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Studies on the Immuno – Stimulant Properties of *Asparagus racemosus*

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ABSTRACT

The objective of the present investigation was studies on the immuno-stimulant properties of *Asparagus racemosus*. The immuno stimulant activity was evaluated by Chemotaxy (Neutrophil adhesion test) and complement system alternative pathways. Albino rabbits of either sex weighing 2.5Kg were divided into three groups consisting of three animals each. Drug was administered as aqueous extract of fresh plants continuously for 14 days. The blood samples were taken first (control), 7th and 14th day. Then chemotaxy and complement activity alternate pathway were evaluated. There was mean increasing of 44.84, 41.04 & 40.28%. After 7th day and 58.81., 55.10., and 50.57%., after 14th day for roots, leaves and stems respectively. The increase was more first seven days than for next seven days. Similarly chemotaxy also exhibited a mean increase of neutrophil adhesion 14.76, 14.32 and 10.23 % after 7th day and 40.11, 34.40, 24.76 % after 14th days for roots, leaves and stems respectively. The study reveals that *Asparagus racemosus* has significant complement alternate pathway activity and chemotaxy.

Key words: *Asparagus racemosus*, Immuno-stimulant, Chemotaxy.

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INTRODUCTION

Management of infectious disease is still a major problem for health care administration throughout the world more particularly in third world countries due to socio economic factors even after in event of potent chemotherapeutic agent, immunization techniques and improvement in social factors affecting health of most various terminologies are use to describe to extent, type and severity of infections.

In Ayurveda, an ancient system of Indian medicine, gastric disorders are classified as *sula*, *parinamasula* and *amlapitta*, which are very similar to modern classification of clinical conditions of peptic ulcer and functional dyspepsia (non-ulcer dyspepsia)[1], [2]. *Asparagus racemosus* Willd. (family Liliaceae) is a well-known Ayurvedic rasayana, and its root paste or root juice has been advocated in various ailments including in the treatment of peptic ulcer [3]. The Ayurvedic concept of rasayana consists of specialised class of drugs which prevent ageing, increase longevity, impart immunity, improve mental functions and add vigor and vitality to the body [3] and are akin to modern classification of adaptogens. According to Lozavev, the medical substances, which increase non-specific resistance, are termed as adaptogens [4]. In consonant, *A. racemosus* (AR) has been reported to have potent adaptogenic activity [5]. AR has also been reported to decrease gastric emptying time [6]. Change in gastric emptying has been reported to be responsible for genesis of ulcers [7]. Free radicals have been reported to be responsible for many ailments including gastroduodenal ulcers [8]. Polysaccharide fraction of AR has been reported to possess significant anti-oxidant activity in vitro [9]. Recently, fresh root juice of *A. racemosus* with honey was reported to be effective in dyspepsia [10]. AR was quantified for total saponins and was reported to contain active saponins, shatavarin I–IV [11].

Literature survey revealed that there is a lack of enough scientific reports regarding immuno – stimulant properties of *Asparagus racemosus*. Hence the objective of the present investigation was to evaluate immuno – stimulant properties of *Asparagus racemosus*.

MATERIAL AND METHODS

Plant materials

The fresh roots, leaves and stems of *Asparagus racemosus* were collected from Kadapakkam, Kanchipuram District of Tamilnadu, India. The plant material was identified by Dr.V.Venkatesalu, Dept. of Botany, Annamalai University, Tamil Nadu, India.

Preparation of Extracts

Fresh roots, leaves and stems of *Asparagus racemosus* was washed and cut into small pieces. Then 10 gm of each part was taken with 100 ml of water then stirred well and boiled for 10 min and filtered. The filterate was concentrated to 25 ml.

Experimental Animals

Three groups of three animals (Albino rabbits) each of either sex at a weight of about 2.5 kg was selected for the study. They were provided with a standard diet supplied by Pranav Agro industries Ltd, India and water ad libitum. The experimental protocol has been approved by Institutional Animal Ethics Committee, Rajah Muthiah Medical College, Annamalai University and animals were maintained in central animal house.

