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Evident experimental data of *Habenaria longicorniculata* J Graham on Cell mediated immunity - Promising source of Herbal immunomodulator

Research Article

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Abstract

Habenaria longicorniculata J. Graham from family *Orchidaceae*, are tuberous herbs, widely used by traditional physicians in the treatment of malignancy as a rejuvenator, immune boosters. Protocol designed to evaluate its *In-vivo* Immunomodulatory activity by cell mediated immunity. Materials and methods: Tubers were collected, cleaned, used for further study. *In-vivo* Immunomodulatory activity on rats for Assessment of immunity by Cell mediated Immunity, conducted as per the standard protocol. Results: Analysis of the results of the study indicates that the test drug Tubers possesses very good immunological oedema suppression effect. Immunological oedema, represent expression of cell mediated immunity hence based on the results obtained these proved as best immunomodulators..

Key Words: *Habenaria longicorniculata J. Graham,* Immunomodulatory activity, Cell mediated immunity, In-vivo Animal experiment.

Introduction

Priority, preference on potentiality of plants as source of medicine is undisputed. Medicinal plants have played a key role in maintenance of health (1). Immune enhancing drugs of herbal origin have a lot of concern in current scenario, in relation to present epidemics (2). Immunity is defined as the state of resistance or insusceptibility exhibited by the host towards injury caused by microorganisms and their products. Immunity, in general, is concerned with the reaction of the body against any foreign antigen (3).

Habenaria longicorniculata J.Graham a terrestrial orchid the tubers of which are used since centuries in malignancy, immune modulators, in traditional practices(4). These tubers are said to be antioxidant, rich with trace elements and are said to be beneficial in form of powder with adjuvants like milk. These also proved as antioxidant and with rich nutritional factors (5).

In-vivo Animal experiments provide important preliminary data to help select plant extracts with potential Immunomodulatory activity (6). Cell-mediated immunity involves cytotoxic T cells recognizing infected cells and bringing about their destruction. Just as the humoral immune response has B cells which mediate its response, the cellular immune response has T cells, which recognize infected cells and destroy them before the pathogen inside can replicate and spread to infect other cells. Unlike B cells, T lymphocytes (T cells) are unable to recognize pathogens without assistance (7).

Hence with all this background it is planned to conduct an animal experiment to assess the *In-vivo* Immunomodulatory activity on tubers of *Habenaria longicorniculata* J. Graham by Cell mediated Immunity in Wistar Albino rats.

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Materials and methods

Drug source

Tubers of *Habenaria longicorniculata* J. Graham procured from their natural habitat cleaned properly, authenticated and deposited at SDM centre for Research in Ayurveda and Allied sciences (8). These tubers were subjected for *In-vivo* Assessment of Cell mediated Immunity in Wistar Albino rats.

Animal grouping

Wister strain albino rats of either sex weighing between 140 – 250 grams were procured. The selected animals were grouped into three groups randomly irrespective of sex, each group comprising six animals. Group 1 administered with water control and Triple antigen sensitization; Group 2 with 1% C.M.C [Carboxy Methyl Cellulose] (vehicle, control) solution and Triple antigen sensitization; and Group 3 with test drug solution and Triple antigen sensitization (9).

Animal rooms were targeted at a temperature of 22–24°C and a relative humidity of 40–60%, and were artificially illuminated (fluorescent light) on a 12-h light/dark cycle. Throughout the test period, food and

water were made available adequately as per the standards (10).

Cell mediated Immunity

Tubers of *H. longicorniculata* J Graham weighing around 400mg and diluted in 20 ml distilled water were administered consecutively for seven days. The dose selection was done on the basis of body surface area ratio using the table of Paget and Bames (1969) (11).

Procedure

Test drug powder dilution was freshly prepared each day and administered for 07 consecutive days. pH of the reagent (that is potash alum adjuvant) was maintained between 5.6 – 6.8 using 10% sodium carbonate. 1% CMC solution was administered for seven days in vehicle control group (12). On seventh day the initial paw volume of left hind paw was noted and 0.1ml of above solution was injected into plantar aponeurosis of left hind paw, volume of Immunological oedema thus produced will be measured by volume displacement method, after 24 hours and 48 hours with Plethysmograph. Percentage increase in paw volume, which was induced for oedema formation over initial value, was calculated. The values from control (Group1 and Group 2) was compared with the values from test drug administered groups(Group 3) to assess the Cell mediated Immunity response of the drug (13).

