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Research Article

EVALUATION OF IMMUNOMODULATION ACTIVITY OF SOMANATHI TAMRA BHASMA

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ABSTRACT

Somanathi Tamra Bhasma is a special method of preparation of tamra bhasma by using shudha tamra, parada, gandhaka, haritala and manashila and which is considered as more effective and less toxic. Tamra possess properties like brimhana, pramehagna and hridya. Therefore it is expected to have immunomodulatory effect. Evaluation of Immunomodulation activity of somanathi tamra bhasma was carried out in S.D.M. Centre for Research in Ayurveda and Allied Science Udupi, Karnataka, India through animal experimentation. Effect of somanathi tamra bhasma on cell mediated Immunity were evaluated by using Immunological paw edema method in albino rats and effect of somanathi tamra bhasma on humoral antibody formation were evaluated by noting Antibody titer and Biochemical, Haematological, and Histopathological findings. Analysis of the results obtained from the Immunomodulation study, clearly shown that, the test drug somanathi tamra bhasma has CMI suppression effect. This effect is produced without affecting anti-body formation indicating specific nature of the observed effect. It is suggested that this effect may be due to modulation of the Th-1 pathway of adaptive immune reaction. Haematological parameter assessment did not reveal any remarkable change. Among the biochemical parameters elevation of SGOT was observed after SRBC injection and was moderately reversed by the test formulation. Thus it can be suggested that this formulation can be employed in clinical conditions involving inappropriate increase in CMI.

Keywords: Somanathi Tamra Bhasma, Immunomodulation activity, Cell mediated immunity, Humoral antibody formation.

INTRODUCTION

The term immunomodulators is being used more and more by nutritionists and health professionals'. In general immunomodulators are responders to the immune system, Immune system dysfunction leads to many infective diseases and other diseases like arthritis, ulcerative colitis, asthma and cardiovascular diseases. Many medicinal plants and minerals are known to have immunomodulatory properties¹. Some of these are believed to promote positive health and maintain organic resistance against infection by re-establishing the bodies' equilibrium and conditioning the body tissue. Rasayanas and bhrimhaniya dravyas are a group of non-toxic herbal as well as mineral drug preparation which are used to improve the general health by stimulating the body immunity. Numbers of immunomodulator single drugs or herbo mineral formulation are mentioned in Ayurvedic classical literature. Tamra (Copper) is also one among them, which possess properties like Brimhana, Pramehagna and Hridya². Therefore it is expected to have immunomodulatory effect. Somanathi Tamra Bhasma³ is a special method of preparation of tamra bhasma by using shudha tamra, parada, gandhaka, haritala and manashila and which was considered as more effective and less toxic.

MATERIALS AND METHODS

Preparation of Somanathi tamra bhasma

Tamra and other required materials for the preparation of somanathi tamra bhasma were collected from SDM Ayurveda pharmacy, udupi, Karnataka, India and somanathi tamra bhasma was prepared at rasashastra and bhaishajya kalpana practical hall of SDM College of Ayurveda, Udupi, Karnataka, India

Immunomodulatory study

All the materials required for the study are collected from SDM centre for research in Ayurveda and allied science, udupi, Karnataka, India and the study was conducted in the same centre. The ethical clearance number of article is 558/02/C/CPCSEA.

Preparation of Somanathi tamra bhasma

Somanathi tamra bhasma was prepared as per the reference of Rasaratnasamuchchaya³, which was prepared by using, one part of shudha tamra, parada, gandhaka, half part of haratala and one fourth part of manashila by using Garbhayantra for marana process by using teevraagni for one yama. After swangasheeta, it was added to khalwayantra and made in to fine bhasma form

Immunomodulatory study

Wistar strain albino rats of either sex weighing between 140-280 g either sex was used for experimental study. Somanathi Tamra Bhasma was subjected for evaluation of immunity by following assessment of cell mediated immunity by noting effect on immunological paw edema in rats and Humoral anti- body formation⁴.

