Physiological Effects of Sericea Tannin in Rats (33127)

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Bell *et al.* isolated a condensed tannin from sericea (*Lespedeza cuneata Don*) which effectively inhibits the fungal hydrolytic enzymes that cause softening of brined cucumber (1). Sericea is a forage crop grown in the southeastern part of the USA. The isolated material from sericea, which inhibits pectinase and cellulase enzymes, is a high molecular weight (14,000–20,000) polymeric leucoanthyocyanidin (1), and is referred to herein as sericea tannin.

The rat feeding studies reported here were designed to determine any possible health hazards to man from the ingestion of pickles made from cucumbers treated with this softening-enzyme inhibitor during the brine fermentation process (2). Other food applications using sericea tannin may also develop.

Methods and Materials.[3] The material fed was isolated from freshly harvested sericea leaves and stems as described by Bell *et al.* (3). The sericea tannin (pectinase inhibitor) was then incorporated into a commercial rat diet at a level of 2%. Ten rats of each sex, from our inbred albino colony, were started on the feeding test at 28 days of age, and after reaching sexual maturity 92 days later, were used to study reproductive performance while continuing on the tannin diet. Equal numbers of rats fed the unsupplemented diet were included as controls. The males were then autopsied after 120 days on test which included a 20-day mating period, and the females were autopsied at the end of 150

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[3] Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

TABLE I. Effect of 2% Sericea Tannin in Diet on Body Weight and Feed Efficiency in Rats.

Group	Sex	No. of rats	Days:	Mean body wt. gains (gm) 92	119	156	Feed efficiency[b] (wt. gain/feed intake)
Purina control	M	10[a]		238	245	—	0.32
+ 2% sericea tannin	M	10		243	251	—	0.29
Purina control	F	10		147	—	172	0.23
+ 2% sericea tannin	F	10		149	—	178	0.22

[a] One rat died after 55 days on test.
[b] During first 35 days of experiment.

days which included a 21-day gestation period, and a 21-day lactation period.

Data were collected on growth, feed intake, hematology, thymus involution, reproductive performance, metabolic fate, and acute toxicity. After autopsy, organ weights, gross pathology, and histopathology data were obtained.

Results. In Table I the growth and feed efficiency values are presented for control and tannin-fed rats. No evidence of undesirable effects due to tannin ingestion is suggested from these data. Shown in Table II are the hematology data of rats fed a diet containing 2% sericea tannin for 85 days. The values all appear to be within normal limits. In Table III the reproductive performance is summarized. Again, there is no suggestion of any abnormal effects due to the ingestion of tannin during the stress of reproduction. Shown in Table IV are the organ weights of control and tannin-fed rats; all values are within normal limits. All rats survived the experimental regimen with the exception of one male rat on the unsupplemented diet which died on day 55 from a respiratory infection.

At autopsy the following tissues were saved for histopathological examination: liver, kidneys, spleen, heart, testes, ovaries, intestine, pancreas, stomach, adrenals, lungs, bladder, thyroids, pituitary, and esophagus. There were no specific gross pathological findings which could be associated with the ingestion of tannin. Also, according to our veterinary–pathologist, Dr. W. E. Gagné, there were no detectable histopathological lesions which could be specifically related to the rats fed the sericea tannin (pectinase inhibitor).

Since the enzyme inhibitor is a polymer of an anthocyanidin, and since various flavonoid compounds have been implicated in the acceleration of thymus involution in rats, mediated through the pituitary–adrenal axis (4), the effect of sericea tannin on thymus involution was investigated. The results were negative in that the thymus weights of a group of 10, 4-week-old male rats fed a semisynthetic diet containing 4% sericea tannin for a period of 2 weeks averaged 0.35 gm/100 gm of body weight, while the mean thymus weight of 10 control rats was 0.30 gm. The mean body weight gain of the rats fed 4% sericea tannin for 2 weeks was also comparable to the control group.

