Immunomodulatory activity of Guduchi Ghana (Aqueous Extract of Tinospora Cordifolia Miers)

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Abstract : In the present work, *Guduchi Ghana* (concentrated form of aqueous extract of *Guduchi*) was prepared according to the method described in classical text – *Sidhdha Yoga Samgraha* and the other sample of aqueous extract was purchased from the market for the assessment of the immunomodulatory activity. It was done by haemagglutination antibody titre method for humoral immunity and footpad swelling method for cell mediated immunity on wistar albino rats. Results of present studies suggest that *Guduchi Ghana* prepared by classically was found to possess significant immunostimulatory action on immune system but market sample of it exhibited significant immunosuppression effect in dose dependent manner when compare with control group at a dose of 50 mg/kg orally. [Umretia B et al NJIRM 2013; 4(3) : 90-96]

Key Words: Guduchi (Tinospora Cordifolia); Ghana Kalpana (aqueous extract); Immunomodulatory activity; Cellular mediated immunity; % Haemagglutination antibody titre

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Introduction: Many plants used in traditional medicines are reported to have immunomodulatory activity. Some of these stimulate both humoral and cell mediated immunity, while others active only the cellular components of the immune system i.e. phagocytic function without affecting the cell mediated immunity or humoral[1]. In Ayurveda, Many herbal plants entitles as *Rasayana, which is used for enhance the body resistance. T. cordifolia* is categorized as '*Rasayana*'[2] and used for its Immunomodulatory activity. *Tinospora cordifolia* (Willd.) Mierrs (family Menispermaceae[3]) is an important medicinal plant recognized as *Guduchi*. The species are widely distributed in India, extending from the Himalayas down to the southern part of peninsular India[3]. Many research works have been carried to find out the immunomodulatory activity of alcoholic and aqueous extracts of *T. cordifolia*. The alcoholic and aqueous extracts of *T. cordifolia* are reported to have beneficial effects on the immune system[4,5] and have been tested successfully for their immunomodulatory activity.[6,7,8,9,10,11,12] The aqueous extract of *T. cordifolia* was found to enhance phagocytosis *in vitro*. The aqueous and ethanolic extracts also induced an increase in antibody production *in vivo*[13]

The present investigation is aimed at studying the immunomodulatory activity of the *Guduchi Ghana* (Concentrated form of decoction), the secondary formulation derived from the primary formulation i.e. *Kwatha* (decoction) in order to justify the traditional claims as a *Rasayana* and compared this activity in market sample of *Guduchi Ghana*.

Materials And Methods: Triple antigen, 10% potash alum, sodium carbonate and normal saline were used for cell mediated immunity test, while SRBC was used for immunomodulatory activity.

Preparation of the *Ghana:* For the preparation of *Guduchi Ghana*[14], the stem was collected from Jamnagar and authentified by botanist. The freshly collected stem was soaked in four times of water and made decoction of it. The decoction was reheated till it became semisolid and dried in oven at 55°C. The second sample was purchased from the market.

Animal and dose selection: Charles Foster strain albino rats of either sex weighing between 180 were obtained from the animal house (Pharmacology department, I. P.G.T. & R.A., Jamnagar, India). They were housed at 22 ± 2ºC with constant humidity 50 – 60%, on a 12 h natural day and night cycles. They were fed with diet

Amrut brand rat pellet feed (Pranav agro industries, Jamnagar, India) and tap water *ad libitum*. All the experiments were carried out after obtaining permission from "Institutional Animal Ethical Committee". The selected animals were grouped into three groups of 6 animals each and were given the following treatments. The classical dose of Ghana is 2 *Ratti* i.e.250 mg/day[15]. The dose for experimental animals was calculated by extrapolating the human dose to animals based on the body surface area ratio by referring to the standard table of Paget and Bernes[16]. The stock solution was prepared freshly by mixing adequate quantity of water with both the samples and used for all the experimental purpose. Group A received *Guduchi Ghana* prepared from *Kwatha*, Group B received Guduchi Extract market sample at the dose of 50 mg/kg orally and Group C received tap water as a control. The drugs were administerd to overnight fasted animals in the dose of 1 ml/ 100g body weight with the help of gastric catheter sleeved to syringe.

