and to the Henry Doubleday Research Association without whose lively co-operation this work could not have been undertaken.

References

T. Furuya and K. Araki (1968) Studies on Constituents of Crude Drugs 1 Alkaloids of *Symphytum officinale* Linn. *Chem. Pharm. Bull.16* 2512–2516.