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The Effect of Different Storage Conditions on the Chemical Stability, Laxative Effect and Acute Toxicity of Sennoside Solutions

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Abstract: This paper investigates the effect of different storage conditions on the chemical stability, laxative effect and acute toxicity of sennoside solutions. The variables in storage conditions were pH, time and temperature (room temperature or 100°). The chemical stability of sennosides in aqueous solutions was found to be pH-dependent, with the best stability at pH 6.5 ($t_{90\%}$ = 8.4 months) and the poorest at pH 8.0 ($t_{90\%}$ = 2.5 months). Two years of storage at room temperature did not reduce the laxative potency in mice, regardless of the pH. After 4.25 years of storage the potency declined in alkaline solutions only. The degradation products with laxative potencies are chemically unknown. The acute toxicity of sennoside solutions increased with time during storage, the acid solution being more toxic than either the neutral or alkaline ones.

Modern methods of chemical analysis have shown that sennosides A and B are not stable in water solutions (Lemli 1963; Merle & Barthes 1984; Lemli 1986), regardless of whether the solutions are derived from senna extracts or from pure sennosides. Cascara extracts containing anthraquinones related to sennosides have also been found to be stable in simple liquid preparations for a few months only (Crippa 1980). Nevertheless senna products are widely marketed in liquid form and many pharmacopoeias still contain liquid senna preparations (e.g. USP XXI, BP 1980).

The acceptability of liquid senna preparations probably lies in the fact that the majority of chemical assay methods for senna products are based on colorimetry. These methods, however, are not sufficiently specific (Lemli 1976). Many of the decomposition products of sennosides can also react with the colour reagent or have light absorption maxima within the same range as sennosides. This may well have led to incorrect conclusions being drawn from stability tests with liquid senna products.

Studies on the laxative effect of senna products have shown it to be even higher than expected on the basis of the chemically measured content of sennosides. The crude senna drug, for instance, has been found to be 1.7 times as active as pure sennosides (Fairbairn & Moss 1970; Fairbairn 1980). Thus the decomposition products or other constituents may also have a laxative effect, providing misleading evidence as to the stability of the liquid senna products. The therapeutic response of the product may be maintained during storage although the sennoside content falls.

Toxicity tests with senna products have shown that many components of senna extracts are clearly more toxic than pure sennosides (Marvola *et al.* 1981; Hietala *et al.* 1987). It is therefore probable that the decomposition products in liquid senna preparations are toxic, and that a product stored for some years is more toxic than a fresh one.

The aim of the present study was to study the effect of different storage conditions on the stability of pure sennosides A and B in water solutions (conventional storage tests and heating at 100°), using an HPLC method and colorimetry, to study possible changes in the biological responses, laxative potency and acute toxicity of stored solutions, and to compare the biological effects with the chemical stability of the sennosides.

Materials and Methods

Drug. Sennosides A + B (Oy Extracta Ltd., Helsinki, Finland) were used in the experiments. Detailed sennoside contents of the batches are given below.

Storage in water solutions at different pH. McIlvaine citrate-phosphate buffer solutions were used as vehicles in the stability tests. pH values of 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 were selected. Methyl-p-hydroxybenzoate (2 mg · ml^{-1}) was added as a preservative. 12.5 mg · ml^{-1} of sennosides were dissolved into these solutions. The original composition of the drug batch used was: sennoside A 63.5%, sennoside B 33.0% and sennosides A_1 + C 3.5%. The solutions were stored in amber glass bottles at room temperature away from direct sunlight.

Effect of heat. In order to study the effect of heat on the stability of sennosides, 5% water suspensions were prepared. The measured pH of this suspension was 5.0.

The suspensions were heated at 100° under reflux for 1, 2, 4, 8 and 16 hours.

Subsequently the suspensions were stored at −20° until tested. The original composition of the drug batch used was: sennoside A 62.2%, sennoside B 33.2% and sennosides A_1 + C 4.5%.

Laxative effect. The laxative potency of the stored sennoside solutions and heated sennoside suspensions was studied by the method according to Lou (1949). Ten female NMRI mice (Animal House, School of Pharmacy, University of Helsinki) weighing 20–30 g were used for each single dose tested. The tests were repeated to give results from five parallel experiments at each dose level. The doses were administered in randomized order. Drugs were administered (10 ml · kg^{-1}) into the stomach by means of a polyethylene tube.

Table 1.

