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DIFFERENTIAL SOLUBILISATION OF THE PROTEINS OF ABRUS PRECATORIUS LINN

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Abstract. The solubilisation of the proteins of the seeds of *Abrus precatorius* (Linn) by various anions and cations and at different pHs has been studied. Except for the amount of protein extracted the curves for water and buffers show an identical pattern. Cations seemed to have little effect on solubilisation of protein. Slight variations were observed in the proportion of protein extracted with different anions. The physiological properties of these extracts showed that both the toxin and the agglutinin were extracted to almost the same extent. However, the electrophoretic studies revealed that the toxin and the agglutinin belonged to two different protein components.

The seeds of *Abrus precatorius* Linn. (commonly known as jequirity) have received much attention on account of their toxicity[1–4] and the property to agglutinate blood cells of various species *in vitro*.[5–7] It has also been reported that the seed extracts have anticancerous properties[8] while the oil from the seeds appears to have antifertility effects on laboratory animals.[9–11]

Various attempts have been made to isolate the toxin which has been referred to both as an albumin and globulin.[3,4,12–14] Using standard methods of protein purification Humphreys noticed that the toxin was distributed in all the fractions[15] although electrophoretic studies of the seed extracts by Khan *et al.*[6] had shown that the toxin and the agglutinin are associated with two different fractions, which constitute almost 40% of the total protein of the seeds.

Djang *et al.*,[16] Giri and Tawde[17] have reported the various factors involved in the peptization of the proteins of *Phaseolus aureus* and *Cajanus indicus* respectively. Djang *et al.*[18] were able to purify globulins of mungbeans by fractional peptization. Taking advantage of these results attempts were made to separate the toxin and the agglutinin from each other and also from other protein fractions of the seeds.

The present communication, therefore, relates to a study whereby proteins of *Abrus precatorius* seeds were solubilized under a variety of conditions and the extracts thus obtained were studied for their physiological properties to see if there was any differential solubilization.

Experimental

The scarlet variety of *Abrus precatorius* seeds were procured from the local market, decorticated, ground to 40 mesh and then defatted with other at room temperature (20–25°C). The proximate analysis of the defatted meal gave the following results: protein (N×6.25), 20.9%; fat, 1.2%; moisture, 10.3%; ash, 4.7%.

Extraction of the Meal. The degree of solubilization of *Abrus* seed proteins was determined from the total protein solubilized using (a) various concentrations of anions and cations (b) water adjusted to pH 2–12 and (c) buffers (μ=0.1) of pH 2–12.

Preliminary experiments on the extraction of meal showed that maximum extraction could be obtained by shaking the samples in a Griffin shaker for 120 min. employing a meal to solvent ratio of 1:20 (w/v). Residue was removed first by filtration through cotton and then by centrifugation. The percentage solubilization was determined by thrice washing the residue with the solvent and adding the washings back to the supernatant. For the determination of toxicity and agglutination, however, the residue was not washed. With slight modification proteins were determined according to the method of Lowry *et al.*[20]

The effects of cations and anions on the extractibility of the seed proteins were studied by using 0.1M solutions of the chlorides of lithium, sodium, potassium, magnesium, calcium, cadmium, mercury(ic), nickel(ous) and cobalt(ous), and also by using 0.1M, 0.01M and 0.001M solutions of chloride, fluoride, bromide, iodide acetate, carbonate, bicarbonate, sulphite, sulphate, thiosulphate, monohydrogen and dihydrogen phosphates of sodium.

The effect of varying the pH of the extraction on the solubilization of proteins was investigated by changing the pH of water from 2 to 12 by adding either dilute acid or alkali. Buffers[21] of ionic strength 0.1 in the

range of pH 2–12 were also used for the extraction of the meal. The pH of the contents was checked and readjusted once, during the two-hour shaking.

Paper Electrophoresis. The extracts were subjected to paper electrophoresis on an Eel electrophoretic chamber, using Veronal buffer pH 8.6 (μ=0.05). Each strip was given 1.5 ma current. After a 20–24 hr run the papers were dried in an oven at 110°C for 15 min and dyed in a 0.2% bromphenol blue solution in methanol. The relative amounts of protein in each band were determined by scanning the dyed papers on a Beckman Analytrol.

