ANTI-ALLERGIC ACTIVITY OF PLANT SAPONINS IN RELATION TO THEIR HISTAMINE RELEASING AND ANTICHOLINESTERASE EFFECTS*

By

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Saponins in general are known to cause disruption of mast cells and release histamine (4). The bronchoconstrictor responses to the intravenous administration of saponins of *Solanum xanthocarpum* and *Clerodendron serratum* in anaesthetized dogs were reported from our laboratory (5). It was of interest to note that on repeated administration of the saponin, the bronchoconstrictor and hypotensive responses decreased, and the bronchoconstrictor responses to compound 48/80 were blocked. These and other observations after the administration of saponins suggested that the pharmacological responses were due to the release of histamine from the tissues. This was further substantiated by the fact that there was a marked reduction in histamine content after chronic treatment with these saponins, similar to that observed after glucocorticoids and chloroquine which have been found to produce beneficial effects in chronic bronchial asthma (1, 7 and 14). Moreover the saponins on repeated injections or on prolonged administration have been found to cause development of general resistance(13) and inhibit esterase activity in the serum and like di-isopropyl fluorophosphate (DFP) may influence histamine release from tissues and mast cells (10). It was therefore of interest to investigate the antiallergic activity of the saponins in relation to their histamine releasing and anticholinesterase effects in experimental animals.

The protective effect of intraperitoneal administration of the saponins against histamine and acetylcholine micro-aerosols was investigated in guinea pigs. No protection was observed against histamine or acetylcholine aerosol after 2 hr of administration of the saponin of *Clerodendron serratum*, though the saponin of *Solanum xanthocarpum* did increase the preconvulsive time in guinea pigs on exposure to histamine micro-aerosol (Table I).

TABLE I

Effect of the saponins of Clerodendron serratum and Solanum xanthocarpum against histamine induced micro-aerosols in guinea pigs.

Drug	*Mean preconvulsive time in sec.*			± *S.D.*	*% Protection*
	Pretreated control (A)	*Treated (T)*	*Posttreated control (B)*	*Average of controls A & B (C).*	$\frac{(1-C)}{T} \times 100$
Control (8)	129.26 ± 40.88	148.71 ± 23.11	132.50 ± 26.15	130.18 ± 33.51	12.0
Saponin of *S. xanthocarpum* 20 mg/kg (5)	129.40 ± 13.47	245.80 ± 18.26	142.00 ± 18.06	135.70 ± 11.21	44.5
Saponin of *C. serratum* 20 mg/kg (5)	139.00 ± 23.29	170.66 ± 15.77	136.00 ± 17.47	132.50 ± 19.45	22.47
Antazoline hydrochloride 2 mg/kg (5)	125.20 ± 13.70	380.85 ± 65.95	133.00 ± 21.56	129.20 ± 8.57	63.80

(Figures in parentheses indicate the number of animals.)

*Presented at the Symposium organised under joint auspices of the Association of Physiologists and Pharmacologists of India and Indian Council of Medical Research held at Kanpur in December, 1969.

Guinea pigs sensitized with egg albumin 3 weeks earlier, were treated with the saponin of *Clerodendrone serratum* for six weeks and were exposed at weekly intervals to the antige (egg albumin) micro-aerosols. It was observed that the chronic treatment caused gradual dev lopment of resistance against the micro-aerosol (Table II and Fig 1).

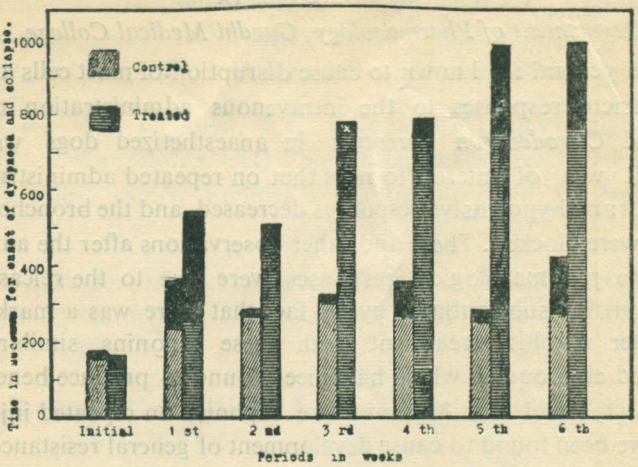

Fig 1

Effect of saponin of Clerodendron serratum on the onset of dyspnoea (hatched bars) and collapse (dark bars) in sensitized guineapigs exposed to egg albumin (antigen) micro-aerosols.