Stability of sennosides in different water solutions stored at room temperature. The figures indicate the contents of total sennosides A+B+C+A_1 in the solutions (mg · ml^{-1}) measured by the HPLC method.

pH of solution	Storage time, years							Regression line	Correlation coefficient r	$t_{90\%}$ months
	0	0.25	0.4	1.0	1.9	2.4	4.25			
5.5	12.9	11.9	11.6	10.3	8.9	8.3	4.4	ln y = 2.57 − 0.24x	0.983	5.3
6.0	12.7	12.2	11.9	10.8	9.6	8.7	5.5	ln y = 2.57 − 0.19x	0.989	6.6
6.5	12.6	11.9	11.7	9.9	10.3	8.9	6.4	ln y = 2.54 − 0.15x	0.987	8.4
7.0	12.9	12.2	11.8	10.8	9.2	8.5	4.6	ln y = 2.59 − 0.23x	0.984	5.5
7.5	12.0	11.3	10.8	8.7	7.1	5.6	2.8	ln y = 2.52 − 0.34x	0.996	3.7
8.0	12.3	10.4	9.9	7.0	5.0	4.5	1.3	ln y = 2.52 − 0.51x	0.989	2.5

Each animal was placed in a separate steel cage (6 × 18 cm) with a wire-grid floor and was supplied with a food preparation made by mixing 10 parts of powdered ratcubes and 7 parts of water. Laxative effect was measured by counting the total number of wet faeces per kg of body weight. The final count was carried out 24 hrs after the single dose drug administration. The same group of mice was used four to five times with intervening rest periods of one week. No weight loss in the animals was noted during the experimental period.

The laxative effect of the sennoside solutions was determined after 2 years and 4.25 years of storage. At 2 years, three different dose levels (12.5, 50 and 100 mg · kg^{-1}) were used but at 4.25 years, to decrease the number of animals, only a dose of 100 mg · kg^{-1} was used. The solutions were used as such or, if necessary, diluted with 1.4% $NaHCO_3$ solution. The heated sennoside suspensions were diluted with 1.4% $NaHCO_3$ solution before administration.

Standard preparations consisted of freshly made solutions of sennosides in 1.4% $NaHCO_3$. The regression lines of the dose-response curves were calculated for each test and their parallellism determined by comparing the regression coefficients (P = 0.05). The relative potency of each solution measured against the freshly made sennoside solutions was calculated at a dose level of 100 mg · kg^{-1}.

Intravenous toxicity. The acute intravenous toxicity of the stored sennoside solutions and the heated sennoside suspensions was studied in female NMRI mice weighing 20–30 g. Food and water were withdrawn the morning before experiments, but after drug administration the animals were allowed free access to food and water.

To eliminate the effect of inorganic buffering salts on the toxicity tests, the drugs were precipitated from the solutions with 5 M H_2SO_4. The precipitated anthraquinones were separated and washed with water. They were then suspended in water and the pH was adjusted to 7.3–7.5 with $NaHCO_3$. Subsequently all drugs were dissolved to give clear solutions for intravenous administration. By this method small amounts of anthraquinones were left in the mother liquid but the measured amounts were less than 1% of the precipitated anthraquinones.

Table 2.

Stability of sennosides in various water solutions stored at room temperature, evaluated by the colorimetric method.

pH of solution	Original concentration mg · ml^{-1}	Drug concentration at 2.2 years mg · ml^{-1}	Drug concentration at 4.6 years mg · ml^{-1}
5.5	12.9	15.3	14.4
6.0	12.7	15.5	14.9
6.5	12.6	14.8	15.3
7.0	12.9	17.4	15.7
7.5	12.0	15.4	12.7
8.0	12.3	15.9	13.1

The heated sennoside suspensions were diluted with 1.4% $NaHCO_3$ solution to give clear solutions.

Volumes of 10–20 ml · kg^{-1} were administered. Ten mice were used at each dose level and the number of dead animals was counted 24 hrs after administration. LD50 values were calculated according to the Nordic Pharmacopoeia.

Oral toxicity. The acute oral toxicity was tested only with the heated sennoside suspensions. The animals were fasted overnight but allowed free access to water, also after drug administration. A volume of 20 ml · kg^{-1} was used, meaning that not all the drug was in dissolved form in the dose. Other details of the oral tests were as in the intravenous toxicity tests.