Effect on humoral antibody formation: The effect of test drugs on anti-body formation against SRBC against sheep red blood cells (SRBC)[17] was studied as described by Puri *et al*. The drugs were administered for 10 days to respective Groups; on 3rd day of dosing schedule, sheep blood was collected in a sterilized bottle containing Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride) aseptically so that agglutination of blood does not take place. The collected sheep blood was thoroughly washed with sterile normal saline through repeated centrifugation until the supernatant fluid became colorless and made to 30% SRBC solution. This sensitizing agent was injected subcutaneously in the dose of 0.5ml/100 g of body weight to the rats. On the 11th day, rats were sacrificed by cervical dislocation and blood was collected in separate test tubes. Blood was used for both sensitization and to determine antibody titre. From the collected blood, serum was separated and incubated in a serological water bath for 30 min at 55°C to inactivate complement in it.

Estimation of antibody titre: Serial two fold dilutions of the serum in sterile saline solution were made in the volume of 0.1ml of micro titre plate. 0.1 ml of thrice saline washed 3% SRBC was added to each well of the tray.

Blood from the same animal sheep) was used for both sensitized and to determine antibody titre. The trays were covered and placed in refrigerator overnight. Antibody titre (heamagglutination titre) was noted on the next day. The titre value was converted to log $_2$ values for easy comparison[18]. Rat spleen and thymus (including lymph nodes) were dissected out and their weight was also recorded. Tissues were transferred to 10% formalin solution for fixation and later on processed for histopathological studies by referring standard procedure[19]. The slides were vied under binocular research Carl-Zeiss's microscope (Germany) at various magnifications to note down the changes in the microscopic features of the tissues studied.

Effect on cell mediated immunity: The test drugs were evaluated to assess their effect on cell-mediated immunity by following the procedure of Bhattacharya[20]. The rats were sensitized subcutaneously (0.5 ml/100 g body weight), on first day of drug administration, by following solution; Triple antigen(DPT) – 1 ml, normal saline (0.9%) – 4 ml and potash alum (10%) – 1 ml. The pH of the above solution was maintained between 5.6-6.8 using 10 % sodium bicarbonate. On 7th day, the initial paw volume of right hind paw was noted and then 0.05 ml of above solution was injected into planter aponeurosis of right hind paw. Volume of immunological oedema thus produced was measured by volume displacement method[21] after 24 hr and 48 hr with plethysmograph. The % increase in paw volume which is the index of edema formation over initial value, was calculated. The values from control group were compared with test drug administered group to assess the cell mediated immunity response of the drug.

Statistical Analysis

All the values were expressed as mean ± SEM. The data was analyzed by unpaired Student's't' test[22]. A level of $p<0.05$ was considered as statistically significant and the value of $p<0.01$ was considered statistically highly significant. Level of significance was noted and interpreted accordingly.

Results: The effect of test drugs on antibody formation against SRBC is represented in Table 2. In Group A, in which *Guduchi Ghana* prepared from *Kwatha* was administered, 22.34 % increase in antibody titre was observed and Group B, in which *Guduchi Ghana*- Market sample was administered, 12.85 % increase in antibody titre was observed. However, only the increase observed in the group A is found to be statistically significant.

Table- 1: Effect of test drugs on antibody formation against SRBC in albino rats

Group	Dose (mg /kg)	Anti body titre (Log_2 values)	% Change
W.C.	Q.S	03.58± 0.11	-
Group A	50	04.38 ± 0.14***	22.34 ↑
Group B	50	04.04 ± 0.21	12.85 ↑

Each value is expressed as mean antibody formation ± SEM ,
***P< 0.001 is considered to be statistically highly significant with respect to control.

Table- 2: Effect of test drugs on Absolute and relative weight of Spleen

Group	Dose (mg/kg)	Weight of Spleen			
		Absolute (mg)	% Change	Relative (mg/100g body Weight)	%Change
W.C.	Q.S	475.0 ±41.23	-	214.0 ± 13.18	-
Group A	50	589.5± 45.72	24.10↑	260.8 ±25.60*	21.86↑
Group B	50	558.3 ± 32.49	17.53 ↑	260.9 ± 21.21*	21.91↑

Each value in parenthesis is expressed as mean absolute and relative body weight ± SEM. *P> 0.01 is considered to be statistically non significant

The effect of test drugs on spleen weight is shown in Table 3. An apparent increase in the weight of spleen was observed in both the test drug administered groups in comparison to control group, however, the observed increase in both the groups were found to be statistically non-significant.