Haemagglutination and Toxicity. Agglutination of either human 'O' group or dog's erythrocytes by the various fractions was carried out by the method described earlier.[6]

Toxicity was estimated by subcutaneous injections of the various fractions in doses of 0.4 mg/kg in full grown mice (25–29 g) or 6-week old rats (125–150 g).

Results and Discussion

The amount of protein solubilized at different pHs is shown in Fig. 1. Except for the amount of protein extracted the curves for water and the buffers show an identical pattern, with a minimum at pH 4. Cations, in general, seemed to have very little effect on the solubilization of proteins—sodium being the best while mercury was the poorest extractant. Slight variations were observed in the proportion of protein extracted when different anions were used (Table 1).

To determine whether the protein solubilized under different conditions represented different fractions of protein, the extracts were subjected (a) to toxicity and haemagglutination tests and (b) to paper electrophoresis in Veronal buffer pH 8.6 (μ=0.05).

From the groups of buffer extractants, buffers of pH 3, 6 and 9.0 were used, so as to cover the whole range. From the cationic group, only mercuric chloride was used while acetate and sulphite were selected among the anions. They were so selected as to form a group of extractants giving(a) higher,(b) similar and (c) lower amounts of proteins when compared with 10% NaCl extract of the seeds. The results are given in Tables 2 and 3. It appears that there are no marked differences in the activity of the extract: the activities being slightly less in pH 9 extracts than those observed in others. Both the toxic and agglutinating properties were absent from the mercuric chloride extracts. This is probably due to the inhibitory action of Hg(III) ions which are reported to be enzyme inhibitors.[19] The results obtained with anions could be explained on the basis of the pH of their solutions. Maximum amount of protein is extracted by sulphite having a pH around 8.5, but

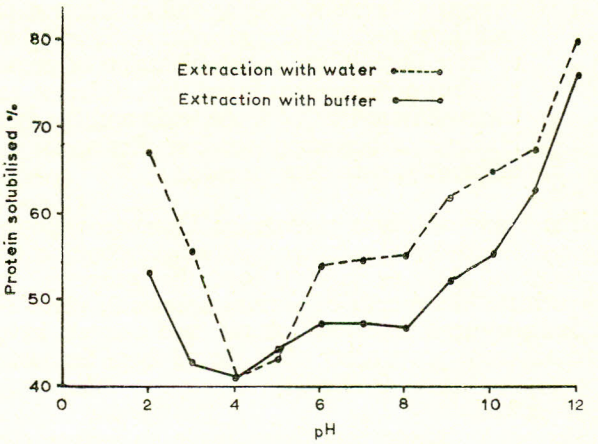

Fig. 1. Solubilization of the proteins of *Abrus precatorius* with water adjusted to pH 2–12 and also with buffers (μ=0.1) of the same pHs.

TABLE 1. EFFECT OF DIFFERENT ANIONS AND CATIONS ON THE EXTRACTABILITY OF PROTEINS OF *Abrus precatorius* SEEDS.

Effect of cation		Effect of anions			
Cations as chloride conc 0.1M	Protein solubilized (%)	Anions as Na-salt	Protein solubilized (%) 0.1M	0.01M	0.001M
Li^{+1}	52.38	Chloride	55.95	50.00	48.20
Na^{+1}	55.95	Acetate	46.43	42.86	44.05
K^{+1}	52.38	Carbonate	65.43	65.42	61.83
Mg^{+2}	53.57	Bisulphate	51.19	48.81	48.81
Ca^{+2}	53.57	Sulphate	54.76	53.33	52.38
Cd^{+2}	44.04	Sulphite	63.09	61.83	55.86
Hg^{+2}	25.71	Dihydrogen phosphate	44.05	36.90	45.23
Ni (ous)	51.19	Monohydrogen phosphate	47.62	52.38	50.00
Co(ous)	44.04	Bicarbonate	58.30	57.11	52.38
		Thiosulphate	63.05	51.19	47.59
		Fluoride	57.10	53.50	58.30
		Bromide	51.11	52.32	66.53
		Iodide	54.76	50.00	61.90

TABLE 2. PHYSIOLOGICAL PROPERTIES OF THE SEED EXTRACTS AT DIFFERENT pH.

