IMMUNOMODULATING EFFECT OF JULIFLORINE ON THE ANTIBODY RESPONSE TO *LISTERIA* HEMOLYSIN
Preliminary Report

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SUMMARY: Juliflorine, an antimicrobial alkaloid, isolated from Prosopis juliflora is shown to possesses some immunomodulating activity. This activity was tested in rabbits and compared with Freund's complete adjuvant. Listeria hemolysin (antigen) was injected intramuscularly along with varying concentrations of juliflorine; and a dose related immune response was noted. After four weeks, following weekly doses of 30 mg/Kg of juliflorine, the antihemolysin titre was found high (1:1280) and more than that with Freund's complete adjuvant; but matches at 20 mg/Kg of juliflorine with Freund's complete adjuvant. On the other hand repeated injections of 30 mg/Kg of juliflorine were found toxic and produced tissue degeneration, increased lymphocyte count as high as 95%; and high level of lactate dehydrogenase, cholesterol and triglyceride.

Key Words: Juliflorine, prosopis juliflora, immunomodulator, alkaloid, adjuvant, listeria hemolysin.

INTRODUCTION

Juliflorine, the main alkaloid of *Prosopis juliflora*, was isolated (1) and reported its partial structure by Ahmad *et al.* (2). Later Hesse *et al.* (3) reported the complete structure of juliflorine (juliprosopine) from *P. juliflora* (Figure 1).

Juliflorine has been reported to possess significant antidermatophytic (4) and antibacterial (5) activity. It also possesses a differential characteristic, which could be used as a presumptive test for the identification of *Campyelobacter* from other enteropathogens (6).

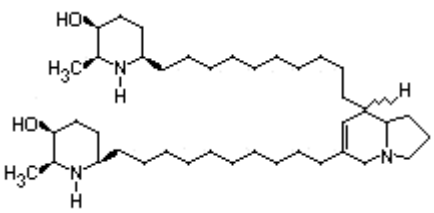
Alkaloids are known to enhance immune response, hence act as adjuvant. Since 1960s, adjuvant chemotherapy has appeared to be the best method of reducing breast cancer (7). Studies on immunotherapy using BCG after remission in acute non-lymphocytic leukemia in adults have been reported (8). More recently a large number of alkaloids have been investigated for their immunomodulating properties (9-11) and are also being studied to determine their therapeutic potentials (12,13).

The present work deals with the study of immunomodulating activity of juliflorine. Efficacy of this alkaloid compared to Freund's complete adjuvant has been evaluated.

MATERIALS AND METHODS

The juliflorine, an antimicrobial alkaloid of *P. juliflora* has been studied for its immunomodulating property by using *Listeria* hemolysin as a weak antigen.

Figure 1: Juliflorine.



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1. Preparation of *Listeria* hemolysin

Listeria monocytogens 5124 was grown in Tryptose Soya Phosphate broth (Oxoid) at refrigerated temperature. After one week incubation, bacteria and cellular debris were removed by centrifugation at 5000 rpm for 30 minutes. Supernatant was collected in sterile flask. The hemolysin was precipitated by $(NH_4)_2 SO_4$ (Merck) (34 g/100 ml) and kept for overnight refrigeration. Precipitate was separated by centrifugation at 5000 rpm for 30 minutes and then dissolved in Phosphate buffer saline (pH. 7.4). Same procedure was repeated three times after which it was dissolved in smaller volume of PBS.$(NH_4)_2 SO_4$ was removed by dialysis in PBS. Hemolysin was filtered sterilized and titrated to determine the unit of hemolysin.

2. Titration of hemolysin

Serial dilution of hemolysin was prepared 1:2 - 1:2048 in PBS. Each tube containing 0.5 ml diluted hemolysin, 1 ml 0.2% cystein (as reducing agent) and 0.5 ml of 0.5% Sheep RBC suspension. The tubes were incubated in 37°C water bath for 2-3 hours. The hemolysin titre causing complete lysis of sheep RBC suspension was identified.

3. Antihemolysin production in rabbits

Rabbits were injected intramuscularly with 1 ml/Kg (1024 units) hemolysin in triplicate along with Freund's complete adjuvant (1 ml/Kg) and various concentrations of juliflorine (10, 20, 30 mg/Kg). Three booster doses were also injected weekly.

4. Titration of antihemolysin (antibodies)

Two fold dilution of serum was prepared in PBS (each tube containing 0.5 ml). To each tube 0.5 ml containing 2 units of hemolysin was added and incubated in 37°C water bath for 1 hour. Then 0.5 ml of 0.4% cystein and 0.5 ml of 0.5% Sheep RBC suspension were added and incubated in 37°C water bath for 2 hours. Titre of hemolysin was determined as the highest dilution showing no lysis of RBC.