Dose fixation

The dose selection was done on the basis of body surface area ratio using the table of Paget and Barnes (1969) and it was calculated as; Therapeutic human dose × body surface area ratio (convertible factor for animal). Conversion of the dose obtained above to dose in ml/kg /day by multiplying with suitable conversion factor based on the average weight of the animal.

Route of drug administration

The test drug, vehicle and control would be administered according to the body weight of animals by oral route.

Assessment of cell mediated immunity by Paw edema method

In this study three groups were used as following;
Group 1 - water control + triple antigen sensitization

Group 2 – 1 % C.M.C (vehicle, control) solution + triple antigen sensitization

Group 3 - SomanathiTamraBhasma solution + triple antigen sensitization

Above mentioned groups were administered for seven days. The rats were sensitized subcutaneously (1 ml /100 g body weight) on first day of drug administration with the following solution;

Triple antigen (D.P.T) = 1 ml

Normal saline (NS) = 4 ml

Potash alum (10%) = 1 ml

pH of the above reagent (that is potash alum adjuvant) will be maintained between 5.6 – 6.8 using 10 % sodium carbonate. On seventh day the initial paw volume of left hind paw were noted and 0.1 ml of above solution were injected into plantar aponeurosis of left hind paw, volume of immunological edema thus produced was measured by volume displacement method, after 24 hours and 48 hours with plethysmograph. Percentage increase in paw volume, which was the induced edema formation over initial value, was calculated. The values from control group were compared with the values from test drug administered groups to assess the cell mediated immunity response of the drug.⁵



Figure 1: Measuring paw edema



Figure 2: T.P.A Induced paw edema

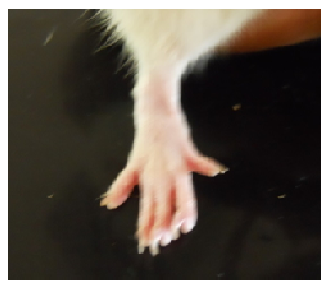


Figure 3: Decreased paw edema in test group

Assessment of immunity by Humoral anti-body formation

In this study also three groups were used with following specifications

Group 1 – water control (SRBC control)

Group 2 – 1 % C.M.C (vehicle, control) solution

Group 3 – SomanathiTamraBhasma solution

The test drug and vehicle were administered for 10 consecutive days. On third day of test drug administration, the animals were sensitized with 30 % SRBC solution. For this purpose the sheep blood was collected from city slaughterhouse 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 become colourless and made to 30 % SRBC solution. This sensitizing agent was injected sub- cutaneously in the dose of 0.5 ml/100 g of body weight to the rats. On the 10th day the animals were sacrificed by stunning and

severing the neck vessel and the blood was collected in sterile test tubes. Serums were separated from it and the components were inactivated by incubating for 30 minutes at 56 °C temperature in a serological water bath. The micro-titer plates were filled with 0.1 ml sterile normal saline and serial two fold dilutions of 0.1 ml of the serum in sterile saline solution. 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 sensitization and to determine antibody titer. The trays were covered and placed in refrigerator overnight. Antibody titer (haemagglutination titer) was noted on the next day. The titers were converted to log₂ values for easy comparison. Spleen and thymus were dissected out from the sacrificed animals and their weight was recorded. Tissues (including lymph node) were transferred to 10 % formaldehyde solution for fixation and later on processed for histological studies. Students "t" test for unpaired data has been used. "P" value less than 0.05 is considered statically significant. The level of p < 0.01 or p < 0.001 is considered. Level of significant were noted by interpreted according

OBSERVATION AND RESULTS

Effect on cell mediated immunity (triple antigen method)