High-pressure liquid chromatography (HLPC). The HPLC analysis was performed in the reversed phase manner and with gradient elution (Hietala *et al.* 1988).

Apparatus: Hewlett-Packard liquid chromatograph 1084 B equipped with 79859 LC terminal. Column: Hewlett-Packard RP-8. Detection: Hewlett-Packard VW-detector, measuring wavelength 270 nm.

As solvents were used: Solution A: 5.00 ml 85% phosphoric acid + 12 ml 5 M sodium hydroxyde solution diluted to a volume of 1000 ml. Solution B: Methyl alcohol. Temperature of solution A + 70°, solution B + 40°, oven temperature + 40°.

Attenuation: 8. Paper velocity: 1 cm · $min.^{-1}$. Slope sensitivity: 0.5. Elution programme: 0–18 min. 9–42% B; 18–19 min. 42–60% B; 19–23 min. 60–90% B. Total solvent flow 3 ml · $min.^{-1}$.

Pure rhein, sennoside A, sennoside B, sennosides A_1 + C, sennoside D and sennidins A and B were used as references in the analysis. Methylhydroxybenzoate and the buffers used did not influence the results.

Standard curves were determined for different substances by chromatography of 0–20 µg quantities. The substance quantity in the analysed probe is directly proportional to the number of area units of the corresponding peak of the elution curve as determined by the integrator of the chromatograph.

Colorimetry. An aliquot of the alkaline solution to be analysed, containing sennoside derivatives corresponding to a maximum of 0.4 mg sennoside B, was diluted with 1 M sodium hydroxide in a graduated test tube to a volume of 10 ml (Hietala *et al.* 1988). The test tube was immersed in a boiling water bath for 60 min. The tube was cooled to room temperature and the absorbance of the solution measured with a photoelectric spectrometer at a wave length of 530 nm. Standard curves for each substance were determined by carrying out the reaction with 0–0.4 mg of each. Methylhydroxybenzoate and the buffers used did not influence the results.

Results

Effect of storage solution pH.

Chemical stability. The decrease in total sennoside content in the storage solutions measured by the HPLC method is

Table 3.

Effect of pH on the laxative effect of sennosides stored in water solutions for 2 years. Linear regression lines given for the dose response curves; relative potencies calculated at a dose level of 100 mg · ml^{-1}. Three different dose levels (12.5, 50 and 100 mg · ml^{-1}) administered to five groups of ten mice. Freshly prepared solution of sennosides used as standard.

Test solution	Regression line	Correlation coefficient r	Number of wet faeces · kg^{-1} at 100 mg · kg^{-1}	Relative potency at 100 mg · kg^{-1}
Standard	$y = 54 \ln x - 93$	0.999	156	100
pH 5.5	$y = 73 \ln x - 150$	0.991	186	119
pH 6.0	$y = 72 \ln x - 144$	0.998	188	120
pH 6.5	$y = 66 \ln x - 126$	0.998	178	114
pH 7.0	$y = 66 \ln x - 131$	0.969	173	111
pH 7.5	$y = 64 \ln x - 127$	0.980	168	108
pH 8.0	$y = 73 \ln x - 160$	0.991	176	113

shown in table 1. Regression lines for curve fitting according to first-order kinetics are also given in table 1. A clear relationship is seen between the pH of the solution and the stability of the drug. The optimum pH value for stability was 6.5, but even under these circumstances $t_{90\%}$ was only 8.4 months. At pH values of 5.5 and 8.0 the corresponding times were as short as 5.5 and 2.5 months, respectively.

There was also a clear difference in the disappearance rate between sennoside A and sennoside B. The initial ratio of sennoside A to sennoside B was 1.90 and the final ratio after 3.5 years was 1.66 at pH 5.5 and 2.42 at pH 8.0.

The drug concentrations of the storage solutions were measured by the colorimetric method at 2.2 and 4.6 years (table 2). The colorimetric method invariably detected drug concentrations 6–35% higher after storage than the initial concentration.

Laxative effect. The effect of pH of the solution on the laxative effect of sennosides is shown in table 3 (storage time 2 years) and table 4 (storage time 4.25 years). None of the regression lines for the storage solutions in table 3 were parallel to that for the freshly made sennoside solution ($P < 0.05$), the slope invariably being steeper for the storage solutions. The regression lines for the storage solutions at pH 6.5, 7.0 and 7.5 were parallel to each other ($P < 0.05$). The same result was obtained for the storage solutions at pH 5.5, 6.0 and 8.0.