5. Haematological and Biochemical studies of rabbit blood

The following investigations were also made at the beginning and end of experiment.

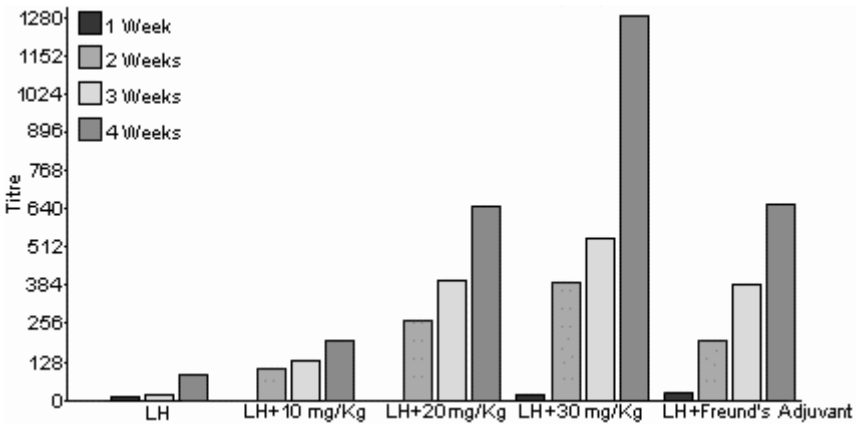
A. Haematological tests

(1) Haemoglobin percentage
(2) Erythrocyte sedimentation rate (ESR)
(3) Erythrocyte count
(4) Leucocytes count
(5) Differential count
(6) Plateletes count
(7) Packed cell volume.

Table 1: Titre of antihemolysin in rabbits injected with Listeria hemolysin along with Freund's complete adjuvant and various concentration of the Juliflorine.

Haemolysin 1 ml of 1:1 with	1 Week		2 Week		3 Week		4 Week	
	Titre	Average	Titre	Average	Titre	Average	Titre	Average
Control	NIL		1:8		1:16		1:40	
Listeria hemolysin		NIL		1:8		1:24		1:100
	NIL		1:8		1:32		1:160	
Freund's	1:8		1:128		1:512		1:640	
complete adjuvant		1:12		1:192		1:384		1:640
1 ml	1:16		1:256		1:256		1:640	
Juliflorine 10 mg	NIL		1:128	1:128	1:256		1:160	
		NIL				1:192		1:240
	NIL		1:128		1:128		1:320	
20 mg	NIL		1:256		1:256		1:640	
		NIL		1:256		1:384		1:640
	NIL		1:256		1:512		1:640	
30 mg	1:8		1:512	1:389	1:512		1:1280	
		1:4				1:512		1:860
	NIL		1:256		1:512		1:640	

Figure 2: Titre of Anti-Listeria Hemolysin in rabbit injected with Listeria Hemolysin (LH), Listeria hemolysin with different concentration of Juliflorine (LH+10, 20, 30 mg/Kg of Juliflorine) and Listeria Hemolysin with Freund's complete adjuvant.



B. Biochemical tests

(1) Lactate Dehydrogenase
(2) Cholesterol
(3) SGOT
(4) SGPT
(5) Alkaline phosphatase
(6) γ-glutanyl transferase
(7) Glucose
(8) Triglyceride
(9) Urea
(10) Uric acid
(11) Calcium
(12) Creatinine
(13) Total bilirubin
(14) Total protein
(15) Phosphates

Table 2: Blood picture of rabbit injected with hemolysin, Freund's complete adjuvant and various concentrations of alkaloids.

Rabbits injected with	RBC Morphology	Differential Count					WBC Per cu mm	RBC in million per cu mm	Haemo globin %	Packed cell volume	Platel-lets	ESR
		Neutro-phil	Eosino-phil	Baso-phil	Mono-cyte	Lympho-cyte						
Saline	Normal, Leptocytes, Microcytes and anisocytosis	42	NIL	NIL	02	56	8200	4.11	8.3	25.0	2.9	1
Haemolysin	Microcytes, Anisocytosis	53	02	NIL	04	41	4800	4.05	9.4	29.0	3.29	1
Adjuvant + Haemolysin	Leptocytes, Polychromasia	49	01	NIL	NIL	50	5400	3.4	8.0	22.0	3.85	2
Juliflorine 10 mg	Leptocytes, Polychromasia	24	01	NIL	NIL	75	2800	3.09	7.8	22.0	3.78	2
20 mg	Leptocytes, Poly-chromasia, Aniso-cytosis; Target	06	04	NIL	0.1	89	5100	2.68	6.4	20.0	3.15	4
30 mg	Leptocytes, Poly-chromasia, Micro-cytes, Anisocytosis, oval, Hypochromia	03	01	NIL	0.2	94	3250	1.27	3.9	18.5	1.27	42

*All values are means of duplicate.