TABLE II

Gradual development of resistance against antigen (egg albumin) micro-aerosols given weekly in senitized guinea pig after prolonged treatment with saponin of C. serratum (20mg/Kg)

Group		*Time in sec ± S.D. for onset of dyspnoea/collapse on exposure to histamine micro-aerosol*						
		Initial	*1st week*	*2nd week*	*3rd week*	*4th week*	*5th week*	*6th wee*
Control (11)	Dyspnoea	165.5 ± 51.1	242.7 ± 38.5	277.1 ± 38.2	308.6 ± 48.3	279.5 ± 91.0	257.0 ± 51.7	275.0 ± 74.0
	Collapse	179.0 ± 15.6	377.0 ± 49.2	292.3 ± 38.2	326.1 ± 71.3	360.1 ± 38.2	282.9 ± 50.9	428.1 ± 49.1
Treated (8)	Dyspnoea	139.3 ± 38.5	265.7 ± 56.1	469.3 ± 35.8	760.5 ± 50.6	744.3 ± 52.9	756.0 ± 40.8	767.5 ± 59.2
	Collapse	168.1 ± 25.6	550.0 ± 36.3	520.6 ± 92.7	783.7 ± 81.7	793.3 ± 98.3	983.3 ± 52.5	989.2 ± 89.5

(Figures in parentheses indicate the number of animals.)

A group of rats received the saponin of *Clerodendrone serratum* for three weeks, following which the animals were sacrificed and the bronchoconstrictor responses of the isolated air

perfused lung to the graded doses of actylcholine and histamine were recorded (2) till maximum bronchoconstrictor response was elicited.

The percentage of bronchoconstrictor response was plotted against the millimolar doses of acetylcholine and histamine perfused through the pulmonary arteries of the lungs obtained from the control and treated groups of rats. It was observed that the sensitivity of the lung tissue to histamine was significantly diminished after treatment with the saponin of *Clerodendron serratum*, (Fig 2). The sensitivity to acetylcholine was, however, not significantly changed.

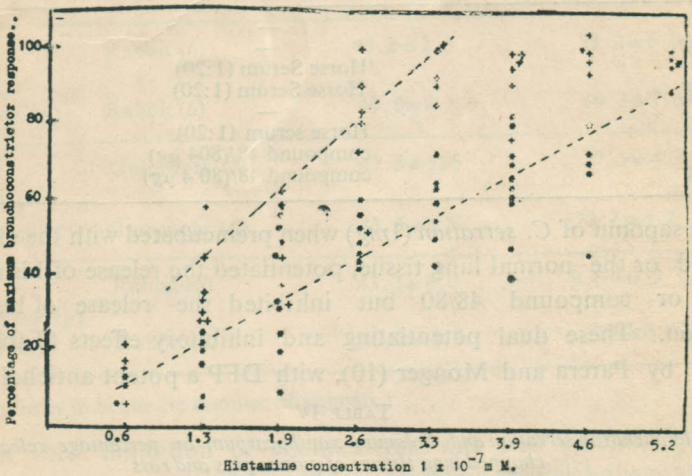

Fig 2

Percentage of the maximal bronchoconstrictor responses to histamine perfused through the pulmonary arteries of air insufflated isolated lungs of control rat (+) *and of those treated with saponin of C-serratum.* (*)

In the set of experiments in which isolated lung from the sensitized rat or guinea pig was perfused with the antigen (horse serum/egg albumin), it was observed that the amount of slow-reacting substance (SRS-A) was significantly less in the lungs obtained from the animal treated for a period of 21 days, as compared to those of untreated animals. The lung tissue of a few animals, which had developed marked resistance against antigen, were extracted successively with hydrochloric acid, petroleum ether, ethyl acetate and carbon tetrachloride according to the technique of Francis *et al.* (3). The lung extract, from the treated animals were found to inhibit the constrictor response to standard doses of histamine for longer time (24.5 min) as compared to the lung extracts from control animals which caused inhibition for 11.2 min only.

