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## Laxative Action in Mice of Tinnevelly and Alexandria Senna, and of Several Botanically Related Plants\*

By I. W. Grote and Maribelle Woods

Senna consists of the dried leaflets of *Cassia acutifolia* Delile, known in commerce as Alexandria senna, sometimes called Egyptian senna; and of *Cassia angustifolia* Vahl, commercially known as Indian or Tinnevelly senna (1). In materia medica, senna is placed in the anthraquinone-containing group of vegetable purgatives which act on the large intestine, "and is one of the most efficacious drugs of this class" (2).

Alexandria senna is collected mostly from wild plants growing in the Nile Valley from Assouan to Kordofan. Usually it is shipped in loose bales or bags containing many broken leaves. For domestic sale in small packages, some whole leaves are separated, but the commercial grades consist largely of mixed or broken leaf, half leaf, fourth leaf, and siftings.

The greater amount of the commercial senna supply consists of Tinnevelly senna collected from *Cassia angustifolia* plants in the Tinnevelly section of southern India, grown originally from seed obtained from wild growth in Arabia. The cultivation, collection and grading of Tinnevelly senna is much more carefully done than with Alexandria senna. Most of the leaflets are received in this country in unbroken form, having been shipped in hard-pressed bales of approximately four hundred pounds each. These are graded in the country of origin as No. 1, No. 2 and No. 3. "The grading is done," according to a letter to the authors from S. B. Penick & Co., importers, "entirely by physical characteristics and by the size of the leaf. Color is not usually considered. For instance, even though a No. 1 is faded in color it would still be classified No. 1 because of its size. The actual size of the leaf included in each grade is variable and depend somewhat on the crop con-

ditions from year to year. The grading is not done by measurement but by sight."

In general, all three grades of Tinnevelly senna are cleaner and less likely to include brown leaves, stems and pods than is the case with Alexandria senna, although both drugs are remarkably clean and free from adulterants.

Notwithstanding the better and more uniform appearance of Tinnevelly senna, Alexandria senna traditionally is more highly regarded and usually brings a higher price than Tinnevelly. Both contain anthraquinone derivatives, and the statement is variously made that Alexandria senna contains more of these derivatives than does the Tinnevelly. According to Maurin, quoted by Trease (3), Alexandria senna contains 1.55 per cent of anthraquinone derivatives while Tinnevelly contains 1.35 per cent, the chief derivatives being aloemodin and rhein. Both derivatives exist in the free state and as glycosides and were isolated by Tutin in 1913 (4).

We were unable to find a comparison of the relative laxative action of Alexandria and Tinnevelly senna. Therefore, we conducted such an investigation, using the mice assay method of Geiger (5) with modifications by Hazleton and Fortunato (6) and with a few modifications by our own staff.

### EXPERIMENTAL

The mice were kept in colony cages in a room in which the temperature and ventilation were controlled, and fed a constant diet of specially prepared mouse pellets. No essential difference was found between the laxative dose required for adult male and female mice, but the sexes were kept separated to avoid possible variations due to pregnancy complications. Mostly male mice were used in this work. The mice were weighed previous to dosage and were then placed in individual wire cages raised above an absorbent paper so protected by wire mesh as to prevent destruction by the animal. The animals used were mature mice weighing between 18 and 28 Gm. At the end of a thirty to

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forty-five minute observation period, mice showing no signs of soft stools or diarrhea characterized by fecal stains appearing through the underside of the paper were employed for the tests. The animals were not used more than once a week.

The senna test samples were prepared by grinding the leaflets to completely pass an 80-mesh sieve and were made into suspensions with boiling distilled water. One-half cubic centimeter doses of such suspensions were given the mice by stomach tube and the mice were then placed in individual wire cages elevated over paper as before and left overnight. Fecal stains on the paper from the animals showing laxative action were easily distinguished from urine stains by reading the stains on the underside of the paper. At least ten mice

form in producing a laxative effect. A dose of 6.3 mg. produced a laxative effect in 54 per cent of 980 mice, while 8.3 mg. produced such an effect in 72 per cent of 570 animals, and a dose of 5.0 mg. produced a laxative effect in 39 per cent of 683 animals. Dosages of plus or minus 20 per cent above or below those necessary to produce a M. La. D.<sub>50</sub> effect are readily detected. The test therefore seems sufficiently sensitive and reproducible to be of a quantitative bioassay nature. The Tinnevely senna used as a standard when bioassayed in another laboratory by this same method gave practically identical results. Mice seem to be slightly more sensitive to the action of senna during weather in which the barometer is rapidly changing, but no seasonal variation has been found and the animals treated with the refer-

