

EFFECT OF SAPONIN FROM *CESTRUM NOCTURNUM* ON ERYTHROCYTES

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ABSTRACT

Saponin from *Cestrum nocturnum* was isolated, were tested for their purity. Human red blood corpuscles and Wistar rat erythrocytes in a phosphate buffered saline was used for calculating the hemolytic effect. Spectroscopic method was used for estimating the haemolysis caused by different concentration of the saponin. Phospholipids, cholesterol and sialic acid were estimated from the haemolysed solution along with lipid peroxidation index and glutathione concentration. The haemolysed solution shows structural component of membrane and the oxidative damage cause increased lipid peroxidation with decrease level of glutathione. Haemolysis caused in present study may be due to the presence of functional hydroxyl group in the saponin of *Cestrum nocturnum*. Further study on these saponin may lead to the discovery of novel medicine. Experiments are in progress to investigate effect of isolated purified saponin on various biological systems.

KEY WORDS: *Cestrum nocturnum*, Erythrocytes, Glutathione, Haemolysis, Lipid peroxidation, Sialic acid, Saponin.

INTRODUCTION

Cestrum nocturnum belongs to genus *Cestrum* and tribe *Cestreae* in the family Solanaceae. *C. nocturnum* is said to be poisonous (Watt and Breyer-Brandwijk, 1962) and reported to be used for epilepsy and as a stupefying charm medicine in West Indies. Aqueous extract of *C. nocturnum* shows excellent piscicidal properties (Patil and Jawale, 2000). The presence of saponins, Alkaloids, Hydrocarbons, Fatty acids and essential oil has been reported in the various species of *Cestrum* (Van Jhank Chan and Murovea, 1990; Viqar Uddin *et al.*, 1991, 1993, Halim *et al.*, 1971; Karowya *et al.*, 1971; Yoshihiro Mimaki, 2001, 2002), and their bioactive compounds showed Piscicidal, Larvicidal, Insecticidal potential activities (Jawale *et al.*, 2010; Jawale and Dama, 2010a; 2010b; Jawale *et al.*, 2012).

Despite the poisonous nature of the plant, the compound(s) responsible for the toxicity and their mode of action in biological system(s) is hopeful. Pharmacological study on the isolated heart of guinea pig and Frog shows its cardiotonic property. The two saponin from *C. nocturnum* were studied for toxicity and for effect on cardiac, smooth and skeletal muscles. They do not show emetic action. Both the saponins exhibit cardiotonic and cardiotoxic properties. They cause no spasm of the smooth muscles, no decreases in contraction of diaphragm following stimulation and no spasmogenic action on guinea pig ileum. *C. nocturnum* was extremely studied for its calcinogenic properties (Mello and Habemebl, 1998). The haemostatic system depends on normal function and integration between the vascular wall, platelets, the coagulation system and the fibrinolytic system. The toxic effect of these factors results in a bleeding tendency, thrombosis or disseminated intravascular coagulation (Chris *et al.*, 1998). Identification of toxic effects in these complex interactive pathways is at present confined to ex-vivo techniques with animal subjects (Theus and Zbinden, 1984). Toxicity towards the erythrocyte in the peripheral circulation is mediated by an effect either on the cell membrane, hemoglobin, red cell antioxidant protective mechanism or a combination of all three sites, resulting in loss of erythrocyte integrity or oxygen transport capability. Since saponin is the most interesting pharmacological active compounds. We have isolated and tested their action in Haemic system. Primarily, to acquire information about the effect of this saponin on blood elements. In-vitro hemolytic effect of saponin *C. nocturnum* on both human and rat erythrocytes has been selected. Our observation may help to explain the mode of action of *C. nocturnum* saponin on Haemic system.

MATERIALS AND METHODS

The saponin of *C. nocturnum* was isolated by the method of Viqar Uddin Ahmed *et al.*, (1991) with slight modifications. The leaves of *C. nocturnum* were extracted with ethanol. The extract was hydrolyzed with NaCl and HCl, mixed with aqueous butanol and separated the butanol extract then repeatedly recrystallized with aqueous alcohol to yield crystalline saponin of *C. nocturnum*. The saponin freed from other compounds were tested for their chemical purity using Liebermann-Burchard reaction (Ikan, 1969). 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 0.1M phosphate buffer, pH 7.4) system was used for the experiment. The percentage haemolysis was measured by the modified method of Krzyzaniak *et al.*, (2000). To 3.5 ml of 6% cell suspension in phosphate buffered saline, different concentration of saponin of *C. nocturnum* added and the volume made up to 5 ml with the buffer. The mixture was incubated for hour at 37°C and centrifuged. The supernatant was read at 530nm in Shimatzu spectrophotometer. Appropriate controls were maintained without the saponin. The percentage of haemolysis was calculated by keeping hypotonic haemolysis as 100%. Aliquots of the supernatants were subjected to extraction of phospholipids, cholesterol and sialic acid which were estimated by the methods of Fiske and Subba Rao (1925), Parekh and Jung (1970) and Warren (1959), respectively Lipid peroxidation index and glutathion concentration in the haemolysis were estimated by the methods of Ohkawa *et al.*, (1979) and Moron *et al.*, (1979) respectively. Hemoglobin content of the blood was estimated by the method of Drabkin and Austin (1935).