Table 1: Effect of Somanathi Tamra Bhasma on Triple antigen induced Paw oedema in rats

| Group | Dose (ml/kg) | Change in paw volume in ml | | | | | |
|-----------------------------|--------------------------|----------------------------|----------|-------------------------------|----------|----------------------------------|----------|
| | | Basal Mean ± SEM | % Change | 24 th h Mean ± SEM | % Change | 48 th hour Mean ± SEM | % Change |
| Normal control | Normal tap water 5 ml/kg | 0.88 ± 0.02 | - | 1.49 ± 0.07** | - | 1.27 ± 0.05** | - |
| Vehicle control (0.5 % CMC) | 10 ml/kg | 0.775 ± 0.0367 | 11.93↓ | 1.501 ± 0.0742** | 0.738↑ | 1.348 ± 0.0724** | 5.78↑ |
| Test | 67.5/kg | 0.8 ± 0.04 | 9.09 ↓ | 1.05 ± 0.03** | 29.5 ↓ | 0.91 ± 0.05** | 28.346↓ |

Data in Mean = SEM, **P < 0.01 in comparison to normal control group

Table 2: Effect of Somanathi Tamra Bhasma on Percentage changes in Paw volume

| Group | 24 h | 48 h |
|-----------------|----------------|----------------|
| | Mean ± SEM | Mean ± SEM |
| Normal control | 68.65 ± 9.4 | 44.05 ± 4.98 |
| Vehicle control | 97.15 ± 10.69* | 75.2 ± 9.78* |
| Test | 32.92 ± 4.87** | 14.73 ± 5.17** |

*P < 0.05, **P < 0.01

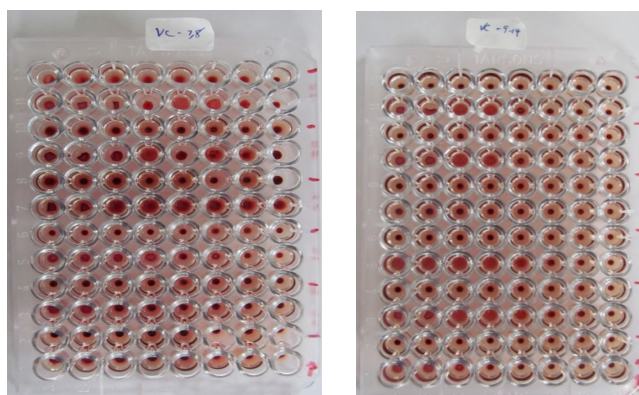


Figure 4: Antibody titer plates of vehicle control group

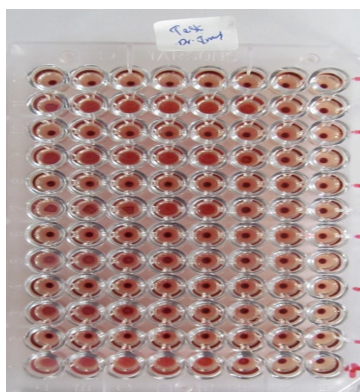


Figure 5: Antibody titer plate of test group

Effect of somanathi tamra bhasma on humoral antibody formation

Antibody titre

Table 3: Effect of Somanathi Tamra Bhasma on Antibody formation (SRBC)

| Group | Dose | Log ₂ value Mean ± SEM |
|--------------|---------|-----------------------------------|
| SRBC control | | 4.71 ± 0.51 |
| Test | 67.5/kg | 4.50 ± 0.25 |

Table 4: Haematological test reports

| Group | Hb (g/dl) Mean ± SEM | % change | WBC count (10 ³ /cu mm) Mean ± SEM | % change | RBC count(10 ⁶ /μl) Mean ± SEM | % change | PCV% Mean ± SEM | % change |
|----------------|-------------------------|-------------|--|----------|--|----------|--------------------|----------|
| Normal control | 16.38 ± 0.31 | - | 12600 ± 1122.2 | - | 8.178 ± 0.266 | - | 44.5 ± 0.839 | - |
| SRBC control | 15 ± 0.59 | 8.42↓# | 6940 ± 890.84 | 44.92↓# | 7.214 ± 0.214 | 11.78↓# | 40.62 ± 1.545 | 8.71↓# |
| Test | 13.81 ± 0.55 | 7.93↓@ | 10716.66 ± 2083.8 | 54.41↑@ | 6.96 ± 0.3433 | 3.520↓@ | 38.98 ± 1.537 | 4.03↓@ |