TABLE I.—LAXATION IN MICE PRODUCED BY DIFFERENT GRADES OF TINNEVELLY AND ALEXANDRIA SENNA

Test Material	Dose, 12.5 Mg. <sup>a</sup>			Dose, 8.3 Mg. <sup>a</sup>			Dose, 6.3 Mg. <sup>a</sup>			Dose, 5.0 Mg. <sup>a</sup>			Calculated Mg. Dose, M. La. D. <sub>50</sub>	Percentage of Ref. Standard Senna
	Number of Mice	Positive Response	Positive Percentage	Number of Mice	Positive Response	Positive Percentage	Number of Mice	Positive Response	Positive Percentage	Number of Mice	Positive Response	Positive Percentage		
Reference Standard Select No. 1 Tinnevely	...	...	...	570	411	72	980	525	54	683	266	39	5.9	100
No. 1 Tinnevely	50	45	90	75	27	36	75	22	29	50	12	24	8.8	67
No. 2 Tinnevely	...	...	...	50	31	62	50	21	42	50	15	30	6.9	86
No. 3 Tinnevely	50	45	90	125	79	63	125	70	56	75	25	33	6.1	97
5-Yr.-Old Tinnevely No. 2 Leaves	...	...	...	50	38	76	50	30	60	50	17	34	5.8	102
5-Yr.-Old Tinnevely No. 3 Broken Leaves	...	...	...	50	40	80	50	27	54	50	18	36	5.9	100
5-Yr.-Old Tinnevely Nos. 1 and 2 Powdered Leaves	...	...	...	50	35	70	50	32	64	50	14	28	5.9	100
Whole Leaf Alexandria	130	85	65	130	40	31	130	13	10	...	...	...	10.4	58
Half Leaf Alexandria	50	33	66	50	21	42	50	11	22	...	...	...	9.4	63
Fourth Leaf Alexandria	...	...	...	75	57	76	75	51	68	150	65	43	5.3	111
Fine Siftings Alexandria	20	15	75	120	76	63	100	56	56	100	48	48	5.2	113
Mixed Leaf Alexandria	90	74	82	175	101	58	145	71	49	75	16	21	6.5	91
Siftings Alexandria	90	71	79	100	51	51	90	31	34	...	...	...	8.1	73

<sup>a</sup> Dose was suspended in 0.5 cc. distilled water.

were treated with each of three dilutions of a Tinnevely senna used as a standard, and usually twenty-five on each of three dilutions of the "test" senna on the same day. The dilutions were then adjusted on successive days so that preferably approximately 75 per cent, 50 per cent and 35 per cent positive laxative results were obtained, respectively on each of the three dilutions of the reference standard senna and the "test" material. The results were then interpreted according to standard procedure. The dosage required to produce laxation in 50 per cent of the animals is called the M. La. D.<sub>50</sub>. At least a total of fifty animals was used on each of the three experimental doses for the "test" sennas. A 0.5-cc. dose of distilled water, under identical conditions, was found to be without effect.

In order to have a reference standard for comparison of laxative action, several pounds of exceptionally bright green Grade 1 Tinnevely senna were ground to pass an 80-mesh sieve and packed carefully into small tightly sealed bottles. This reference standard has been used throughout our work for the past year, and has proved remarkably uni-

formity in producing a laxative effect. A dose of 6.3 mg. produced a laxative effect in 54 per cent of 980 mice, while 8.3 mg. produced such an effect in 72 per cent of 570 animals, and a dose of 5.0 mg. produced a laxative effect in 39 per cent of 683 animals. Dosages of plus or minus 20 per cent above or below those necessary to produce a M. La. D.<sub>50</sub> effect are readily detected. The test therefore seems sufficiently sensitive and reproducible to be of a quantitative bioassay nature. The Tinnevely senna used as a standard when bioassayed in another laboratory by this same method gave practically identical results. Mice seem to be slightly more sensitive to the action of senna during weather in which the barometer is rapidly changing, but no seasonal variation has been found and the animals treated with the refer-

ence standard senna during the tests herein reported check closely the results obtained during the entire one-year period. Representative samples of Alexandria and Tinnevely sennas were obtained from several sources and ground to pass an 80-mesh sieve, and were administered under conditions identical with those of the reference standard senna. All samples examined met the U. S. P. specifications for senna. The results of the animal tests are given in Table I.

