STUDY ON THE IMMUNOMODULATORY ACTIVITY OF *KSHIRVIDARI* (*Ipomoea digitata* Linn) IN ALBINO MICE

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Abstract

The study aimed to assess the Immunomodulatory activity of the tuberous root of *Kshirvidari* (*Ipomoea digitata* Linn.) *Kwath* (decoction) and *Churna* (powder) in experimental animals by following standard procedure. Randomly selected mice were divided into five groups of six animals each. The root of *Ipomoea digitata* Linn.) was administered orally in the form of *Kwath* and *Churna*. Parameters like Haemoglobin, RBC count, WBC count, DLC, Platelet count, and measurement of Paw thickness were studied. *Kwath* (10.4 ml/kg) and *Churna* (1300mg/kg) of *Kshirvidari* showed significant improvement in paw volume. From the present study, it can be concluded that the root of *Kshirvidari* has Immunomodulatory activity.

Introduction

Kshirvidari (Ipomoea digitata Linn.), of the Convolvulaceae family, is distributed throughout India's warm and moist regions[1]. It was mentioned in various *Ganas* (groups of drugs) of *Ayurvedic* classical *Samhitas* viz. *Madhurskandha*, *Vidarigandaadi Gana, Balya Gana, Varnya Gana, Bruhaniya Gana etc*. As per *Ayurvedic* literature, *Kshirvidari* mainly works as *Vrishya, Mutral, Swarya, Varnya, Rasayana, Jeevaniya, Stanyajanan, Bruhna,* etc.[2,3] Conventionally, the root of this plant has been used in various formulations like *Sivagutika, Chawanprash, Mahamash* oil, etc. Also, the roots have shown Hepatoprotective activity, Hypoglycaemic activity, etc.[4] Hence, this study was carried out to assess the Immunomodulatory activity of tuberous root of *Kshirvidari* (*Ipomoea digitata* auct.) in the form of *Kwath* (decoction) and *Churna* (powder) in experimental animals by Delayed-Type hypersensitivity (DTH) Method.

Materials and Methods

Animals: Swiss albino mice were taken of either sex weighing approximately 20 to 25 grams and selected from the animal house of the National Toxicology Centre (NTC), Pune. Animals were maintained at room temperature, with 12 hours of the day and dark cycles. Standard laboratory diets were given an unlimited water supply of drinking water. The animal experiment was carried out at National Toxicology Centre (NTC), Pune; after the approval of the Institutional Animal Ethical Committee. Sheep RBCs were used as an antigen for the challenge to get an immune response.

Drug preparation: The tuberous root of the plant *Kshirvidari* (*Ipomoea digitata* Linn.) was self-collected from *Marunji*, Pune. Authentication of the plant was done at the **Botanical Survey of India, Pune (voucher number- BSI/WRC/100-2/Tech./2017/24)**. The tuberous root of the plant was washed thoroughly in tap water, cut into small pieces, and dried under

shade. The weight of fresh tuberous root was 2kg approximately. After complete drying for 15-20 days weight of the dry tuber root was 750 grams. Two dosage forms of *Ipomoea digitata* Linn (*Churna and Kwath*) were prepared by the following methods given in *Sharangdhar Samhita* [5]. Dried tuber roots were powdered on the grinder. The mesh size of the powder was 100. Fresh *Kwath* was prepared every day during the experiment.

Pharmacognostic studies: Physiochemical analysis, preliminary phytochemical analysis of *Churna* and *Kwath,* and T.S. of tuberous root of *Kshirvidari Kand* (*Ipomoea digitata* Linn.) were done at **Bhide Laboratory, Pune**.

Dose selection and schedule: Two dose forms i.e. *Churna* and *Kwath* were used in the experiment. The study was carried out at two dose levels, for *Kwath* namely T1 (low dose - 5.2 ml/kg) and T2 (high dose -10.4ml/kg) respectively; while for *Churna* namely T3 (low dose – 650mg/kg), and T4 (high dose -1300mg/kg). The dose in mice was calculated by using factor 0.0026. The test drugs were administered orally.[6]

Study protocol: Cell-mediated immune response was studied using Delayed-Type Hypersensitivity (DTH) model [7, 8]. The selected animals were divided into five groups. The first group (Group 1) was kept as Diseased control (DC), whereas the second (Group 2- T1), and third (Group 3- T2) were administered with *kwath* at a dose of 5.2 ml/kg and 10.4 ml/kg respectively. While the fourth group (Group 4- T3) and the fifth group (Group 5- T4) were treated with *churna* at doses of 650mg/kg and 1300mg/kg respectively.

