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The effect of raw material purity on the acute toxicity and laxative effect of sennosides

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All commercial sennoside preparations contain chemically unidentified impurities besides sennosides A and B, and these may be responsible for many of the side effects of senna preparations (Schmid 1952; van Os 1976; Breimer & Baars 1976). We have compared the acute toxicity and laxative effect of pure sennoside glycosides with those of commercial senna extracts (containing 20–80% sennosides) not only by mouth but intravenously because although the drugs are never administered by that route it is possible that some systemic side effects of senna preparations are due to absorbed impurities (van Os 1976; Breimer & Baars 1976).

The following commercial sennosides or senna extracts used were: (1) sennoside A 99% (Salco Ltd), (2) sennoside B 99% (Salco Ltd), (3) sennosides A + B 99% (Salco Ltd) (I), (4) calcium sennosides A + B 82% (Medica Ltd) (II), (5) calcium sennosides A + B 60% (Andard-Mount Ltd) (III), and (6) calcium sennosides A + B 20% (Indian origin, supplied by Lehner AG) (IV). The percentage contents are those given by the manufacturers. As vehicles, 0·9% NaCl solution for calcium salts and 1·4% $NaHCO_3$ solution for free glycosides were used.

Acute toxicity was studied by intravenous and oral administration of the drugs. Female NMRI mice (20–25 g) were used. The mice for the oral toxicity test were fasted overnight but allowed free access to water, otherwise food and water were withdrawn on the morning before the experiments. Volumes of 10–50 ml kg^{-1} (according to the solubility) were used in the i.v. tests and 20 ml kg^{-1} in the oral tests. All solutions for i.v. injection were clear but, in oral tests, suspensions were also used. Ten mice were used for each dose level, dead animals were totalled 24 h after the drug had been given. The LD50 values were calculated according to the Nordic Pharmacopoeia and expressed as the amount of sennosides in the drug.

The laxative effect of the drugs was studied by the method of Lou (1949). Ten mice (20–30 g) were used for each test. 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 rat-cubes and 7 parts of water. Drugs were administered (10 ml kg^{-1}) into the stomach. Laxative activity was measured by counting the total number of wet faeces produced by the group of 10 mice over 24 h and expressed as the number of wet faeces kg^{-1} of mouse. The animals were used 4 or 5 times with intervening rest periods of one week. The drugs were administered in randomized order. The test were repeated so that results from five parallel experiments at each dose level were available. The regression lines for the dose response curves of each drug were calculated and the parallelity of the lines determined by comparing the regression coefficients ($P = 0.05$).

The results of the toxicity tests are seen in Table 1. No oral dose was lethal. Calcium sennosides A + B 20%

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Table 1. The acute toxicity of pure sennosides and sennoside extracts (calculated as sennosides) in mice.

Drug	LD50 mg kg^{-1} (mean ± 95% conf. limits) Oral	Intravenous
Sennosides A + B 99%	>5000	4100 (3700–4500)
Calcium sennosides A + B 82%	>5000	865 (760–980)
Calcium sennosides A + B 60%	>5000	945 (865–1030)
Calcium sennosides A + B 20%	>2500	172 (154–191)

could not be administered orally at doses higher than 2500 mg kg^{-1}. The intravenous LD50 values of the 82% and 60% extracts did not differ significantly ($P > 0.05$) from each other. All the other differences were significant. When either sennoside A or sennoside B were injected at 4100 mg kg^{-1} into 10 mice half of the animals died in each group. Thus, no difference in acute toxicity between the two isomers of pure sennoside was evident. There was a clear difference in the time of death between the intravenously administered pure sennosides and the sennoside extracts. Animals given the pure sennosides at sufficiently high doses died after some hours, while those given the sennoside extracts died after some minutes.

The results of the laxative effect studies are seen in Table 2. The dose response curves of the drugs were parallel except for that of the 20% extract. The relative potencies of the drugs calculated at 50 mg kg^{-1} and at 100 wet faeces kg^{-1} mouse are seen in Table 2. In spite of the differences in potency at higher doses the minimum effective dose was approximately the same for all drugs. Laxative action started about 2 h after the administration and was maximal at about 4 h. No differences in the rate of laxative action between the drugs were noted.

The oral toxicity of pure sennosides and sennoside extracts was low and LD50 values could not be obtained which agrees with earlier results (Godding 1976). As far as we know the intravenous LD50-values of sennoside preparations have not been reported, the values we obtained show that acute toxicity decreases as the purity of the drug increases. The intravenous LD50 for pure sennosides was 4100 mg kg^{-1} while that for the 20% extract was 171 mg kg^{-1}, a difference of about 24-fold.

We found the sennoside extracts to be more potent laxatives than the pure sennoside. This is in agreement with earlier observations (Fairbairn 1976), and the relative potencies determined for sennoside A (69–84%) are close to the findings of 60–68% by Fairbairn (1965) and Fairbairn & Moss (1970). The two isomers sennoside A and sennoside B were of approximately equal potency and did not seem to be synergistic with each other either in laxative effect or in toxicity. The dose

Table 2. Relative potencies and linear regression lines for the dose response curves of pure sennosides and sennoside extracts in mice (** = $P < 0.01$, *** = $P < 0.001$).

Drug and Regression line	r	Minimum effective dose mg kg^{-1}	Relative potency at 50 mg kg^{-1}	Relative potency at 100 wet faeces kg^{-1}
Sennoside A $y = 152 \ln x - 358$	0·9949***	~10	69	84
Sennoside B $y = 133 \ln x - 299$	0·9931***	~10	64	85
A + B 99% (98%) $y = 160 \ln x - 393$	0·9961***	~10	68	75
A + B 82% (59%) $y = 152 \ln x - 335$	0·9928***	~10	75	100
A + B 60% (61%) $y = 164 \ln x - 351$	0·9942***	~10	84	112
A + B 20% (15%) $y = 232 \ln x - 564$	0·9875**	~10	100	100

The figures in parentheses are the measured sennoside contents by a h.p.l.c. analysis (Huovinen et al 1980).

response curve of the 20% extract was not parallel with those of the other drugs, which indicates that the pharmacodynamic effect was not the same for all grades of purity. This difference was particularly evident at higher doses (100 and 200 mg kg^{-1}). In all, the differences in laxative effect between the drugs were not dramatic.

It is evident that the drugs studied do not contain sennosides, just as declared by the manufacturers. According to a recent h.p.l.c. analysis the drugs used contained sennosides (A + A_1 + B + C + D) as follows: (1) I (99%) 98%, (2) II (82%) 59%, (3) III (60%) 61%, and (4) (20%) 15% (Huovinen et al 1980). This means that the relative laxative potencies of II and IV (calculated as sennosides) are a little higher than is shown by the present results, but also that the i.v. acute toxicities of II and IV are higher.

The intravenous acute toxicity of pure sennosides is significantly lower than that of senna extracts. The laxative potency of pure glycosides especially at higher doses, is also lower, but the difference is not nearly so great as between the acute toxicities. It is evident that the commercial senna preparations contain, besides sennosides A and B, undeclared compounds that are more toxic and have a laxative potency higher than sennosides, or which are synergistic with sennosides.

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