Samples larger and smaller than the doses shown in Table I were also given in many cases, with results agreeing with those obtained from the more significant medial doses. To compute the M. La. D.<sub>50</sub>, the logarithms of the dose in milligrams were plotted against the percentage of response to laxative action and the M. La. D.<sub>50</sub> obtained by interpolation. Two thousand two hundred doses of the reference standard Tinnevely senna demonstrated by statistical interpolation that a dosage of 5.9 mg. was required to produce a laxative effect in 50 per cent of the mice, and this dosage result has been called 100 per cent activity. Three thousand two

hundred additional doses were given from the twelve samples of senna listed in Table I. The results may be summarized as follows:

An unselected No. 1 Tinnevely senna was 67 per cent as active as the reference standard; a No. 2 Tinnevely senna was 86 per cent as active; a No. 3 was 97 per cent as active. A mixed No. 1 and No. 2 Tinnevely senna which had been powdered more than five years ago was 100 per cent as active as the reference standard. Another No. 2, over five years old, was 102 per cent; and a No. 3 over five years old, was 100 per cent of the standard. With the Alexandria senna samples, one of whole leaf was 58 per cent of the reference standard; a half-leaf grade was 63 per cent of the standard; a fourth-leaf grade was 111 per cent; a mixed leaf was 91 per cent, while two separate samples of siftings showed 113 per cent and 73 per cent of the reference standard strength.

If the six samples of each of the two species are added, a dose of 6.6 mg. of Tinnevely and 7.4 mg. of Alexandria would be required to produce the same laxative action as that obtained from 5.9 mg. of the reference standard Tinnevely.

#### COMPARATIVE LAXATIVE ACTIVITY OF SEVERAL SENNA-RELATED PLANTS

War shipping difficulties, threatening to decrease importations of Tinnevely and Alexandria senna, suggested that our investigation be extended to include several of the domestic, botanically related *Cassia* plants. The genus *Cassia* is represented by at least five species which may be found growing wild in Tennessee; namely, *Cassia marilandica* L. (Maryland senna), *Cassia medsgeri* Shafer (wild senna), *Cassia chamaecrista* L. (partridge pea), *Cassia nictitans* L. (wild sensitive plant) and *Cassia tora* L. (low senna or coffee weed). Because of their immediate taxonomic relationships, leaves of *Cercis canadensis* L. (red bud) and of *Gleditsia triacanthos* L. (honey locust) were also included.

Except that *Cassia marilandica* is said by some authorities to have laxative properties, the scientific literature has but scant references to these wild "sennas" beyond describing their botanical characteristics and occasionally speculating as to possible laxative effects of some of them. Slama (7) made an excellent morphological study in 1934 of the first four of the *Cassias* just mentioned. *Cassia marilandica* was listed under this name as a drug in the United States Pharmacopœia from the first edition in 1820 through the 1873 edition, although it was listed separately from the official sennas and it was not indicated to be used as a source thereof or as of equal value. Its therapeutic action was understood to be "similar to the Alexandrian senna, but less active" (8), and scattered references can be found to its supposedly "cathartic acid" content. The dose, according to U. S. P. II, was one ounce of the dried leaf in in-

fusion while the U. S. P. II dose of the official senna of 1830 *Cassia acutifolia* was one scruple to one dram, or not more than 10 per cent as much as was required of *C. marilandica*. It is doubtful if Maryland senna was ever much used, since Oldberg and Wall (9) in a contemporary commentary on the sixth revision of the Pharmacopœia stated: "We have vainly endeavored to obtain some of it on the market, which proves it is not now used, if it ever was."