Table 4.

Effect of pH on the laxative effect of sennosides stored in water solutions for 4.25 years. 100 mg · kg^{-1} administered to five groups of ten mice. Freshly prepared solution of sennosides used as standard (table 3).

Test solution	Number of wet faeces · kg^{-1} at 100 mg · kg^{-1} (mean ± S.D., n = 5)	Relative potency
Standard	156	100
pH 5.5	197 ± 101	126*
pH 6.0	168 ± 72	108
pH 6.5	182 ± 90	117
pH 7.0	199 ± 55	128*
pH 7.5	135 ± 81	87
pH 8.0	76 ± 63	49*

* $P < 0.05$, Student's *t*-test.

Table 5.

Effect of pH on acute intravenous toxicity of sennosides stored in water solutions for 2.4 years. Freshly prepared solution of sennosides used as a standard. 3–5 different dose levels administered to groups of ten mice.

Test solution	LD50 mg · kg^{-1} Calculated from original sennoside content: mean	95% conf. limits	Calculated from sennoside content measured by HPLC at 2.4 years: mean
Standard*	4100	3700–4500	4100
pH 5.5	570	555–585	292
pH 6.5	1190	1110–1275	648
pH 7.5	1337	1228–1455	480

* Marvola *et al.* (1981).

The relative laxative potency of the storage solutions at 2 years was always 8–20% higher than that of the standard. At 4.5 years the mean potency of the solutions with pH values of 5.5 to 7.0 was consistently 8–28% higher than that of the freshly made solution. However, at 4.5 years the mean potency of the alkaline solutions (pH 7.5 or 8.0) was below 100% (87% and 49%, respectively).

Acute toxicity. The acute intravenous toxicity of the solutions with pH values of 5.5, 6.5 and 7.5 was studied after 2.4 years of storage. The results are listed in table 5. All storage solu-

Table 6.

Effect of heat (100°) on sennoside and other anthraquinone concentration of a 5% suspension of sennosides, determined by the HPLC method.

Heating time hrs	Sennosides A, B, A_1 + C mg · ml^{-1}	Rhein mg · ml^{-1}	Other anthraquinones mg · ml^{-1}	Total anthraquinones mg · ml^{-1}
0	49.5	–	–	49.5
1	39.0	–	–	39.0
2	23.5	–	1.6	25.1
4	14.5	–	1.6	16.1
8	3.3	–	5.1	8.4
16	0.0	3.1	1.2	4.3

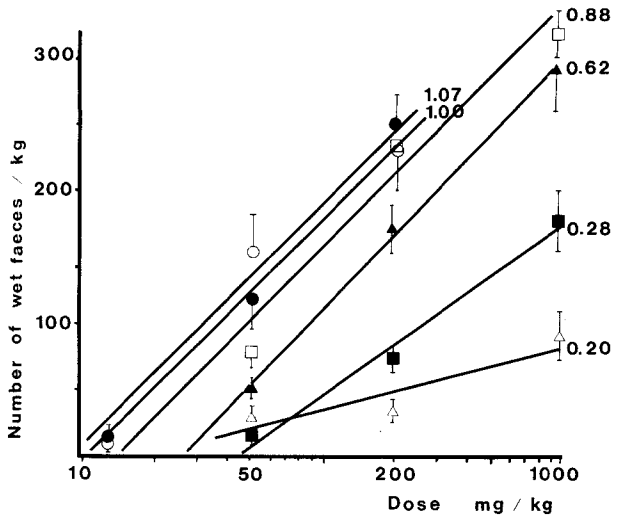

Fig. 1. Laxative effects and relative potencies of heated sennoside A+B solutions, 0 hr (○), 1 hr (●), 2 hrs (□), 4 hrs (▲), 8 hrs (■) and 16 hrs (△). Each point represents the mean ± S.E.M. of five parallel experiments. The figures give the relative potencies at a dose level of 100 mg · kg^{-1}.

