HEMOLYTIC EFFECT OF *PROSOPIS JULIFLORA* ALKALOIDS

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PROSOPIS JULIFLORA A Dc. (Leguminosae) is a poisonous plant widely distributed all over the world[1]. The crushed leaves of *P. juliflora* are commonly used as a suicidal agent and the bark of the plant is employed for the preparation of illicit arrack. The incidence of *P. juliflora* poisoning is common in rural areas due to its abundant availability. The presence of glycosides, tannins, flavonoids and highly complex alkaloids in the plant has been reported[2-7]. Despite the poisonous nature of the plant, the compound(s) responsible for the toxicity and their mode of action in biological system(s) is enigmatic. Since alkaloids are the most interesting pharmacologically active compounds, we have isolated and tested their action in biological system. Primarily, to acquire information about the effect of these alkaloids on blood elements, *in vitro* hemolytic effect of alkaloids *P. juliflora* on both human and rat erythrocytes has been chosen. Our observations may help to explain the mode of action of *P. juliflora* alkaloids on erythrocyte system.

The total alkaloids of *P. juliflora* were isolated by the method of Ott-Longoni *et al*[6] with slight modifications. The leaves of *P. juliflora* were collected, dried and powdered and were then defatted by cold hexane extraction. The defatted powder was subjected to repeated methanol extraction and the combined extracts were concentrated *in vacuo*. The concentrated residue was stirred with 0.2 N HCl and filtered after 16 h. The aqueous solution was shaken with methylene chloride to remove the non-basic material. Then they were made alkaline with ammonium hydroxide in cold. The alkaline solution was extracted with methylene chloride and the organic extract was passed through anhydrous sodium sulphate. The resulting solution was evaporated to dryness to yield total alkaloids of *P. juliflora*. The alkaloids freed from other compounds like flavonoids, glycosides and saponins were tested for their purity using their respective identification test by adopting the method of Paech and Tracey[8].

Whole blood from normal human volunteers and from male Wistar rats was collected in heparinized tubes. The blood was centrifuged to remove plasma and the packed erythrocytes were washed thrice with saline. A cell concentration of 6% in phosphate-buffered saline (9:1 ratio of 0.9% NaCl and 0.1 M phosphate buffer, pH 7.4) system was used for the experiment. The percentage hemolysis was measured by the modified method of Trotta *et al*[9]. To 3.5 ml of 6% cell suspension in phosphate-buffered saline, different concentrations of total alkaloids of *P. juliflora* were added and the volume made up to 5 ml with the buffer. The mixture was incubated for 1 h at 37°C and centrifuged. The supernatant was read at 530 nm in a Shimadzu spectrophotometer. Appropriate controls were maintained without the alkaloids. The percentage of hemolysis was calculated by keeping hypotonic hemolysis as 100%. Aliquots of the supernatant were subjected to extraction of phospholipids, cholesterol and sialic acid which were estimated by the methods of Fiske and Subba Row[10], Parekh and Jung[11] and Warren[12] respectively. Lipid peroxidation index and glutathione concentration in the hemolysates were estimated by the methods of Ohkawa *et al*[13] and Moron *et al*[14] respectively. Hemoglobin content of the blood was estimated by the method of Drabkin and Austin[15].

Alkaloids of *P. juliflora* produced significant hemolysis and the percentage hemolysis in both the rat and the human erythrocytes is presented in table 1. The percentage hemolysis increased in a dose-dependent fashion and, at a concentration of 150 mcg, produced more than 90% hemolysis in both the rat and the human erythrocyte. It is evident that human erythrocyte is more susceptible to hemolysis. Since hemolysis occured due to membrane injury[16], *P. juliflora* alkaloids might be damaging the erythrocyte membrane.

The important membrane components, namely phospholipids, cholesterol and sialic acid enter the supernatant, as a result of membrane injury. The values are presented in table 2. In biological membranes, the levels of cholesterol, phospholipids and glycoproteins are biologically regulated and any

Table 1 *Hemolytic effect of total alkaloids of P. juliflora*

Alkaloid concentration (mcg)	Percentage hemolysis	
	Rat erythrocyte	Human erythrocyte
50	36.04 ± 2.12	41.71 ± 2.94
100	48.21 ± 2.84	60.18 ± 3.16
150	92.03 ± 4.83	96.41 ± 3.07
200	98.26 ± 2.04	100

Values are expressed as mean ± SD from six individual experiments.

Table 2 *Levels of phospholipids, cholesterol and sialic acid release during in vitro hemolysis by P. juliflora alkaloids*

Alkaloid concentration (mcg)	Phospholipid (mc mole Pi/g Hb)		Cholesterol (mc mole/g Hb)		Sialic acid (mc g NANA/g Hb)	
	RE	HE	RE	HE	RE	HE
50	0.52 ± 0.036	0.68 ± 0.047	0.38 ± 0.018	0.49 ± 0.020	2.41 ± 0.17	3.07 ± 0.22
100	0.74 ± 0.043	0.82 ± 0.058	0.45 ± 0.021	0.60 ± 0.039	3.63 ± 0.21	4.72 ± 0.31
150	1.26 ± 0.058	1.57 ± 0.076	0.79 ± 0.040	0.98 ± 0.076	7.37 ± 0.49	8.95 ± 0.53
200	1.44 ± 0.081	1.69 ± 0.081	0.87 ± 0.053	1.08 ± 0.091	8.04 ± 0.56	9.31 ± 0.64

Values are expressed as mean ± SD from six individual experiments; NANA, N-acetyl neuraminic acid; RE, Rat erythrocyte; HE, Human erythrocyte.