Protein extract	Agglutination against dog's blood erythrocytes			Toxicity*
	0.2	0.5	1.0	
NaCl extract (control)	+	++	++++	3/3 18hr†
pH 3	+±	++++	++++	3/3 20 hr
pH 6	+++	++++	++++	3/3 18 hr
pH 9.0	±	±	+++	3/3 27 hr

* Subcutaneous injections in a dose of 0.4 mg/kg were given to 6-week-old rats weighing 125 g.
† 18 hr calculated as the animals were dead when checked in the morning; they could have died a bit earlier too.

TABLE 3. PHYSIOLOGICAL PROPERTIES OF THE SEED EXTRACTS IN PRESENCE OF DIFFERENT IONS.

Protein extract	Agglutination against dog's blood erythrocytes			Toxicity
	0.2	0.5	1.0	
NaCl extract (control)	+	++	++++	3/3 18 hr
Acetate (pH 8)*	+	+++	++++	3/3 20 hr
Sulfite (pH 8)*	—	±	+±	3/3 36 hr
Hg†	—	—	—	Only one died out of 3 after 72 hr.

* 0.1M solution as sodium salts were used.
† 0.1M solution as $HgCl_2$ was used.
The method of extraction was the same as given in Experimental for toxicity and agglutination tests (Table 2).

TABLE 4. CONCENTRATION AND PHYSIOLOGICAL PROPERTIES OF VARIOUS FRACTIONS SEPARATED ON PAPER ELECTROPHORETOGRAMS USING 10% NaCl EXTRACT.

Fraction No.	Protein (%)	Physiological activity		Mobility in cm
		Toxicity	Haemagglutination*	
I	10.22	+ve	—	−1 to +1
II	16.13	—	—	+1 to +3.5
III	29.57	—	+ve	+3.5 to +6.2
IV	44.08	—	—	+6.2 to +9.8

*For agglutination human blood O group (4%suspension) was used.

the protein shows less activity due to the presence of extraneous proteins. On the other hand, lesser amounts of proteins are extracted by acetate and other anions having a pH around 5.0, and the proteins are comparatively more active physiologically.

During the present study, four bands were located when a 10% NaCl extract of the seeds or extracts at pH3, 4 and 5 were subjected to paper electrophoresis in Veronal buffer pH 8.6 (Fig. 2). In all these electrophoretograms toxicity was confined to band I and agglutination to band III. The other two bands showed none of these activities. Electrophoresing any of the above extracts in acetate–acetic acid buffer resulted in the spreading and overlapping of the bands and this might be the reason for Humphreys' observation that the toxicity is associated with each component on the electrophoretogram.[15]

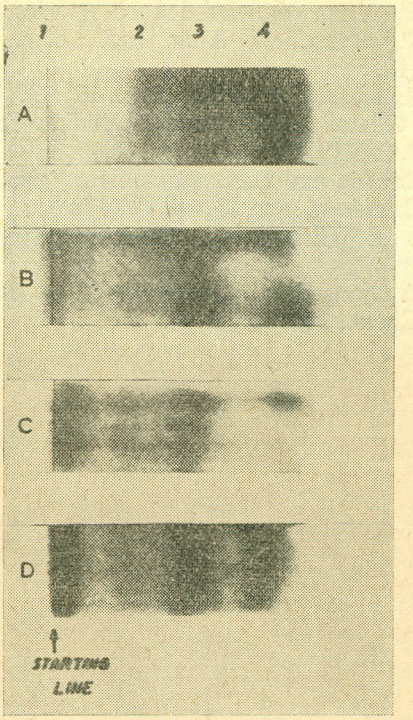

Fig.2. Electrophoretograms of (A) sodium chloride extract, (B.C.D) proteins extracted with water adjusted to pH 5.4 and 3.

Although extraction of proteins of *Abrus precatorius* under various conditions did not result in the differential solubilization of the toxin and the agglutinin, electrophoresis at pH 8.6 of any of these extracts has shown that the toxin and agglutinin belong to two different protein components. Partial separation of these two properties has, however, been obtained in our laboratory by filtration of 10% NaCl extract through Sephadex G-100 (unpublished data).

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