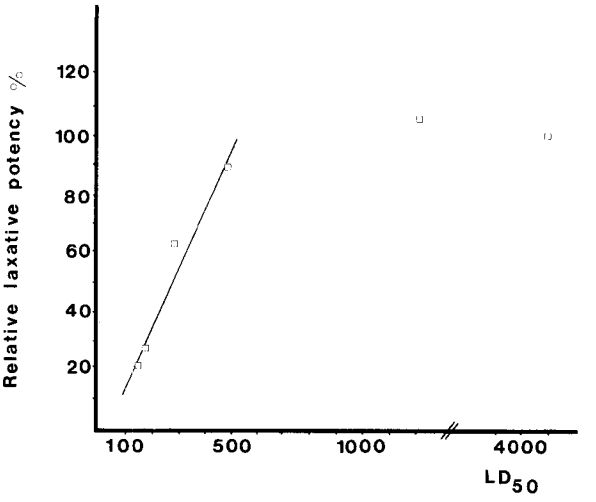

Fig. 2. Relative laxative potencies of heated sennoside suspensions as the function of LD50.

tions were clearly more toxic than the freshly made standard solution of sennosides. The toxicity of the acid solution was enhanced more than that of the alkaline solution.

Effect of heat.

Chemical stability. The effect of heating time on the stability of sennosides in water suspension is shown in table 6. Curve fitting according to first-order kinetics yields the following regression line: $\ln y = 3.93x$, $r = 0.997$, which gives a $t_{50\%}$ value of 2.0 hrs and a $t_{90\%}$ value of 0.31 hrs.

Laxative effect. Fig. 1 shows the laxative potency of the heated suspensions. When heated for 2 hrs or longer the potency declined clearly. The dose-response lines for 8 and 16 hrs were no longer parallel to that of the standard (untreated sennoside solution).

Acute toxicity. The effect of heating time on the acute intravenous toxicity of the sennoside suspension is shown in table 7. When heated for 2 hrs or longer a clear linear correlation ($y = -0.14 + 0.19x$, $r = 0.963$) between the laxative potency and toxicity existed: the higher the toxicity the lower the laxative potency (fig. 2).

The highest possible single dose of heated suspensions administered orally to a mouse was 1500 mg · ml^{-1}. Table 8 shows the number of dead mice after administration of this maximum dose.

Discussion

Our stability tests based on HPLC quantitation of the drug (table 1) confirm the earlier findings that sennosides are not stable in aqueous solutions (Lemli 1963; Merle & Barthes 1984; Lemli 1986). The stability of the solution is affected by the pH. The highest stability exists at pH 6.5 (table 1) but the $t_{90\%}$ value, which is commonly used as an expiration time for drug products, is only 8.4 months, even under these circumstances. The chemical stability of sennosides is lower still, in more acidic, and particularly in alkaline, solutions.

If evaluation of the stability of sennosides is based on colorimetric measurement, no decline in the drug content is observed during the 4.6 years of storage (table 2). It is obvious that the transformation products also have light absorption at the same wavelength as sennosides. The pharmacopoeial assay methods for senna products have so far been based on colorimetry, which has most obviously led to the aproval of a shelf-life of 5 years for many liquid senna products.

When the laxative effect of sennoside solutions is evaluated after 2 years of storage, no decline in potency is observed. On the contrary, somewhat higher mean potencies are found (table 3). The pH of the solutions seems to have no effect on laxative potency. The calculated sennoside con-

Table 7.

Acute intravenous toxicity of sennosides heated in a 5% water suspension.

Heating time hrs	LD50 mg · kg^{-1} Calculated from original sennoside content mean	95% conf. limits	Calculated from sennoside content measured by HPLC mean
0	4100*	3700–4500	4100
1	1200		946
2	480	455–507	228
4	257	241–275	75
8	171	159–183	11
16	125	113–137	0

* Marvola *et al.* (1981).

Table 8.

Effect of heat (100°) on the oral toxicity of sennosides A+B in mice. Test suspension: 5% of sennosides in water, pH 5.

Heating time hrs	Dose $mg \cdot kg^{-1}$	Dead mice/tested mice
4	1500	0/10
8	1500	1/13
16	1500	3/13

tents at 2 years decreased to between 34% (pH 8.0) and 75% (pH 6.5). It is evident that the degradation products have a laxative effect which compensates for the decrease in sennoside content.

Since the dose-response curves for the storage solutions are invariably steeper than those for the freshly prepared sennoside solution, it is even possible that the new compounds formed in the solutions potentiate the purgative effect of the sennosides. This is consistent with our previous finding that senna extracts may be more potent than pure sennosides (Hietala *et al.* 1987).