Table 3: Effect of test drugs on Absolute and Relative weight of thymus

Group	Dose (mg/kg)	Weight of Thymus			
		Absolute (mg)	% Change	Relative (mg/100g body weight)	% Change
W.C.	Q.S	353.3 ±19.22	-	160.6 ± 9.83	-
Group A	50	428.3 ±24.41*	21.2↑	186.4 ±6.14*	16.06↑
Group B	50	391.6± 27.37	10.8 ↑	182.5 ± 15.91	13.63 ↑

Each value is expressed as mean absolute and relative body weight ± SEM
*P< 0.05 is considered to be statistically non significant.

Table 4: Effect of test drugs administration on body weight of rats

Group	Dose (mg/kg)	Body weight			
		Initial body weight	Weight at the end of study	Actual change in weight (g)	% Change
W.C.	Q.S	196.5 ± 9.32	221.6 ± 11.37	25.16 ± 5.10	-
Group A	50	231.3 ± 16.14	245.8 ± 12.80	17.16 ±7.35*	31.79 ↓
Group B	50	216.6 ± 9.09	228.3 ± 7.92	25.00 ± 7.30	00.63 ↓

Each value is expressed as mean absolute and relative body weight ± SEM,
*P> 0.01 is considered to be statistically non significant.

The effect of test drugs on thymus weight is shown in Table 4. Both the trial groups showed an apparent increase in the thymus weight in comparison to the control group. However, only the increase observed in Group A was found to be statistically significant irrespective of presentation of values as either absolute or relative weight.

The effects of test drugs on body weight of albino rats are shown in the Table 5. Body weight gain was observed in both the control and test drug administered groups when the body weight at the end of the experiments was compared to the initial body weights. However, the body weight gain was slightly less in Group-A in comparison to control. The observed difference was found to be statistically non-significant. In Group B the weight gain was similar to control group.

Table 5: Effect of test drug on immunological paw oedema in triple antigen sensitized albino rats

Group	Dose (mg/kg)	Percentage increase in paw volume at different time intervals			
		After 24 hrs	% Change	After 48 hrs	% Change
W.C.	Q.S	60.83 ± 3.16	-	60.28 ±4.99	-
Group A	50	28.17 ±2.89***	53.69 ↓	27.28 ± 3.66***	64.66↓
Group B	50	40.72 ± 4.01**b	33.05 ↓	46.15 ± 4.17[b]	23.49↓

Each value is expressed as mean absolute and relative body weight ± SEM.
***P< 0.001 and ** P< 0.01 , [b] the values are significantly high in comparison to Group-B

In cell-mediated immunity, T-lymphocytes directly react with antigen to cause its destruction, whereas in antibody mediated immune response, the formation of antibodies takes place in the plasma cells. The cell-mediated immunity is also mediated by release of lymphokines; antibody and complements are not involved in these reactions. This phenomenon is responsible for the rejection of foreign cells.

The effect of test drugs on triple antigen induced immunological oedema is summarized in Table 6. In Group A, statistically highly significant decrease in paw edema was observed in comparison to control group at both 24 hr and 48 hr post immunization. An apparent suppression was observed in Group-B rats, too. However, the observed decrease was less in comparison to Group-A and was statistically significant only with respect to the decrease observed at 24 hr. The difference between Group-A and Group-B was found to be statistically significant at p <0.05 level.

Discussion: Between the two different samples of *Guduchi Ghana*, the sample, which was prepared by *Kwatha*, showed highly significant increase in antibody formation against SRBC in comparison to control group. It may be due to the active principle of *Tinospora cordifolia* viz. Syringin, cordiol,

cordioside and coriofolioside[23], which may be present in this sample. In contrast the *Guduchi Ghana* -market sample could not modify humoral antibody formation against SRBC to significant extent.

The test formulation may be producing this effect through different mechanism of action. As already mentioned *Tinospora cordifolia* is reported to stimulate the granulocytes macrophages formation indicating the presence of granulocytes macrophage colony stimulating factor (CGM-SF). It also possess neutrophilia and stimulation of macrophages associated with a significant increase in serum fibrogen, indicating the possibility of increasing IL-1 production. The immunomodulatory activity may be mediated through the spontaneous mitogen activity on splenocytes which potentiated none specifically the responsiveness of splenocytes to mitogen. This may be indicative of stimulation of both B and T lymphocytes.