Drug administration

Albino rabbits of either sex weighing 2.5 kg were used for the study. The animals were divided into three groups consisting of three animals each. Before administration of drug they act as control. The aqueous herbal extract was administered orally at a dose of 5 ml/ day for 14 days to rabbits for assessment of immuno stimulant effect. The blood was drawn on 7th and 14th day. The immuno stimulant activity was evaluated by Chemotaxy (Neutrophil adhesion test) and complement system alternative pathways.

EVALUATION OF IMMUNO STIMULANT ACTIVITY

I) Chemotaxy

Chemotaxy was studied based on the principle that neutrophils tend to adhere to an foreign particle. Here nylon fibre of fixed weight was used as foreign particles.

Neutrophil adhesion test

Before administration of the drug, 7th, 14th of after administration of the drug treatment, blood samples were collected by puncturing the retro-orbital plexus into heparinised vials and analysed for total leucocyte counts (TLC) and differential leucocyte counts (DLC) by fixing blood smears and staining with the field stain I & II – leishamn's stain. After initial counts, blood samples were incubated with 80mg / ml of nylon fibres for 15min at 37°C.

The incubated blood samples were again analysed for TLC & DLC. The product of TLC and % neutrophil given neutrophil index (NI) of blood sample. Percent neutrophil adhesion was calculated as shown below [12].

$$\text{Neutrophil adhesion (\%)} = \frac{NI_u - NI_t}{NI_u} \times 100$$

Where,

NI_u – Neutrophil index of untreated blood sample

NI_t – neutrophil index of treated blood sample.

Total count of white blood corpuscles [13]

The white cells are counted in the fair corners of 1 square millimeter and in the central ruled are on both sides. The counting chambers of the haemocytometer 10 square millimeters in all. The white cells are recognized by the refractile appearance and by the slight colour given to them by the stain contained in the diluting fluid. The cells touching the boundary lines are not counted. The number is multiplied by 10 or 20. The number of leucocytes per cu. mm.

Number of cells counted X Dilution X 10

Number of 1 square millimeter counted

Differential leucocytes counts

A thin blood film is made on a clean dried glass slide. It is dried and stained with leishman's stain solution. The drop of leishman's stain and counted. Wait for 2 minutes. During this time the methyl alcohol fixes the blood films. Fixation means nucleus and various cellular organs are fixed without any damage to the cells of cellular organs. After 2 minutes add double quantity of distilled water over the slide and wait for 7 minutes. In the mean time the stains initiate the chemical reaction, which result in formation of acidic dye eosin and basic dye methylene blue and also some intermediate compounds. The acidic dye eosin stains various acidophyllic structures and some neutrophyllic granules and basic dye methylene blue stains the basophilic structure like nucleus, basophilic granules. Cytoplasm of the lymphocyte and monocyte. After 7 minutes was the slide in a slow stream of water dry it in air. Place one drop of cedar wood oil over the film. Identify the cells and enter into the 100 squares and give reports about the % of different types of leucocytes present in rabbit blood.

II) Complement activity alternative pathway[14]

The blank, sample and 100% lysis are incubate 37°C for 1 hr then make upto 5ml using diluting fluid. Centrifuge and collected to supernatant solution. Measure the absorbance of B and C at 450nm using A is blank. Complement haemolytic activity in serum is most often expressed in CH50 units/ml, where one CH50 unit produces 50% haemolysis of EA under standard conditions. This method is very fully described by Mayer, whose conditions were 5x10⁸ E (Corresponding to 1ml of 2%E) treated with the optimal concentration of serum and lysed at 37°C in CFD in a total volume of 5ml.