The effect of chronic treatment on the percentage disruption of mesenteric mast cells on incubation with the antigen (horse serum) and the compound 48/80 was also investigated according to the technique of Norton (12). The disruption of mast cells with antigen and compound 48/80 was only 13.8 $\pm$ 2.31 and 29.32 $\pm$ 6.32 per cent respectively in treated groups of rats as compared to 31.57 $\pm$ 4.38 and 48.56 $\pm$ 11.36 per cent observed in the untreated control animals. The acute effect of incubation of different concentrations of saponin on the percentage disruption of mast cells was also investigated in a series of experiments. The disruption of

cells increased in proportion to the increase in concentration. It was also interesting to no that in the lower concentration (2×10^{-5}), the saponin inhibited the disruption caused b compound 48/80 significantly, as shown in Table III.

TABLE III

Effect of the saponin of Clerodendron serratum on percentage disruption of mast cells of sensitized rat mesentery incubation with antigen and compound 48/80

Treatment	*Disrupting agent*	*Percentage disruption* ±*S.D.*
Control	—	4.70 ± 2.49
Saponin 25μg	—	13.85 ± 4.40
Control	Horse Serum (1:20)	88.62 ± 5.67
Saponin 25 μg	Horse Serum (1:20)	43.84 ± 7.98
50 μg	—	45.87 ± 15.39
50 μg	Horse serum (1:20)	58.36 ± 5.79
Control	compound 48/(804 μg)	82.85 ± 18.63
Saponin 25 μg	compound 48/(80 4 μg)	26.66 ± 5.38

Similarly, the saponin of *C. serratum* (1*mg*) when preincubated with the chopped lung tissu from the senstized or the normal lung tissue, potentiated the release of histamine in respons to either antigen or compound 48/80 but inhibited the release of histamine followin lower concentration. These dual potentiating and inhibitory effects (Table IV) were simila to those reported by Parera and Monger (10), with DFP a potent anticholinesterase drug.

TABLE IV

Effect of saponins of Clerodendron serratum and Solanum xanthocarpum on percentage release of histamine fro chopped lung tissues of guinea pigs and rats

Tissue	*Saponin*	*Releaser*	*Percentag release*
Guinea pig chopped lung.	Control	—	3.12
	Saline	compound 48/80 (1 *mg*)	31.82
	C. serratum (1 *mg*)	—	18.91
	C. serratum (1 *mg*)	compound 48/80 (1 *mg*)	45.14
	C. serratum (0.1 *mg*)	compound 48/80 (1 *mg*)	11.83
Sensitized guinea pig chopped lung.	Control	—	3.31
	Saline	Egg albumin (1 *mg*)	11.00
	C. serratum (50 μg)	—	8.27
	C. serratum (50 μg)	Egg albumin (1 *mg*)	23.13
Sensitized rat chopped lung.	Control	—	5.38
	Saline	Horse serum (1:4)	30.12
	C. serratum (50 μg)	—	8.24
	C. serratum (50 μg)	Horse serum (1:4)	8.23
Guinea pig chopped lung.	Control	—	2.90
	Saline	compound 48/80 (2 *mg*)	37.80
	S. xanthocarpum (0.4 *mg*)	—	30.10
	S. xanthocarpum (0.4 *mg*)	compound 48/80 (2 *mg*)	57.40
	Saline	Octylamine (0.2 *mg*)	45.20
	S. xanthocarpum (0.4 *mg*)	Octylamine (0.2 *mg*)	28.70

The blood serum of the guinea pigs which were treated with saponins of *C. serratum* or *S. xanthocarpum* manifested significant anticholinesterase activity (Table V) measured according to the method of Rappaport *et alo* (11).

TABLE V

Effect of saponins on serum cholinesterase in vive

Drugs	*Animals*	*Serum cholinesterase activity in units (± S.D.)*		
		Normal	*After drug*	*Percentage inhibition*
Saponin of *C. serratum* (0.3 *mg/kg*)	Rabbit (6)	45.2±2.5	22.2±6.8	50.6±7.2
Physostigmine (0.04 *mg/kg*)	Rabbit (6)	40.6±3.8	19.3±7.6	52.4±5.7
Saponin of *C. serratum* (0.3 *mg/kg*)	Guineapig (5)	47.5±5.6	27.5±4.5	49.0±6.1
Physostigmine (0.04 *mg/kg*)	Guineapig (5)	43.3±6.8	22.2±4.7	48.8±3.6
Saponin of *S. xanthocarpum* (0.05 *mg/kg*)	Rabbit (6)	43.2±6.0	9.6±0.9	77.5±3.2
Neostigmine (0.05 *mg/kg*)	Rabbit (6)	43.2±4.3	13.6±4.0	68.2±8.1

(Figures in parentheses indicate the number of animals.)