Both the samples showed increase in the weight of the thymus; especially *Kwatha Ghana* sample in comparison to others. Increase in the weight of the

thymus is the indication of stimulation of the activity of the gland. The immunological component, T lymphocytes migrates from the bone marrow to the cortex of the thymus where they greatly proliferate in number and from the cortex they go to the medulla of the thymus where they undergo further processing and become immunological component. This proliferation may be the cause for the observed increase in the weight of the thymus and also anti-body titre showed highly significant increase in the anti-body level in the same test drug administered group.

Just like the weight of the thymus both the samples showed increase in the weight of the spleen but to the non-significant level. Spleen is a contractile sponge, which may rapidly undergo marked variation in size. The observed increase may be due to stimulation of the organ.

Histopathological study showed features of stimulation in spleen sections especially in Group A and the response was of higher magnitude in comparison to the stimulation response observed in Group-B. No significant changes could be observed in thymus and lymph node. (Plates 1-2).

Thus, all the three parameters- anti-body titre, weight changes and histopathological changes features of immunostimulation are observed in trial drug. The response was of much higher magnitude in Group-A. This clearly shows presence of immunostimulation activity in the anti-body formation part of immune system. The stimulation may involve one or more than one of the mechanisms mentioned earlier.

Group-A suppressed cell mediated immunity in a highly significant manner at both 24 hr and 48 hr time intervals, while Group B exhibited only a marginal effect. This CMI suppression may be one of the contributing factors for the benefits observed with the formulation. In cell mediated immunity different set of chemical mediators play a role. It is responsible for delayed type hypersensitivity, in which T-lymphocytes play an important role**.** The first step, which is common to both types of immune mechanisms, is recognition of the antigen. The second step is the activation of cells involved in the CMI through elaboration of cytokines. The final mediator of this activity is the activated macrophage.

The activated T cells secrete cytokines, such as gamma interferon, which activates macrophages, and tumor necrosis factor (TNF), which stimulates an inflammatory response. Cell mediated immunity is amplified by γ-interferon by enhancing the process of antigen processing by macrophages. Macrophage migration inhibition factor inhibits movements of macrophages from the affected site. Interleukin-2 (IL_2) acts on the activated T-lymphocyte and helps in their clonal expansion. It also activates cytotoxic lymphocytes and B-lymphocytes. The activated cytotoxic T cells take part in the neutralization of the antigen[24].

Th-1 T-lymphocyte pathway controls Cell mediated immunity. The first step in this reaction is the processing and presentation of antigen by macrophages and other related antigen presenting cells followed by differentiation of T-cells into different types including Th-1 type. Th-1 cells produce IL-$_{2,}$ Tumor Necrosis Factor-β (TNF-β) and γ-interferon (IFN-γ). These cytokines activate macrophages enhancing their phagocytosis capacity and stimulate another sub set of T-lymphocytes known as CD8+, which mature into cytotoxic cells, which will neutralize the foreign substance responsible for the initiation of the reaction. Activation of macrophages leads to generation of large amounts of chemical mediators, reactive oxygen metabolites and neutral proteases, which are responsible for the inflammation observed during this reaction.

In the context of the above enumerated background, it can be suggested that the test drugs may produce their effect through the below mentioned probable mechanisms: interference with induction stage, the activation of Th-1 cells, CD8+ cells, macrophages, inhibition of synthesis and release of cytokines, inhibition of synthesis and release of phlogistic factors from the activated cells, interference with the activity of the phlogistic mediators.

Another interesting point to be noted is that normally a drug affecting one limb of the immune response is likely to produce opposite effect in the other limb. In the present study, the test drug, which has produced immunostimulation with respect anti-body formation, produced significant

CMI suppression. This profile clearly matches the profile of immunomodulator hence it can be suggested that one of the main mechanisms of action of the test drug is to set right the altered immune response.

Histamine is the local hormone synthesized by mast cells in the tissue and basophiles in the blood. It is released as part of the inflammatory reaction increasing capillary permeability and dilation. It also causes contraction of smooth muscles of intestine.

Conclusion: It was observed that *Guduchi Ghana* prepared from *Kwatha* base produced significant anti-body formation stimulation and CMI suppression effect. This provides clear basis for the therapeutic efficacy of this preparation. The observed clinical efficacy may be attributed to the presence of immunomodulatory effect especially CMI suppression effect of the preparation. Though market *Guduchi Ghana* exhibited similar activity tendency the magnitude of the effect was much less. Classical method has been found much better in comparison to the market sample.

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