A NOTE ON THE ACUTE TOXICITY OF HYDROLYSABLE AND CONDENSED TANNINS

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In the course of an investigation into the toxicity of the acorn, Clarke and Cotchin[1] found that acorn tannin had a toxicity comparable with that of tannic acid, but were unable to trace experiments on the toxicity of other tannins. Although these substances had long been regarded as the toxic principles of certain plants, little interest had been shown in their toxicity to man until numerous fatalities were reported after the use of tannic acid in the treatment of burns. Cameron, Milton and Allen[2] and others then showed that tannic acid was extremely toxic by injection in laboratory animals, death following severe liver damage. It is important to note, however, that except for some work by Hartman and Romence[3] on quebracho tannin, all these experiments were carried out with tannic acid. This substance, usually obtained from galls growing on the oak (*Quercus* spp.), is but one of many tannins.

Broadly speaking, these substances may be divided into two classes—hydrolysable tannins and condensed tannins. The former on treatment with mineral acids are readily hydrolysed to sugars and gallic acid or ellagic acid, while the latter condense to yield ill-defined insoluble bodies called phlobaphenes. Tannic acid is a member of the first group. Whether the difference in chemical properties between these two groups of tannins was paralleled by difference in toxicity is the subject of this note.

Experimental Procedure

In addition to tannic acid it was decided to use acorn, myrabolam, sumac and chestnut tannins to represent the hydrolysable, and spruce, quebracho, mimosa, mangrove and gambier to represent the condensed tannins. The material was injected parenterally by four different routes in experiments limited to mice.

The tannic acid was of B.P. grade, the acorn tannin was prepared by the method described by Clarke and Cotchin[1] and the other tannins were commercial samples. All solutions were made in distilled water, and were sterilised by autoclaving, which had the additional effect of redissolving any tannin precipitated by dilution. The volume of solution injected was 0·25 ml. Two-fold serial dilution was employed, the largest dose being 1600 mg./kg.

Results

Table I gives the values of the median lethal doses for the various tannins used. They were calculated by Kärber's method[4]. The use of this method was considered to yield values sufficiently accurate for purposes of comparison in view of the wide range of dose used. This

TABLE I

MEDIAN LETHAL DOSES OF CERTAIN TANNINS (Mg./kg.)
KÄRBER'S METHOD

Hydrolysable tannins	Intravenous	Intraperitoneal	Intramuscular	Subcutaneous
Acorn	150 (8)	100 (8)	75 (8)	100 (8)
Myrabolam	150 (9)	150 (8)	150 (8)	150 (8)
Sumac	150 (8)	150 (8)	150 (8)	150 (8)
Valonia	50 (16)	110 (38)	280 (20)	170 (20)
Chestnut	50 (22)	150 (22)	120 (20)	140 (20)
Tannic acid	75 (22)	50 (22)	350 (20)	240 (20)
Condensed tannins				
Spruce	400 (30)	800 (26)	>1600 (30)	>1600 (30)
Quebracho	130 (17)	360 (12)	>1600 (10)	>1600 (10)
Mimosa	130 (45)	320 (32)	>1600 (30)	>1600 (30)
Mangrove	100 (8)	400 (12)	>1600 (10)	>1600 (10)
Gambier	370 (37)	950 (24)	>1600 (24)	>1600 (24)

The figure in parenthesis after each dose indicates the number of mice used in the determination of that dose.

Table, however, does not adequately express the toxicity of these tannins, as it leaves out of account the time factor. This is shown more clearly in Table II, which gives the number of instantaneous and delayed deaths, and the number of survivals, in groups of 20 mice injected with decreasing amounts of various tannins, four mice being injected with each dose. The instantaneous deaths occurred within a few seconds, while the delayed deaths usually took place between the first and the fifth day. Animals that appeared normal on the seventh day were counted as survivors.

TABLE II

TOXICITY OF CERTAIN TANNINS FOR MICE

	Total number of mice injected	Instantaneous death	Delayed death	Survivors
Intravenous:				
Tannic acid	20	16	4	0
Valonia	20	12	8	0
Chestnut	20	16	4	0
Spruce	20	9	3	8
Mimosa	20	15	1	4
Gambier	20	9	0	11
Intraperitoneal:				
Tannic acid	20	0	20	0
Valonia	20	0	18	2
Chestnut	20	0	18	2
Spruce	20	0	6	14
Mimosa	20	0	10	10
Gambier	20	0	5	15
Intramuscular:				
Tannic acid	20	0	13	7
Valonia	20	0	12	8
Chestnut	20	0	17	3
Spruce	20	0	1	19
Mimosa	20	0	4	16
Gambier	20	0	0	20
Subcutaneous:				
Tannic acid	20	0	13	7
Valonia	20	0	15	5
Chestnut	20	0	16	4
Spruce	20	0	0	20
Mimosa	20	0	1	19
Gambier	20	0	2	18

Discussion

With intravenous injection, there is little difference in the lethal dose between the two groups of tannins. For the intraperitoneal route, however, the lethal dose is clearly smaller for the hydrolysable tannins. For the intramuscular and subcutaneous routes the difference between the groups is very marked; in fact with some of the condensed tannins all mice survived the largest dose used (1600 mg./kg.).

Intravenous injection not only failed to show the difference in lethal dose that was shown by injection by other routes, but also appeared to kill in a different fashion. Most of the mice died more or less instantaneously, apparently from embolism due to the precipitating action of the tannins on the blood, and not from any systemic toxic action.

After subcutaneous and intramuscular injection, tannins of both groups caused local necrotic and inflammatory lesions, but histological examination of a limited number of mice suggested that only the hydrolysable tannins produced centrolobular liver necrosis of the kind described by Cameron[2] and others in experiments with tannic acid. This suggests that the condensed tannins are held in combination with protein at the site of injection or at any rate do not diffuse away quickly enough to reach the liver in sufficient quantity to cause necrosis. In this connection Gustavson[5,6] has shown that the condensed tannin from mimosa combines with protein at physiological pH to a much greater extent than does tannic acid, which scarcely combines at all.

On intraperitoneal injection the hydrolysable tannins produced centrolobular necrosis of the liver but there was no clear evidence that the condensed tannins did so. The cause of death in the latter case thus remains undetermined.

These results were obtained in mice. It should, however, be noted that, with dogs, Hartman and Romence[3] found centrolobular necrosis of the liver in two out of four animals after the subcutaneous injection of quebracho tannin.

It seems, then, that one may conclude that the condensed tannins when injected subcutaneously or intramuscularly are much less toxic than the hydrolysable. If this were found to hold for tannins applied to a burned surface, there might be a case for investigating the use of condensed tannins in the treatment of burns.

Summary

1. Differences in chemical properties known to exist between the hydrolysable and the condensed tannins were found to be paralleled by differences in toxicity.

2. Condensed tannins were much less toxic by intramuscular or subcutaneous injection than were hydrolysable tannins such as tannic acid.

3. It is suggested that the use of condensed tannins in the treatment of burns merits experimental investigation.

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