RESULTS & DISCUSSION

In present study, as far as possible the procedure normally adopted in ayurveda and siddha was followed without incorporating usual phytochemical techniques. The results are shown in the tables 1, 2, 3, 4, 5, 6, 7, 8, and 9. The complement activity alternate pathway was determined as described and the results are given in table 10, 11 and 12. There was mean increasing of 44.84, 41.04 and 40.28%. After 7th day and 58.81, 55.10, and 50.57% after 14th day for roots, leaves and stems respectively. The increase was more first seven days than for next seven days. Similarly chemotaxy also exhibited a mean increase of neutrophil adhesion 14.76,

14.32 and 10.23 % after 7th day and 40.11, 34.40, 24.76 % after 14th days for roots, leaves and stems respectively. The study reveals that *asparagus racemosus* has significant complement alternate pathway activity and chemotaxy.

Table 1: Neutrophil adhesion Test of ROOTS treated animals – animal I

S. No.	Treatment	TLC (10 ³ /mm ³) (A)		Neutrophil (%) (B)		Neutrophil index (A X B)		Neutrophil Adhesion (%)	(%).Increase Neutrophil Adhesion
		UB	FTB	UB	FTB	UB	FTB		
1	Control	6.45	5.12	54	49	348.3	250.88	27.97	-
2	7 th Day	6.62	5.02	57	51	377.3	250.02	32.14	14.91
3	14 th Day	6.96	4.98	63	53	438.48	263.92	39.80	42.30

Table 2: Neutrophil adhesion Test of ROOTS treated animals – animal II

S. N o.	Treatment	TLC (10 ³ /mm ³) (A)		Neutrophil (%) (B)		Neutrophil index (A X B)		Neutrophil Adhesion (%)	(%).Increase Neutrophil Adhesion
		UB	FTB	UB	FTB	UB	FTB		
1	Control	6.44	5.34	56	51	360.6	272.34	24.47	-
2	7 th Day	6.64	5.45	58	52	385.1	283.4	26.40	7.89
3	14 th Day	6.89	5.64	68	56	468.52	315.8	32.60	33.22

Table 3: Neutrophil adhesion Test of ROOTS treated animals – animal III

S. No.	Treatment	TLC (10 ³ /mm ³) (A)		Neutrophil (%) (B)		Neutrophil index (A X B)		Neutrophil Adhesion (%)	(%).Increase Neutrophil Adhesion
		UB	FTB	UB	FTB	UB	FTB		
1	Control	6.36	4.80	55	51	349.8	244.8	30.02	-
2	7 th Day	6.52	4.54	57	52	371.64	236.1	36.47	21.49
3	14 th Day	6.76	4.85	66	52	446.16	251.2	43.47	44.80

Table 4: Neutrophil adhesion Test of LEAVES treated animals – animal IV

S. No.	Treatment	TLC (10 ³ /mm ³) (A)		Neutrophil (%) (B)		Neutrophil index (A X B)		Neutrophil Adhesion (%)	(%).Increase Neutrophil Adhesion
		UB	FTB	UB	FTB	UB	FTB		
1	Control	6.45	5.40	52	48	335.40	259.20	22.72	-
2	7 th Day	6.80	5.68	54	48	367.20	272.64	25.75	13.34
3	14 th Day	6.95	5.79	61	52	423.95	301.08	28.98	27.55

Table 5: Neutrophil adhesion Test of LEAVES treated animals-animal-V

S. No.	Treatment	TLC ($10^3/mm^3$) (A)		Neutrophil (%) (B)		Neutrophil index (A X B)		Neutrophil Adhesion (%)	(%)Increase Neutrophil Adhesion
		UB	FTB	UB	FTB	UB	FTB		
1	Control	6.15	4.96	54	50	332.10	248.00	25.32	-
2	7 th Day	6.35	4.98	56	51	355.60	254.00	28.57	12.84
3	14 th Day	6.65	5.12	59	50	392.35	256.00	34.75	37.24