Mice were primed with 0.1 ml of SRBC suspension containing $1X10^8$ cells intraperitoneally on Day 0. The *kwath* and *churna* were administered orally on day 0 and continued till day 7 of challenge, to the test drug-treated groups. On the 7th day, the thickness of the right hind foot pad was measured using a Vernier calliper. The animals were then challenged on day 7 with 0.1 ml of SRBC suspension containing $1X10^8$ cells in the right hind paw. A foot pad thickness was measured again after 24 hr, 48 hr, and 72 hr of the challenge. The difference between pre and post-challenge paw thickness expressed in mm was taken as a measure of DTH response. Haematological parameters viz. Hemoglobin %, RBC count, WBC count, DLC, Platelet count**,** and Haematocrit Count were also measured as these are supportive criteria for the immunomodulatory effect.

Table-1 Study design

Group	Day	Procedure
Group 1 Diseased control (DC)	D-0 D1-D6 D7 D8-D9	Immunization of mice by injecting SRBC's I.P. On normal diet Challenge with SRBC's in hind paw Measurement of paw thickness on 24th and 48th hour
Group 2 Treatment group *Kwath* (T 1)	D-0 D1-D6 D7 D8-D9	Immunization of mice by injecting SRBC's I.P. Treated with *Kwath* 5.2ml/kg dose Challenge with SRBC's in hind paw Measurement of paw thickness on 24th and 48th hour
Group 3 Treatment group *Kwath* (T2)	D-0 D1-D6 D7 D8-D9	Immunization of mice by injecting SRBC's I.P. Treatment with *Kwath* 10.4ml/kg dose Challenge with SRBC's in hind paw Measurement of paw thickness on 24th and 48th hour
Group 4 Treatment group *Churna* (T 3)	D-0 D1-D6 D7	Immunization of mice by injecting SRBC's I.P. Treatment with *Churna* 650mg/kg dose Challenge with SRBC's in hind paw

	D8-D9	Measurement of paw thickness on 24th and 48th hour
Group 5 Treatment group *Churna* (T 4)	D-0 D1-D6 D7 D8-D9	Immunization of mice by injecting SRBC's I.P. Treatment with *Churna* 1300mg/kg dose Challenge with SRBC's in hind paw Measurement of paw thickness on 24th and 48th hour

Statistical analysis: Results were presented as Mean ± SEM. Data was quantitative ANOVA test was selected for statistical analysis.

Result:

The organoleptic study of the tuber root of *Kshirvidari Kand (Ipomoea digitata* Linn.) of the drug was done according to *Ayurvedic* parameters. Physicochemical and phytochemical analysis of the drug reveals standardization of the tuber root of *Kshirvidari Kand* (*Ipomoea digitata* Linn.) *Churna* and *Kwath* which includes determination of moisture content, total ash, acid insoluble ash, water soluble ash, T.L.C., pH value, Refractive value, and viscosity. Physicochemical analysis was done according to guidelines **at Bhide Laboratory, Pune**.

Observations during the experimental study show that there is no specific behavioral change. No specific change in the intake and output (urine and stool) of animals. The food intake of animals was stable throughout the experiment. No specific changes were observed.

The cell-mediated immune response of decoction and powder of *Kshirvidari* was assessed by DTH reaction, i.e. foot pad reaction. A dose-dependent suppression of SRBC-induced DTH response at 24, 48, and 72 hrs was observed in *Kwath* low dose (5.2 ml/kg), *Kwath* high dose (10.4 ml/kg), *Churna* low dose (650 mg/kg), *Churna* high dose (1300 mg/kg) treated groups. The result of Delayed type hypersensitivity (DTH) response to SRBC and haematological parameters are given in Table 2.

Table 2: Effect of *Kwath* and Churn of *I. digitata* L. on paw thickness and haematological parameters

Group	**Paw Volume in mm**			**Haematological parameters**				
	At 24hr	**At 48hr**	**At 72hr**	**WBC (10^3/µl)**	**RBC (10^6/µl)**	**HGB (g/L)**	**HCT (%)**	**PLT (10^3/µl)**
DC	3.58±0.134	3.33±0.203	2.99±0.25	7.1167	8.1333	13.09	44.967	577
T 1	2.6066±0.214	2.478±0.216	2.283±0.148	6.94	7.4117	12.807	43.617	579.5
T 2	3.18±0.290	2.915±0.2874	2.523±0.279	7.43	7.53	12.95	43.317	577.67
T 3	2.846±0.20	2.653±0.184	2.305±0.208	7.3667	8.2267	12.867	46.1	580
T 4	2.996±0.10	2.878±0.17	2.686±0.11	6.7833	7.1	12.8	43.933	582.83