Table 3: Blood biochemistry of rabbits injected with the Alkaloids and Freund's complete adjuvant.

Rabbits injected	LDH (U/L)	CHOL (mg/dl)	SGOT (U/L)	SGPT (U/L)	ALP (mg/dl)	GT (U/L)	GLU (mg/dl)	TRIG (mg/dl)	UREA (mg/dl)	U.A (mg/dl)	Calcium (mg/dl)	Crt. (mg/dl)	T.Bil. (mg/dl)	T.P (G/L)	PHOS (mg/dl)
Saline Control	591	19	31.9	89.2	24	27.8	72	42	54	0.6	15.49	0.88	0.37	5.32	2.02
Listeria Haemolysin	463	12	27.4	84.2	18	12.6	72	73	55	0.0	14.42	0.92	0.34	5.87	3.36
Listeria Haemolysin															
+ Julilorine 10 mg	592	5	35.5	71.1	23	6.3	69	45	41	0.1	14.87	0.80	0.34	5.98	2.88
20 mg	904	20	60	73.6	43	16.5	64	58	62	0.1	14.37	0.97	0.37	5.78	4.54
30 mg	1083	49	50.5	44.9	19	18.1	61	132	50	0.2	12.85	0.57	0.26	5.96	3.75
Adjuvant + Listeria Haemolysin	980	15	72.8	85.1	26	4.9	117	83	51	0.2	14.02	1.22	0.29	6.09	3.02

All values are means of duplicate.

RESULTS AND DISCUSSIONS

Juliflorine, an antimicrobial alkaloid of *Prosopis juliflora* has been investigated for its effect on immune response. Rabbits were injected weekly with *Listeria* hemolysin along with Freund's complete adjuvant and with varying concentration of juliflorine (10, 20 and 30 mg/Kg). Antibodies against hemolysin were titrated in serum obtained from injected rabbits (Table 1).

The results show that juliflorine possesses some immunomodulating or immunostimulating activity. Comparing the titre of hemolysin antibodies, a dose related immune response was observed (Figure 2). Significant titre ($p<0.05$) was observed in rabbits injected with juliflorine and Freund's complete adjuvant. Its immunostimulating effect was more than Freund's complete adjuvant in concentration of 30 mg/Kg even though no statistical significance difference was noted between these two groups.

Hematological studies show that subsequent injection of juliflorine resulted in (dose related) anemia, polychromasia, decrease in neutrophil and platelet count indicating its hemolytic activity while with its increased concentrations, the RBC count, Hemoglobin %, packed cell volume decreases. But lymphocyte count was found significantly high (94%, Table 2).

Toxicological studies of juliflorine was also reported (14). Higher concentration of juliflorine (30 mg/Kg) was found toxic and showed sterile cyst formation and degeneration at injected site. It is probable that neutrophil leucocytes attack juliflorine and are killed, but apparently lymphocytes are not so affected. Juliflorine damages the tissue (also blood cells) but on the other hand it probably also promotes immune response that results in the production of antibodies.

Biochemical studies were also performed with *Listeria* hemolysin, and *Listeria* hemolysin along with various concentrations of the juliflorine in rabbits; and monitored their effect on various biochemical parameters in blood, namely LDH, cholesterol, triglyceride, glucose, total protein and liver function test etc. (Table 3).

Our results indicate that injection of *Listeria* hemolysin produces variable effects on different biochemical parameters: it decreases the blood level of LDH, cholesterol, alkaline phosphatase and uric acid; while SGPT, urea and Calcium were found to be elevated. However no significant effect was observed on SGOT, γ-glutamyl transferase, glucose, triglyceride, creatinine, total bilirubin and phosphate, with comparison to normal control rabbits.

When *Listeria* hemolysin and juliflorine in concentration of 10 mg, 20 mg and 30 mg/Kg body weight were injected once again variable results were obtained (Table 3), when compared with normal counterparts. As can be seen from the table that the level of various biochemical parameters mentioned above were affected most with *Listeria* hemolysin + 30 mg/Kg concentration of juliflorine. It can be clearly seen that the values of LDH, cholesterol,

SGOT, triglyceride and phosphates were higher, while the levels of SGPT, alkaline phosphatase γ-glutamyl transferase, glucose and uric acid were lower; and the amount of urea, calcium, creatinine and total bilirubin were almost the same as those of the normal control rabbits. It is noticeable that the most effective dose of juliflorine was 20 mg/Kg + *Listeria* hemolysin because higher concentration (30 mg) did not produce further alterations. Since the experiment was conducted in duplicate using a total of 12 rabbits consistent variations has been observed in the results. This could be due to the procedure normally set for the analysis of human serum and the conditions may not be optimum for rabbits serum. Therefore more work is needed in order to arrive at definite conclusion.

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