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A Plant Extract that Prevents Clotting of Mammalian Blood

by

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INTRODUCTION

Roots of *Terminalia glabra* (Kumbuk—in Sinhala) are used in Ayurvedic medicinal treatment in Sri Lanka, and it has been claimed that a water extract of the roots of *T. glabra* possesses 'blood anti-coagulant characteristics'. No experimental evidence has yet been reported in favour of or against such a claim. The experiments herein reported were carried out in an attempt to find out whether a water extract of *T. glabra* has any 'blood anti-coagulant action'.

METHODS

The water extract of the roots of *Terminalia glabra* was prepared as specified by Ayurvedic Physician Dr. Amaratunga of Lauries Road, Colombo 5, Sri Lanka. Fresh roots were chopped into about half inch pieces, and 55 grams of such pieces were boiled in 1920 ml. (= eight cups)of water until the volume was reduced to 240 ml. (= one cup). The reddish brown supernatent was separated out by centrifugation. This solution (extract) was slightly acidic (pH 5 — 6) and was stored in a refrigerator for later use. In most experiments the extract was neutralized with NaOH solution before use.

One series of experiments was carried out on citrated bovine blood, citration being done with 3.1% Sodium Citrate solution (Bell, Davidson & Scarborough, 1968, page 454) at the time of collection of blood. Citrated blood + extract mixtures were made in test tubes, normally, by mixing four ml. of blood with one ml. of the extract of a known 'concentration'. Concentrations used were 100% (original extract), 50%, 25%, 12% and 6.25% (dilutions were made by using 0.9% Sodium Chloride solution). Citrated blood was mixed with 0.9% Sodium Chloride solution in a sixth test tube. Time taken to form a 'firm clot', following addition of excess Calcium ions (0.2 ml. of 2% Calcium Chloride solution) to one ml. of each of the above six mixtures was determined. The method of determination of clotting time was basically similar to the method of Lee & White (1913) (as given by Tocantins & Kazal, 1964, page 30; Biggs & Macfarlane, 1962, page 380). One ml. of a mixture was pipetted out into a small, clean, dry glass tube (1 cm. diameter and 5 cm. height); 0.2 ml. of $CaCl_2$ was added from a burette, and the tube was stoppered

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with a rubber cork. The contents of the tube were mixed immediately by tilting, and the tube was tilted every 30 seconds thereafter to determine the time taken to form a film clot. Regular checking, in this manner, was continued for at least 20 minutes.

Experiments exactly similar to the above were performed using plasma prepared from citrated bovine blood by centrifugation (4000 r.p.m. for 15 min.).

In another series of experiments, the extract was given orally to rats, rabbits and cats, and the clotting time of their blood was determined. The animal was anaesthetized with an intraperitoneal injection of Sodium-penta-barbitone (40 mg./kg.) and the extract was administered through a polythene or a rubber tube inserted through the mouth into the stomach. At hourly intervals, blood samples (0.2 ml.) were withdrawn into small glass tubes from the tail in rats, from blood vessels in the ear lobes in rabbits, and from the femoral vein in cats. The clotting time was determined by the method described above.

All clotting time determinations were made at room temperature i.e. 28° — 30° C.

RESULTS

Results from three experiments on citrated bovine blood are given in Table 1. The time taken for coagulation to occur, 'Calcium clotting time' (Biggs & Macfarlane, 1962, page 146), upon addition of $CaCl_2$ Solution is about the same for blood + 0.9% NaCl, blood + 6.25% extract, and blood + 12.5% extract mixtures : the range of Calcium clotting times is 2.0 to 6.0 minutes. Blood samples mixed with higher concentrations of the extract (i.e. 25%, 50%, and 100% extracts) did not coagulate to form a firm clot even 20 minutes after calcification. In blood + 25% extract mixture, however, some 'solidification' was seen after normal clotting time, but this was not firm enough to prevent flow of blood upon tilting the tube.

TABLE 1

EXPERIMENT No.	CLOTTING TIME IN MINUTES					
	0.9% NaCl (CONTROL)	6.25% EXTRACT	12.5% EXTRACT	25% EXTRACT	50% EXTRACT	100% EXTRACT
1	4.0	5.0	6.0	No Clot	No Clot	No Clot
	5.0	5.0	4.5			
	4.5	5.0	5.5			
	5.0		6.5			
2	3.0	2.5	3.0	No Clot	No Clot	No Clot
	3.0	2.5	3.0		No Clot	
3	3.0	3.0	6.0	No Clot	No Clot	No Clot
	3.0	3.0	6.0	No Clot	No Clot	No Clot

Table 2 gives results from six experiments where Calcium clotting time determinations were made on citrated plasma. It is seen that a firm clot was formed within ten minutes of calcification in all the mixtures except in those containing 50% and 100% extracts. In the mixtures having 50% extract, a 'soft clot' was observed.