Table 3 *Effect of P. juliflora alkaloids on erythrocyte glutathione and lipid peroxidation levels*

Alkaloid concentration (mcg)	Glutathione mmol/g Hb		Lipid peroxidation nmol/g Hb	
	Rat erythrocyte	Human erythrocyte	Rat erythrocyte	Human erythrocyte
50	38.92 ± 1.78	41.55 ± 3.63	162.76 ± 10.2	190.06 ± 11.6
100	29.17 ± 1.12	32.18 ± 2.28	194.52 ± 12.3	236.64 ± 14.1
150	16.74 ± 0.76	19.56 ± 0.92	285.08 ± 15.6	326.37 ± 18.4
200	13.91 ± 0.67	18.05 ± 0.74	305.23 ± 18.9	354.55 ± 20.7

Values are expressed as mean ± SD from six individual experiments.

change in these structural elements might disrupt the membrane fluidity[17, 18]. Our results indicate that the alkaloids could have perturbed the membrane and resulted in the liberation of these structural compounds into the supernatant. Also, it is interesting that the amount of liberated membrane components is well pronounced at the 150 mcg level and this is the optimum dose for hemolysis of 6% erythrocyte suspension.

The levels of lipid peroxidation and glutathione in hemolysate are presented in table 3. The increased level of lipid peroxidation along with the decreased level of glutathione clearly indicate that the alkaloids of *P. juliflora* could have altered the erythrocyte membrane structure through a spectrum of oxidative damage. Increased lipid peroxidation and glutathione depletion are well documented in hemolysis[19, 20]. The hydroxyl group of some alkaloids like ellipticine and its derivatives has been reported to be involved in the formation of free radicals which has induced the lipid peroxidation causing cell damage[21]. Hence the observed lipid peroxidation increase in *P. juliflora* alkaloids treated erythrocyte may be due to the formation of free radicals from hydroxyl group substituted piperitine moiety of the alkaloids.

Hence, it may be concluded that *P. juliflora* alkaloids induce membrane lipid peroxidation which results in hemolysis and due to extensive hemolysis the membrane components are liberated. Attempts are in progress to determine the effect of these alkaloids on erythrocyte membrane *in vivo*.

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RIBONUCLEASE ISOZYMES AT EARLY GERMINATION STAGES IN LEAF-RUST-RESISTANT ISOLINES OF WHEAT

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ISOZYMES are better genetic markers when compared to traditional morphological markers used by plant breeders because the former are least influenced by environmental conditions[1,2]. Electrophoretic patterns of different enzymes have been employed to characterize cultivars of wheat and triticale[3]. Peroxidase isozymes have been used as markers of ploidy level in crop plants[4] and to screen cultivars for disease resistance at early stages of seed germination[5].

Ribonuclease I plays a vital role in differentiation as it regulates protein and RNA synthesis[6]. Therefore the study of ribonuclease I isozymes is important for elucidating the development of a sporophyte.

The material of the present study consisted of two cultivars, Thatcher (Tc) and Prelude (Pr), and ten isogenic lines of wheat carrying Lr genes in Tc and Pr backgrounds, viz. Lr_1(Tc); Lr_1(Pr); Lr_3bg (Tc); Lr_3ka(Tc); Lr_3ka(Pr); Lr_{10}(Tc); Lr_{10}(Pr); Lr_{16}(Tc); Lr_{16}(Pr); Lr_{17}(Pr) numbered as 'a' to 'l' in the sequence mentioned above.

The seeds were kept for germination under controlled conditions in a BOD incubator at 25°C. Two grams of sample material at 0, 24, 48, 72 and 96 h and one week of germination were homogenized in a pre-chilled mortar and pestle with 4 ml of 0.1 M sodium acetate buffer (pH 5.2). The homogenate was centrifuged at 12,000 *g* for 20 min at 0°C. The clear supernatant was stored at −20°C till further use.

Polyacrylamide gel (7.5%) electrophoresis in Tris–glycine buffer (pH 8.3) was performed. Ribonuclease I isozymes were stained by the procedure of Wilson[7]. Destaining was carried out in 5% acetic acid and gels were preserved in 5% perchloric acid.

Ribonuclease I staining revealed a total of eight bands at different stages of germination. However, the maximum number at one stage, five bands, could be observed at 48 h and one-week stages. All eight bands were never present together at a particular stage and also not in a single genotype. Appearance and disappearance of bands were noticed as the germination advanced. At the zero and 24 h stages, the electrophoretic patterns were identical and consisted of four bands (figure 1a).

At 48 h there were five bands. At this stage band numbers 3, 4 and 8 were absent but four new bands appeared (figure 1b). The disappearance of bands is usually regarded as indicating their utilization by the germinating seeds[8]. The appearance of a new band(s) may be the result of fresh activation of a new gene(s), or conversion of a precursor mRNA present in the seed during early development to active form and its translation[9]. Band number 7, which was present only in isogenic lines Lr_1; Lr_3bg(Tc); Lr_{10} and Lr_{16}, can be efficiently utilized as a biochemical marker to characterize these isolines. It has been reported that the gene combination $Lr_1+Lr_{10}+Lr_{16}$ confers resistance to all Indian races of *Puccinia recondita*[10]. The superiority of this combination may be due to the additive effects of the genes for the production of