Results

Table 1: Comparison of Paw oedema volume at Basal time, Post sensitization after 24hrs and 48hrs Group 1 (water control)

Animal number	Marking	Gender	Paw volume (ml)		
			Basal	After 24 hrs	After 48hrs
1	Head	F	0.80 ml	1.51 ml	1.75 ml
2	Neck	F	0.81 ml	1.63 ml	1.70 ml
3	Body	M	0.79 ml	1.54 ml	1.60 ml
4	Tail	M	0.83 ml	1.35 ml	1.65 ml
5	Fore limb	F	0.82 ml	2.02 ml	2.40 ml
6	No mark	F	0.81 ml	1.61 ml	1.80 ml

Table 2: Comparison of Paw oedema volume at Basal time, Post sensitization after 24hrs and 48hrs in Group 2 (CMC vehicle control)

Animal number	Marking	Gender	Paw volume (ml)		
			Basal	After 24 hrs	After 48hrs
1	Head	M	0.80 ml	1.75 ml	1.52 ml
2	Neck	M	0.76 ml	1.89 ml	1.65 ml
3	Body	F	0.79 ml	1.48 ml	1.22 ml
4	Tail	M	0.82 ml	1.55 ml	1.34 ml
5	Fore limb	M	0.84 ml	1.63 ml	1.38 ml
6	No mark	F	0.81 ml	1.41 ml	1.19 ml

Table 3: Comparison of Paw oedema volume at Basal time, Post sensitization after 24hrs and 48hrs Group 3 (Test drug)

Animal number	Marking	Gender	Paw volume ml		
			Basal	After 24 hrs	After 48hrs
1	Head	M	0.76 ml	1.53 ml	1.28 ml
2	Neck	F	0.30 ml	2.11 ml	1.33 ml
3	Body	F	0.79 ml	1.35 ml	1.21 ml
4	Tail	F	0.84 ml	1.23 ml	1.26 ml
5	Fore limb	M	0.82 ml	1.54 ml	1.61 ml
6	No mark	M	0.81 ml	1.61 ml	1.19 ml

Table 4: Effect of test drug on immunological paw oedema in pre-sensitized albino rats

Group	24 h Paw volume	% change	48 h Paw volume	% change
Group 1 (water control)	58.35± 9.94	-------	70.82± 12.82	-------
Group 2 (CMC vehicle control)	78.65± 8.49	34.79	54.05± 5.98	23.67
Group 3 (Test drug)	48.68 ± 7.23**	38.10	28.84± 6.31**	47.01

Data Mean ± SEM ** p <0.01 in comparison to the normal control group

In Group 1(water control group) paw oedema volume after 24hrs showed 58.35±9.94 with no changes in percentage, and after 48 hrs it has shown 70.82±12.82 with no changes in percentage in pre sensitized Wister albino rats.

In Group 2 (CMC vehicle control group) paw oedema volume after 24hrs showed 78.65±8.49 with 34.79% increase in percentage whereas paw oedema volume after 48 hrs showed 54.05±5.98 with 23.67% decrease in percentage in pre sensitized rats.

In Group 3(test drug group) paw oedema volume after 24hrs showed 48.68 ±7.23 with 38.10% decrease in percentage and after 48 hrs it has shown 28.84±6.31 with 47.01% decrease in percentage in pre sensitized rats. (Table 4)

Table 5: Consolidated statement of Cell Mediated Immunity

Groups	24th Hour	48th Hour
Group 2 (CMC vehicle control)	NSI	NSD
Group 3 (Test drug)	SD	SD

SI- Significant Increase; SD- Significant Decrease; NSI- Non-Significant Increase; NSD- Non-Significant Decrease

There were no significant changes in Group 1(water control) about Paw oedema volume in both post sensitization after 24hrs and 48 hrs respectively. Non-Significant increase in paw oedema volume in post sensitization after 24 hrs and non-significant decrease in post sensitization after 48hrs observed in Group 2(CMC vehicle control). In Group 3(Test drug) Cell mediated immunity showed very significant decrease in the Paw

oedema volume in post sensitization after 24hrs and 48hrs.

Discussion

In total Tubers of *Habenaria longicorniculata* J Graham on Cell mediated immunity showed very significant decrease in the Paw oedema volume in post sensitization after 24hrs and 48hrs respectively when compared to the CMC Vehicle control group which showed non-significant increase after 24hrs and non-significant decrease after 48hrs respectively. Percentage of change in test group showed 47.01% decrease when compared to CMC vehicle control group of 23.67% decrease after 48hrs respectively, which is statistically significant at $P<0.01$.

Cell mediated immunity is an immune response that doesn't involve antibodies (14). It involves an activation of phagocytes, antigen specific cytotoxic T lymphocytes and release of various cytokines in response to an antigen (15).

Analysis of the data show that the Group 2(CMC vehicle control) produced weak suppression of immunological oedema at 24th and 48th hour after injection of the paw oedema, whereas Group 3(Test drug) shows remarkable and statistically very significant suppression of paw oedema after 24th and 48th hour.

Conclusion

In-vivo experimental study of Cell mediated Immunity on albino rats has shown very good immunological oedema suppression on tubers of *Habenaria longicorniculata* J. Graham. Immunological oedema, represent expression of cell mediated immunity. Thus, based on the results obtained these tubers can be claimed as best immunomodulators.

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