#### EXPERIMENTAL

Sufficient material for the proposed laboratory study of *Cassia marilandica* was not obtainable from locally accessible wild growths, and large wild stands of it could not immediately be located elsewhere. An experimental crop of *C. marilandica*, sufficient for the purpose, was produced from rootstocks obtained from the Wayside Gardens of Mentor, Ohio, and divided to propagate approximately five hundred plants. These were set out in Chattanooga, Tennessee, in March, 1942. This material was thoroughly authenticated as *Cassia marilandica* L. by several well-known pharmacognosists. Within the months of July and August, 44 pounds of dried leaves were harvested from 458 plants.

A considerable supply of *Cassia tora*, *Cassia medsgeri*, *Cassia chamaecrista* and *Cassia nictitans* was obtained from wild stands near Chattanooga, and leaves from *Cercis canadensis* and *Gleditsia triacanthos* were similarly obtained and prepared by being allowed to dry spontaneously, ground and passed through an 80-mesh sieve. Table II shows the results of the administration of these seven plant leaves to mice as described in our comparative study of the laxative action of Tinnevely and Alexandria senna.

#### RESULTS AND DISCUSSION

The five "wild sennas" and their two relatives were found to be inert in dosages up to ten times that of the reference standard senna which had been prepared from *Cassia angustifolia*, only a single positive result being obtained with one animal out of the hundreds employed in the study. A dosage greater than 50 mg. per animal could not be easily administered to mice by stomach tube. In the case of the *Cassia marilandica*, which traditionally was supposed to possess laxative property, a 50-50 admixture with reference standard *Cassia angustifolia* (Tinnevely senna) was administered with less laxative effect than that obtained from the *C. angustifolia* content alone. Possible diverse effects of climate, age of leaf when harvested, and of soil characteristics on the laxative actions of the "wild sennas" were eliminated by the fact that *Cassia angustifolia* grown from seed planted in the same local area, cultivated, harvested and dried under similar conditions, possessed a laxative activity substantially equal to some of the imported official Tinnevely senna.

Since "mice are not men," and in view of

the negative results obtained from administration of *Cassia marilandica* to the animals, two male human subjects in apparently normal health volunteered to take test doses of this one wild senna previously reported active (8). Dosages of 0.5 Gm. of *Cassia angustifolia* (Tinnevely senna) had a marked laxative action on these two subjects, and dosages of 2.0 Gm. had a violent purgative action. Dosages ranging up to 5.0 Gm. of *Cassia marilandica* (Maryland senna) were without effect.

As a possible chemical verification, the well-known Borntrager reaction, as well as several recent modifications of it, were also carried out on the series of Cassia under investigation. This reaction consists of extracting an acid infusion of senna with ether or other solvent such as benzine, and then adding an alkali to the ether extract. Both of the official U. S. P. sennas tested give a distinct red color, but all the seven inert species tested yielded only varying shades of yellow without a tinge of red.

TABLE II.—EFFECTS OF THE ADMINISTRATION OF CERTAIN SENNA-RELATED PLANT LEAVES TO MICE

Test Material	Mg./Dose	Number of Mice	Number Showing Laxative Action	Percentage Positive	Approx. Percentage of Ref. Standard Activity
Distilled water	0	50	0	0	0
Reference Standard Leaflets of <i>Cassia angustifolia</i> (Tinnevely senna)	8.3	250	171	68	100
	6.3	430	223	52	
	5.0	350	137	39	
	4.2	200	47	24	
<i>C. marilandica</i>	50.0	50	0	0	Below 10%
	25.0	40	1	2.5	
	12.5	40	0	0	
	8.3	10	0	0	
	6.3	10	0	0	
	5.0	10	0	0	
<i>C. marilandica</i> plus <i>C. angustifolia</i>					
Reference Standard 50-50 mixture	25.0	50	39	78	80-90 based on <i>C. angustifolia</i> alone
	12.5	50	21	42	
	8.3	50	7	14	
<i>C. tora</i>	50.0	50	0	0	Below 10%
	25.0	30	0	0	
	12.5	10	0	0	
<i>C. chamaecrista</i>	50.0	50	0	0	Below 10%
	25.0	20	0	0	
<i>C. nictitans</i>	50.0	50	0	0	Below 10%
	25.0	30	0	0	
<i>C. medsegeri</i>	50.0	50	0	0	Below 10%
	25.0	20	0	0	
	12.5	20	0	0	
	8.3	20	0	0	
	6.3	20	0	0	
<i>Cercis canadensis</i>	50.0	50	0	0	Below 10%
	25.0	20	0	0	
	12.5	20	0	0	
	8.3	20	0	0	
<i>Gleditsia triacanthos</i>	50.0	30	0	0	Below 10%

## SUMMARY AND CONCLUSIONS

1. From experimental administration of the leaflets of *Cassia acutifolia* Delile (Alexandria senna) and *Cassia angustifolia* Vahl (Tinnevely senna) to mice, the laxative actions of the two kinds of senna do not appear to differ widely.