Further, *in vitro* inhibition of cholinesterase was also studied by incubating graded concentrations of the saponin with serum after adding acetylcholine as a substrate. The saponin (8×10^{-5}) caused about 50 per cent inhibition. The concentration of physostigmine for similar inhibition was 3×10^{-5}) (Table VI).

TABLE VI

Effect of saponins on rabbit serum cholinesterase in vitro

Drug	*Serum cholinesterase activity (mean of 5 observations ± S.D.)*				
	Control	*After incubation with the drugs*			
Concentration	—	1.5×10^{-5}	3.0×10^{-5}	4.5×10^{-5}	6.0×10^{-5}
Physostigmine	52.2±4.8	46.4± 5.2	27.1±3.9	5.2±4.6	0.0±0.0
% Inhibition	—	11.1	48.2	90.0	100.0
Concentration	—	6.0×10^{-5}	8.0×10^{-5}	1.0×10^{-5}	1.2×10^{-4}
Saponin of *C. serratum*	47.2± 5.6	43.5± 4.4	23.5± 7.1	3.0± 2.1	0.0±0.0
% Inhibition	—	7.7	50.2	93.6	100.0
Concentration	—	0.8×10^{-5}	2.0×10^{-5}	5.0×10^{-5}	1.3×10^{-5}
Saponin of *S. xanthocarpum*	45.2± 5.1	42.4±4.3	22.4± 7.2	2.8± 2.6	0.0±0.0
%Inhibinoti	—	6.2	50.5	93.8	100.0

From the foregoing, it is clear that the saponins isolated from *Clerodendron serratum* and *Solanum xanthocarpum* caused release of histamine from tissues and inhibited the enzyme cholinesterase both *in vivo* and *in vitro* experiments. In acute experiments on isolated ileum and tracheal chain preparations, the saponins potentiated the responses to histamine and acetylcholine. Intravenous administration of the saponins also caused bronchoconstriction as recorded through the overflow technique of Konzett and Rossler(8), in anaesthetised dogs and guinea pigs. The bronchoconstrictor response was however, found to decrease gradually on repeating the doses. This is known to occur with other histamine releasers and has been attributed either to the development of a sort of tachyphylaxis or to refractoriness of the tissues to histamine(9).

Both the saponins on chronic administration caused significant development of resistance against histamine. This may be attributed to refractoriness of lung tissue to histamine. The saponin of *Clerodendron serratum*, however, also caused development of marked resistance against antigen (egg albumin) micro-aerosols. It is difficult to attribute this protective effect to depletion of histamine alone since the allergic response is not known to be inhibited by chemical histamine liberators(9). Further, as the anaphylactic response in rat was also reduced markedly after chronic treatment with the saponin of *Clerodendron serratum* and the release of SRS-A on perfusion of antigen was much less in treated animals as compared to the untreated sensitized controls, it is likely that the saponin may be interfering with the formation of allergic substance in tissues or helping in the development of antiallergic substances in the tissues.

In view of the marked esterase inhibitory activity of these saponins as observed from the anticholinestrase activity, it is likely that these saponins may inhibit chymotrypsin or other enzymes responsible for the release of histamine, 5-HT and slow reacting substance in guinea pig and rat anaphylaxis. This derives support from observation that like DFP, the saponins also produced dual potentiating and inhibitory effect on the release of histamine from chopped lung tissue(9). That the chronic treatment with the saponins may cause development of antiallergic substances seems to be substantiated from the fact that the lung extracts from treated animals produced inhibition of histamine as well as SRS-A responses of smooth muscle of ileum for considerably longer period than lung extracts from control animals. It is, therefore, likely that plant saponins may cause development of antiallergic substances as result of adaptation of tissues to chemical stress induced by chronic release of histamine. A new approach to the treatment of allergic conditions with the plant saponins known to cause release of histamine is suggested. This may open a therapeutic possibility in use of non-toxic histamine releasers in allergy, as was indicated by Gaddum(6).

ACKNOWLEDGEMENT

The author is indebted to the Indian Council of Medical Research, for providing a grant-in-aid to carry out the above investigations.

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