Group DC-disease control, Group T1: *Kwath* low dose (5.2 ml/kg); Group T2: *Kwath* high dose (10.4 ml/kg); Group T3: *Churna* low dose (650 mg/kg), Group T4: *Churna* high dose (1300 mg/kg)

Discussion:

Immunomodulatory, anti-aging, anti-oxidant, and rejuvenating effects are retained under the *Rasayan* concept of Ayurveda, which has multiple dimensions to promote physical, and mental health and improve the defense mechanisms of the body, and enhance longevity [9, 10]. In classical texts of Ayurveda, *Kshirvidari* is mentioned as *Balya, Bruhaniya, Varnya & Rasayana*. Ayurvedic As per Ayurvedic Pharmacopeia of India (Vol. 5, Part 1) and other modern literature, *Ipomoea digitata* Linn. is considered as *Kshirvidari*. So this study of

Kshirvidari Kand *(Ipomoea digitata* Linn.*)* was carried out to evaluate its immunomodulatory activity.

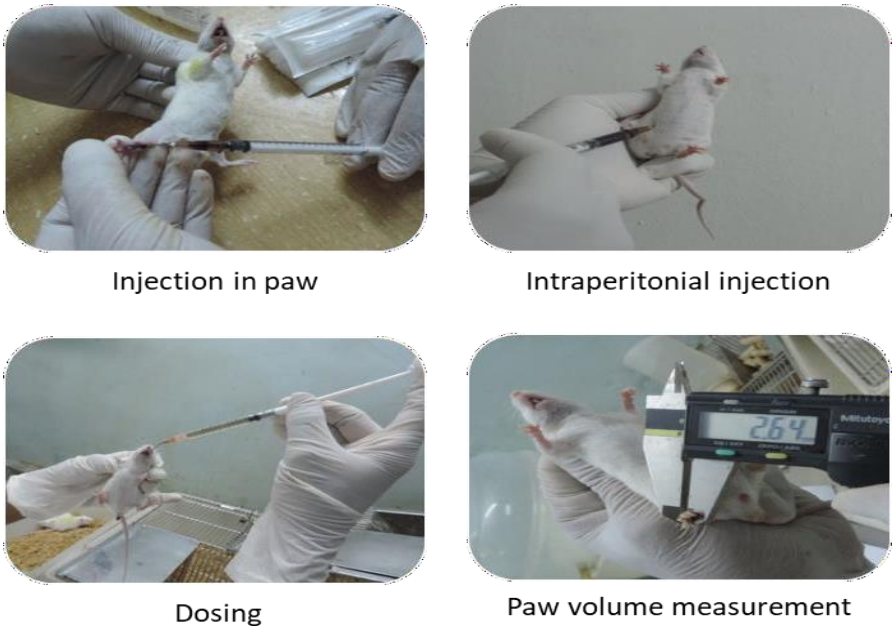
Delayed type hypersensitivity (DTH) is a mechanism associated with T cell-mediated immunity. Delayed type hypersensitivity (DTH) is a part of the process of graft rejection, tumor immunity, and most important immunity to many intracellular infectious microorganisms especially those causing chronic diseases such as tuberculosis and leprosy.

Two dosage forms i.e. *Kwath* and *Churna* were used for this experimental study as it is mentioned in various *Rasayana Kalpana*. Two dose levels were selected for the experiment as mentioned in Samhita and as recommended in its toxicity study.

Results of screening of immunomodulatory activity show that group treated with *Kwath* (10.4 ml/kg), immunity response is highest and slightly lower in *Churna* (1300 mg/kg). Groups treated with low doses of *Kwath* (5.2 ml/kg) and *Chuna* (650 mg/kg), show lower responses than the above two groups.

In hematological parameters, WBC count is a supportive parameter for immunity response. WBC count is also more in the treated group than in the untreated group. It is higher in group treated with *Churna* (1300 mg/kg). There is no significant difference in RBC count. Platelet count in treated groups was slightly low than in the diseased control group, but it is not statistically significant. From the above observations, it is seen that the group treated with a high dose of *Kwath* shows higher overall improvement than other treated groups. These observations are supportive of the immunomodulatory activity of *Kshirvidari kand* and results suggest its action of immunomodulation to control autoimmune diseases. The key phytochemicals identified to date are Beta-sitosterol, scopoletin taraxerol, t-cinnamic acid, and coumarin. Scopoletin [11], which owns immunomodulatory, anti-inflammatory as well as antioxidant activity, and also the presence of Flavonoids are reported to possess immunological effects so it can be said that *Ipomoea digitata* Linn. works as immunomodulatory due to the presence of these chemical constituents.

Fig.1 Induction of *Kwath* and *Churn* of *I. digitate L* and measuring paw volume



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