2. Variations between different test samples within each kind of senna were as great as between representative samples of each of the two kinds of senna.

3. Samples of *Cassia angustifolia* Vahl (Tinnevely senna) from commercial stocks which had been stored in warehouses five

years or possibly longer showed no appreciable loss of activity.

4. Five native "wild sennas" and two related plants in ten times the dosage required of the official sennas were without demonstrable laxative activity when administered to mice.

5. One "wild senna" (*Cassia marilandica*) was found to be inert in two human subjects when administered in dosages up to 5 Gm. while a dose of 0.5 Gm. Tinnevely senna caused consistently a laxative action in the same subjects.

## REFERENCES

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## A Phytochemical Investigation of *Hermidium Alipes*\*†

By Donald W. Buelow‡ and Ole Gisvold§

*Hermidium alipes* (S. Watson) is a North American plant of the Nyctaginaceae or Four O'Clock family and is found on the foothills of Utah, Nevada and California (1). It is a perennial glabrous herb, five to twelve inches high, with entire, opposite, short-petioled leaves. The flowers occur in head-like clusters on the ends of terminal or axillary peduncles. The clusters are six to eight flowered, each flower subtended by a large ovate leathery bract, and the short pedicels united to the midveins of the bract. The most common member of the family is *Mirabilis jalapa* which is widely cultivated as a flowering plant.

*Hermidium alipes* is worthy of a phytochemical investigation because a fluidextract of the plant when injected into the blood stream of experimental laboratory animals produces a marked rise in blood pressure. The plant, therefore, must contain one or more blood pressor substances. The rises produced are similar to those of epinephrine; however, the duration of action is longer than that of epinephrine.

A phytochemical and pharmacological study of *Hermidium alipes* was previously reported (2) in which all attempts to isolate the active principle had failed. A careful study of the techniques employed in this report led to the conclusion that the problem was worthy of reinvestigation. This conclusion was well founded by the isolation of 3,4-dihydroxyphenylethylamine in the free state.

Figure 1 shows the characteristic response obtained by the intravenous injection of a fluidextract of *Hermidium alipes* in the femoral vein of a rabbit. Figure 2 shows the characteristic response obtained from crystalline material (subsequently identified as 3,4-dihydroxyphenylethylamine) obtained from *Hermidium alipes*. Figures 3 and 4 are a quantitative comparison (3) of 3,4-dihydroxyphenylethylamine to epinephrine and indicate that the former has about one-fortieth the activity of the latter. Figure 5 is a similar comparison in which the drum holding the tracing has merely been reduced in speed to show more closely the quantitative and qualitative relationships.

The plant material for this study consisted of roots collected near Reno, Nev., by the Division of Plant Exploration and Introduction, Bureau of Plant Industry, United States Department of Agriculture.

### EXPERIMENTAL

The plant material used was part of that which had been used in the original investigation of this plant. The dried roots had been ground to a No. 60 powder and had been stored in tightly closed tin containers.

*Preliminary Experimental.*—The solubility of the active principle was determined by the preparation of various extracts of the plant. Each of these extracts was tested for its pressor effect by noting the rise in blood pressure obtained by injection into the femoral vein of a rabbit. One hundred grams of the plant material was successively extracted in a Soxhlet apparatus with various solvents. Petroleum ether, diethyl ether, chloroform, ethyl acetate, and 95% ethanol extracts were made. The solvents were removed under reduced pressure and the residues digested with 70% alcohol. When these filtered solutions were injected intravenously into a rabbit, only the original 95% ethanol extract exhibited activity.

The marc which remained after extraction with the above solvents was moistened with 95% ethanol containing 0.5% hydrochloric acid. It was allowed to macerate for twenty-four hours and was then

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