TABLE 2

EXPERIMENT No.	CLOTTING TIME IN MINUTES					
	0.9 NaCl (CONTROL)	6.25% EXTRACT	12.5% EXTRACT	25% EXTRACT	50% EXTRACT	100% EXTRACT
1	3.5	3.5	2.5	3.5	No Clot	No Clot
	3.0	3.0	2.5	3.5		
	3.5					
2	3.0	3.5	4.0	3.5	No Clot	No Clot
	4.5	3.5	3.5	4.0	No Clot	No Clot
	3.5	3.5		4.0		No Clot
		4.0				
3	5.0	6.0	7.0	10.0	No Clot	No Clot
	4.5	5.5	7.0	10.0	No Clot	
	5.0					
4	7.0	4.5	4.5	5.5	6.5	No Clot
	6.0	5.0	5.5	5.0	7.0	
	6.0					
5	3.0	2.0	2.5	2.5	No Clot	No Clot
	2.5	2.5	2.0	2.5	No Clot	No Clot
6	2.5	2.5	2.0	2.5	No Clot	No Clot
	2.5	2.5	2.0	2.5		No Clot

Results from five experiments on rats, where the extract was administered orally, are given in Table 3. Three rats were used in each experiment : two rats (Rats 2 and 3) were each given a certain volume of the extract and the other (Rat 1) was given an equal volume of 0.9% Sodium Chloride solution. The clotting time of the blood withdrawn before oral administration of extract (or NaCl), is given in Column 1, while the clotting times of blood withdrawn subsequently are given in Columns 2, 3, 4, etc.. The results show that the oral administration of the extract has not changed markedly the clotting time of the subsequent samples. Further, clotting times of blood from control rats (Rat 1) and those from others (Rats 2 and 3) fall within the same range (1.0 to 2.5 minutes).

TABLE 3

EXPERIMENT No.	RAT No.	WEIGHT OF RAT	CLOTTING TIME IN MINUTES						
			1	2	3	4	5	6	7
	1	242 g.	1.0	1.0	1.0	1.0	1.0	1.0	1.0
1	2	217 g.	1.5	1.5	1.5	2.0	1.0	1.0	1.0
(5 ml.)	3	232 g.	2.5	1.0	1.0	1.0	1.5	1.0	1.0
	1	242 g.	1.0	1.5	1.5	1.0	1.5	1.0	
2	2	217 g.	2.0	1.5	1.5	1.0	1.5	1.5	
(10 ml.)	3	232 g.	1.0	1.0	1.0	1.0	1.0	1.0	
	1	222 g.	1.5	1.5	1.5	1.0	1.0	1.0	
3	2	192 g.	1.0	1.5	1.5	1.0	1.5	1.0	
(15 ml.)	3	217 g.	1.0	1.5	1.5	1.5	1.5	1.5	
	1	220 g.	1.5	1.5	1.5	1.5	1.5		
4	2	215 g.	1.5	1.5	1.5	1.5	1.0		
(15 ml.)	3	192 g.	1.0	1.0	1.0	1.0	1.5		
	1	215 g.	1.0	1.0	1.0	1.0	1.0		
5	2	230 g.	1.5	2.0	1.0	1.0	1.0		
(20 ml.)	3	259 g.	1.5	1.0	1.0	1.0	1.0		

1. The Volume of extract (or NaCl) given to a rat is given within brackets below each experiment No.

2. Rats used in Experiments 3 and 4 were females, while in other experiments they were males.

3. In each experiment, Rat 1 was given 0.9% Sodium Chloride solution.

4. In all experiments, except in Experiment 4, the blood sample 1 was withdrawn after the intraperitoneal injection of the anaesthetic and before administration of extract (or NaCl). In Experiment 4, blood sample 1 was withdrawn before anaesthetization.

5. Extract (or NaCl) was given 15 – 30 minutes after the anaesthetic.

6. The interval between two consecutive blood samples in each animal was approximately one hour.

N.B. Experiments 1 and 2 were performed on the same animals on two consecutive days.

DISCUSSION

The first observation made during this investigation was that when bovine blood, soon after its isolation from the body, was mixed with about one fourth the volume of the root extract, the blood failed to clot. Subsequently, this observation was confirmed several times on blood withdrawn from rats and rabbits. Similarly, the results presented in Tables 1 and 2 clearly demonstrate, that if citrated blood or plasma is mixed with a 'sufficiently high concentration' of a water extract of the roots of *Terminalia glabra*, the addition of excess Calcium ions does not lead to the formation of firm clot. The samples that did not clot in twenty minutes were kept aside for later observation. 'No clotting' was seen to occur in such samples even nine to ten hours after addition of Calcium ions.

Water extract of roots of *T. glabra* with some other constituents is given orally in Ayurvedic medicine. The results given in Table 3, show, however, that oral administration of the root extract, alone, does not significantly alter the clotting time of blood in rats. Essentially similar experiments were carried out on rabbits and cats. Results from these experiments also suggest that, when administered orally, the extract does not produce a marked change in the 'Clotting time' of blood (at least for five to six hours after administration of the extract).

A few of the basic problems that remain to be solved are listed below :—

1. How does the extract prevent clotting of isolated blood, and which of the clotting factor(s) is (are) put 'out of action' by the extract ?
2. How specific is this effect?
3. Why is oral administration of the extract ineffective?
4. What is the nature of 'soft clot' that forms in samples containing intermediate concentrations of the extract ?

Some experiments carried out more recently indicate that whatever factor responsible for prevention of clotting of isolated blood is not found specifically in the roots of *T. glabra:* for instance, a water extract of the bark removed from the trunk also has very similar characteristics. Further, water extracts of roots of a related species, namely, *Terminalia catappa*, too, possesses 'anticoagulant action'.

Much further work has to be done, however, before the problems mentioned above can be satisfactorily discussed. It is premature to consider, at this stage, whether or not a clinically valuable anticoagulant could be obtained from these extracts.

SUMMARY

A series of experiments was carried out to investigate whether a water extract of roots of *Terminalia glabra* possesses any 'anticoagulant action' on mammalian blood, and the following observations have been made.

1. Isolated mammalian blood remains in 'fluid form' if mixed with the water extract immediately after blood is isolated.

2. Clotting of citrated blood or plasma upon addition of Calcium ions is prevented by mixing the blood or plasma with the extract.

3. Oral administration of the extract to rats, rabbits, and cats does not bring about any significant change in the clotting time of their blood.

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