Table 6: Neutrophil adhesion Test of LEAVES treated animals – animal-VI

S. No.	Treatment	TLC ($10^3/mm^3$) (A)		Neutrophil (%) (B)		Neutrophil index (A X B)		Neutrophil Adhesion (%)	(%)Increase Neutrophil Adhesion
		UB	FTB	UB	FTB	UB	FTB		
1	Control	5.25	4.35	55	48	288.80	208.80	27.70	-
2	7 th Day	5.52	4.42	58	49	320.20	216.60	32.35	16.79
3	14 th Day	5.95	4.46	62	51	3680.90	227.46	38.34	38.41

Table 7: Neutrophil adhesion Test of STEMS treated animals – animal –VII

S. No.	Treatment	TLC ($10^3/mm^3$) (A)		Neutrophil (%) (B)		Neutrophil index (A X B)		Neutrophil Adhesion (%)	(%)Increase Neutrophil Adhesion
		UB	FTB	UB	FTB	UB	FTB		
1	Control	5.47	4.56	54	50	295.40	228.00	22.81	-
2	7 th Day	5.65	4.57	56	51	316.40	233.10	26.33	15.43
3	14 th Day	6.12	4.95	60	51	367.20	252.50	31.24	36.96

Table 8: Neutrophil adhesion Test of STEMS treated animals – animal –VIII

S. No.	Treatment	TLC ($10^3/mm^3$) (A)		Neutrophil (%) (B)		Neutrophil index (A X B)		Neutrophil Adhesion (%)	(%)Increase Neutrophil Adhesion
		UB	FTB	UB	FTB	UB	FTB		
1	Control	5.48	4.56	54	50	295.90	228.00	22.95	-
2	7 th Day	5.94	5.12	59	52	350.50	266.20	24.05	4.80
3	14 th Day	5.99	5.02	60	52	359.40	261.04	27.37	14.46

Table 9: Neutrophil adhesion Test of STEMS treated animals – animal –Ix

S. No.	Treatment	TLC ($10^3/mm^3$) (A)		Neutrophil (%) (B)		Neutrophil index (A X B)		Neutrophil Adhesion (%)	(%)Increase Neutrophil Adhesion
		UB	FTB	UB	FTB	UB	FTB		
1	Control	6.21	4.02	56	50	347.76	251.00	27.82	-
2	7 th Day	6.40	5.03	59	52	377.60	261.56	30.73	10.46
3	14 th Day	6.67	4.96	61	54	406.90	267.84	34.18	22.86

Table 10: COMPLEMENT Activity Alternate Pathway Roots Treated Animals

S.No.	% Haemolysis			% Increasing Activity after	
	Intial (A)	7 th Day (B)	14 th day C	7 th day (B-A)/A ^{x100}	14 th day (C-A)/A ^{x100}
1	45.05	90.26	120.00	50.09	62.60
2	50.22	93.66	123.20	46.38	59.24
3	55.32	89.30	121.83	38.05	54.59
Mean	50.20	91.07	121.68	44.84	58.81

Table 11: Complement Activity Alternate Pathway Stem Treated Animals

S.No.	% Haemolysis			% Increasing Activity after	
	Intial (A)	7 th Day (B)	14 th day C	7 th day (B-A)/A ^{x100}	14 th day (C-A)/A ^{x100}
1	36.36	78.64	118.96	53.76	69.44
2	66.43	98.47	120.19	32.54	44.73
3	59.25	93.79	121.22	36.83	51.12
Mean	54.01	90.30	120.12	41.04	55.10

Table 12: Complement Activity Alternate Pathway Stems Treated Animals

S.No.	% Haemolysis			Increasing Activity after	
	Intial (A)	7 th Day (B)	14 th day C	7 th day (B-A)/A ^{x100}	14 th day (C-A)/A ^{x100}
1	45.60	81.51	112.21	44.06	59.36
2	51.32	84.21	116.72	39.06	56.03
3	56.12	88.13	120.33	37.73	36.32
Mean	51.00	88.00	118.00	40.28	50.57

CONCLUSION

The study reveals that *Asparagus recemosus* has significant complement alternate pathway activity and chemotaxy. Hence further Phytochemical investigation may results is promising immunostimulant drug.

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