At 4.25 years the laxative potency of the solution with a pH value of 8.0 statistically significantly declines below 100% (table 4). At lower pH values no loss in potency is evident. As seen in tables 1, 3 and 4, no correlation between the chemical stability and laxative effect exists at 2 years, while at 4.25 years there is a partial positive correlation. Thus, as far as the laxative effect of aqueous sennoside products is concerned, a shelf-life of 5 years is generally acceptable.

If the toxic effect of the storage solutions is presumed to be due to sennoside content alone, whether the original content or that measured at 2.4 years, the acute toxicity of the storage solutions is 3 to 14 times higher than that measured for pure sennosides (table 5). This is explained by the fact that the degradation products must be clearly more toxic than the sennosides.

The accelerated stability test with the 5% sennoside suspension confirms most of the findings of the conventional stability test. Heating at 100° exponentially reduces the sennoside content, with a half-life of 2.04 hrs (table 6). Although during heating small amounts of other anthraquinones are formed, the greater part of the sennosides change to compounds which are no longer anthraquinones detectable with the present HPLC method.

Heating at 100° for up to 2 hrs does not significantly alter the laxative potency of the sennoside suspension, but beyond 2 hrs a clear decline is evident (fig. 1). The acute intravenous toxicity of the suspension strongly increases as a consequence of heating (table 7). As seen in fig. 2, a linear correlation exists at 2 hrs and thereafter between laxative potency and LD50-value.

It is also noteworthy that several mice in the oral toxicity test die if given a dose of 1500 $mg \cdot kg^{-1}$ from the sennoside suspension heated for at least 8 hrs (table 8). The compounds derived from the sennosides are therefore far more toxic than the original drug.

The following conclusions can be drawn from the present results:

1) Sennosides are not stable in an aqueous milieu, and the disappearance rate is pH-dependent. The colorimetric method frequently used is misleading in that it indicates no decline in the sennoside content of water solutions at different pH values.
2) Storage of the sennoside solutions for 2 years does not reduce the laxative potency, irrespective of the pH. However, 4.25 years of storage does reduce the potency if the pH of the sennoside solution is clearly alkaline (8.0).
3) The acute toxicity of the sennoside solution increases with time during storage.
4) No clear correlation between chemical stability and laxative effect or between chemical stability and toxicity is evident. A negative correlation does exist between laxative potency and toxicity.
5) If we accept the use of aqueous sennoside preparations, their sennoside content will inevitably decrease during the shelf-life of the product. The laxative effect of the preparation may remain unchanged or even improve during that period. However, the toxicity of the product increases during storage and this can be seen in the number and reverity of various side-effects.

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References

Crippa, F.: Problems involved in pharmaceutical and cosmetic formulations containing extracts. *Fitoter.* 1980, **51**, 59–66.

Fairbairn, J. W.: Oxalated, sulphated and primary glycosides. *Pharmacol.* 1980, **20** (Suppl. 1), 83–87.

Fairbairn, J. W. & J. R. Moss: The relative purgative activities of 1,8-dihydroxyanthracene derivatives. *J. Pharm. Pharmacol.* 1970, **22**, 584–593.

Hietala, P., M. Marvola, T. Parviainen & H. Lainonen: Laxative potency and acute toxicity of some anthraquinone derivatives, senna extracts and fractions of senna extracts. *Pharmacology & Toxicology* 1987, **61**, 153–156.

Hietala, P., H. Lainonen & M. Marvola: New aspects on the metabolism of the sennosides. *Pharmacol.* 1988, in press.

Lemli, J.: Densitometrische bepaling van de antraglycosiden in het Blad, de Peulen en de Galenische Bereidingen van Senna. *Verhandel. Konikl. Vlaam. Acad. Geneesk. Belg.* 1963, **25**, 458–472.

Lemli, J.: Chemical assay of anthraquinone drugs. *Pharmacol.* 1976, **14** (Suppl. 1), 62–72.

Lemli, J.: The chemistry of senna. *Fitoter.* 1986, **57**, 33–40.

Lou, T. C.: The biological assay of vegetable purgatives. *J. Pharm. Pharmacol.* 1949, **1**, 673–681.

Marvola, M., A. Koponen, R. Hiltunen & P. Hietala: The effect of raw material purity on the acute toxicity and the laxative effect of sennosides. *J. Pharm. Pharmacol.* 1981, **33**, 108–109.

Merle, J. & D. Barthes: Étude par chromatographie liquide haute performance de l'evolution des sennosides A et B en solution. *Farmaco Ed. Pract.* 1984, **39**, 233–242.