A two-dimensional paper chromatographic technique (5) was used to detect the presence of phenolic degradation products in ether extracts of acidified urine from rats fed tannin. Since no related phenolic degradation

TABLE II. Hematology Data of Rats Fed 2% Sericea Tannin Diet for 85 Days.

Group	Sex	Mean hemoglobin (gm/100 ml)	Mean RBC count ($\times 10^6$/mm^3)	Mean WBC count ($\times 10^3$/mm^3)
Purina control	M	15.6	9.24	15.9
+ 2% sericea tannin	M	16.0	9.13	15.2
Purina control	F	15.1	9.52	11.7
+ 2% sericea tannin	F	15.4	8.97	14.8

TABLE III. Effect of 2% Sericea Tannin on Reproductive Performance in Rats.

Group	Litters	Av pups/litter	Litters weaned at 21 days	Total pups weaned	Mean wt./pup (gm)
Purina only	7/10	7.7	6	50	33.2
+ 2% sericea tannin	7/10	8.0	7	55	35.2

products could be detected, it was tentatively concluded that there was little, if any, absorption of the anthocyanidin polymer per se or degradation products thereof from the intestinal tract.

Acute doses of sericea tannin were given by stomach tube to rats. Deaths were produced within 1 week when rats received 3 daily doses of approximately 3000 mg/kg of body weight of sericea tannin. The cause of death was undoubtedly associated with the finding of the stomach-tubed material in the form of large pellets obstructed in the stomach.

Related to the question of the chronic oral toxicity of sericea tannin, results obtained in an earlier study with grape pomace phlobatannin may be relevant. The grape pomace tannin[4] was fed to rats at a dietary level of 2% for periods from 400 to 700 days. According to Bell *et al.* (1), the condensed tannins isolated from sericea forage and grape leaves give identical color tests and spectral absorbance maxima and hence are probably closely related in chemical structure. There were no histopathological changes observed in the rats fed grape phlobatannin for either 400 or 700 days that were signficantly different from those of the control animals.

Discussion. The absence of specific toxic effects in rats fed a diet containing sericea tannin for up to 150 days was not unexpected since sericea has a history of use as a feed in the southeastern United States in the form of either pasture or hay. That sericea is inferior to alfalfa for growth and milk production in cattle is not surprising in view of its high lignin content, plus the inhibition of cellulose degradation in the rumen by sericea tannin (6). It is also pertinent to mention that numerous foods and beverages consumed by man contain condensed tannins, including tea, coffee, fruits, and vegetables. Hence the relatively small additional intake of tannins associated with the ingestion of cucumbers cured in brine containing sericea tannin would appear to be of negligible consequence.

Summary. The possible toxic effects of a condensed tannin isolated from sericea, which has pectinase and cellulase inhibitor properties, was investigated by feeding this polymeric proanthocyanidin to rats for as long as 150 days at a dietary level of 2%. No effects were observed on growth, feed intake, hematology, reproductive performance, metabolic fate, gross pathology, organ weights, or histopathology. Also included is a summary of the results of feeding a phlobatannin isolated

TABLE IV. Effect of Sericea Tannin on Organ Weights of Rats.

Group	Sex	Mean organ wt./100 gm of body wt.					
		Liver (gm)	Kidneys (gm)	Spleen (gm)	Heart (gm)	Testes (gm)	Adrenals (mg)
Purina control	M	3.14	0.77	0.19	0.38	1.26	11.1
+ 2% sericea tannin	M	3.57	0.77	0.18	0.37	1.18	11.4
Purina control	F	3.84	0.70	0.23	0.40	—	22.4
+ 2% sericea tannin	F	3.97	0.67	0.20	0.38	—	21.5

[4] The grape phlobatannin was extracted from grape pomace with butanol by J. W. Corse and D. C. Patterson of the Western Regional Research Laboratory, Albany, California.

from grape pomace to rats continuously for 700 days. When compared to control rats, there were no toxic effects produced that could be attributed to the ingestion of the isolated sericea tannin or the grape phlobatannin.

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