RESULTS AND DISCUSSION

Saponin of *C. nocturnum* produced significant haemolysis and the percentage haemolysis in both the rat and the human erythrocytes is present in Table 1. The percent haemolysis increased in a dose dependent fashion and, at a concentration of 150 μgm^{-1} produce more than 90 % haemolysis in both the rat and the human erythrocyte. Saponin isolated from *C. nocturnum* shows significant change in the level of the important membrane, namely phospholipids, cholesterol and sialic acid in both the rat and human erythrocytes. The values are presented in table 2. The level of lipid peroxidation and glutathion in haemolysate of rat and human RBCs shows significant changes. The glutathion level decrease with increase in dose concentration, whereas lipid peroxidation level increases in a dose dependent fashion. The values are presented in Table 3.

Table 1 : Hemolytic effect of saponin of *C. nocturnum*

Saponin concentration (μgm^{-1})	Percentage Haemolysis	
	Rat erythrocytes	Human erythrocytes
50	30.01 $\pm$ 3.24	4017 $\pm$ 3.92
100	45.12 $\pm$ 2.15	50.79 $\pm$ 4.83
150	93.43 $\pm$ 1.47	97.19 $\pm$ 2.12
200	97.72 $\pm$ 4.67	100

* Values are expressed as mean $\pm$ S.D from six individual experiments.

Table 2. Changes in levels of phospholipids, cholesterol and sialic acid release during in vitro haemolysis by *C. nocturnum* saponin.

Saponin concentration (μgm^{-1})	Phospholipid ($\mu\text{mole Pi/g Hb}$)		Cholesterol ($\mu\text{mole / g Hb}$)		Sialic acid ($\mu\text{g NANA / g Hb}$)	
	Rat RBCs	Human RBCs	Rat RBCs	Human RBCs	Rat RBCs	Human RBCs
50	0.53 $\pm$ 0.052	0.59 $\pm$ 0.035	0.40 $\pm$ 0.021	0.48 $\pm$ 0.027	1.89 $\pm$ 0.14	2.22 $\pm$ 0.51
100	0.68 $\pm$ 0.038	0.67 $\pm$ 0.047	0.43 $\pm$ 0.037	0.55 $\pm$ 0.040	1.97 $\pm$ 0.25	2.31 $\pm$ 0.41
150	0.87 $\pm$ 0.059	1.02 $\pm$ 0.052	0.61 $\pm$ 0.031	0.86 $\pm$ 0.089	2.04 $\pm$ 0.62	3.16 $\pm$ 0.74
200	1.07 $\pm$ 0.071	1.26 $\pm$ 0.046	0.79 $\pm$ 0.034	1.03 $\pm$ 0.016	2.18 $\pm$ 0.19	3.89 $\pm$ 0.91

- Values are expressed as mean $\pm$ S.D from six individual experiments;
- NANA(N-acetyl neuraminic acid)

The RBCs are structurally and functionally similar in all the vertebrate series and same type of hemolytic effect of plants have been observed by various workers on Rat, Human and fish RBCs (Kandasamy *et al.*, 1989; Bhatt and

Farswam, 1992; Nwude and Pearson, 1978). Human RBCs are found to be more susceptible to haemolysis, since this plant saponin might be damaging the erythrocyte membrane (Brody *et al.*, 1983). The important membrane component such as phospholipids, cholesterol and sialic acid enter the supernatant as a result of membrane injury. In biological membranes, the levels of cholesterol, phospholipids and glycoproteins are biologically regulated and any changes in these structural elements might disrupt the membrane fluidity (Copper *et al.*, 1977; Vander *et al.*, 1974). Experimental results indicates that this plant saponin disturb the membrane moiety and resulted into liberation of these structural compounds in the supernatant. Results show that the amount of liberated membrane component is well pronounced at the 150- 200 μgm^{-1} level. Thus the optimum dose for haemolysis of 6 % erythrocyte suspension lies in between the range. The increased level of lipid peroxidation along with the decreased level of glutathione clearly indicated that the saponin of *C. nocturnum* could have altered the erythrocytes membrane structure through a spectrum of oxidative damage. Increase lipid peroxidation and glutathione depletion are well documented in haemolysis (Das *et al.*, 1987; Ruth and Hana, 1985).

Table 3. Changes in glutathione and lipid peroxidation levels of erythrocytes during haemolysis by *C. nocturnum* saponin.

Saponin concentration (μgm^{-1})	Glutathione $\mu\text{mole/g Hb}$		Lipid peroxidation $\mu\text{mole /g Hb}$	
	Rat RBCs	Human RBCs	Rat RBCs	Human RBCs
50	35.85 $\pm$ 1.25	42.15 $\pm$ 2.12	164.79 $\pm$ 10.4	187.04 $\pm$ 14.3
100	27.45 $\pm$ 1.14	31.81 $\pm$ 1.78	192.48 $\pm$ 11.2	233.21 $\pm$ 16.7
150	14.35 $\pm$ 1.05	18.91 $\pm$ 0.74	277.61 $\pm$ 14.1	314.52 $\pm$ 19.4
200	13.07 $\pm$ 0.89	16.24 $\pm$ 0.46	311.43 $\pm$ 13.6	326.83 $\pm$ 20.7

- Values are expressed as mean $\pm$ S.D from six individual experiments;

It has been reported that functional hydroxyl group of plant saponin involve in the formation of free radical which resulted in lipid peroxidation causing cell damage (Vinod and Pierre, 1986). Hence observed increase lipid peroxidation and Haemolysis in *C. nocturnum* treated erythrocytes may be due to presence of functional hydroxyl group on the these plant saponin. Hence it may be concluded that *C. nocturnum* saponin induce membrane lipid peroxide which results in haemolysis and due to extensive haemolysis the membrane components are liberated. Attempts are in progresses to determine the effect of these plant saponin on various biological systems.

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