Table 5: Haematological test reports

| Group | MCV (fl) Mean ± SEM | % change | MCH (pg) Mean ± SEM | % change | MCHC (g/dl) Mean ± SEM | % change | RDWCV (%) Mean ± SEM | % change |
|----------------|------------------------|-------------|------------------------|----------|---------------------------|----------|-------------------------|----------|
| Normal control | 54.61 ± 1.066 | - | 20.05 ± 0.4209 | - | 36.783 ± 0.0792 | - | 14.5 ± 0.289 | - |
| SRBC control | 56.36 ± 1.274 | 4.028↓# | 20.74 ± 0.443 | 8.42↓# | 36.86 ± 0.1536 | 0.2093↑# | 14.1 ± 0.8591 | 3.09↓# |
| Test | 56.25 ± 1.156 | 0.195↓@ | 19.86 ± 0.2654 | 7.93↓@ | 35.41 ± 0.3664 | 3.91↓@ | 14.83 ± 0.7355 | 5.55↑@ |

Table 6: Haematological test reports

| Group | RDWSD (%) Mean ± SEM | % change | Platelet count (thousands/mm ³) Mean ± SEM | % change |
|----------------|-------------------------|----------|---|----------|
| Normal control | 35.15 ± 3.286 | - | 7.198 ± 0.7855 | - |
| SRBC control | 28.34 ± 1.639 | 19.37↓# | 7.206 ± 0.206 | 0.111 ↑# |
| Test | 29.3 ± 1.395 | 3.387↑@ | 7.073 ± 1.053 | 1.845↓@ |

Data: Mean ± SEM #-compared with normal control, @- compared with vehicle control

Table 7: Biochemical test reports

| Group | SGOT Activity- (IU/L) Mean ± SEM | % change | SGPT (IU/L) Mean ± SEM | % change | Alkaline phosphatase (IU/L) Mean ± SEM | % change | Urea(mg/dl) Mean ± SEM | % change |
|----------------|--|----------|---------------------------|----------|--|----------|---------------------------|----------|
| Normal control | 88.33 ± 5.578 | - | 31.66 ± 2.344 | - | 315.35 ± 81.171 | - | 58.5 ± 2.012 | - |
| SRBC control | 155.24 ± 10.972* | 75.75↑# | 31.44 ± 2.307 | 0.694↓# | 21.6 ± 5.250 | 93.150↓# | 15.8 ± 2.066** | 72.99↓# |
| Test | 180.05 ± 8.638 | 15.981↑@ | 30.566 ± 1.461 | 2.779↓@ | 11.5 ± 1.821 | 46.759↓@ | 20.05 ± 2.82** | 26.898↑@ |

Table 8: Ponderal changes (body weight and organs weight)

| Group | Body weight gain Mean ± SEM | % Change | Group | Spleen weight Mean ± SEM | % change | Thymus weight Mean ± SEM | % change |
|----------------|--------------------------------|----------|----------------|-----------------------------|----------|-----------------------------|----------|
| Normal Control | 6.93 ± 3.66 | | Normal control | 0.815 ± 0.141 | - | 0.625 ± 0.002592 | - |
| SRBC Control | 4.553 ± 1.920 | 34.34↓# | SRBC control | 1.072 ± 0.1108 | 31.53↑# | 0.662 ± 0.04994 | 6.048↑# |
| Test | 1.907 ± 1.089 | 141.91↓@ | Test | 1.088 ± 0.1447 | 1.49↑@ | 0.668 ± 0.02630 | 0.784↑@ |

Data: Mean ± SEM #-compared with normal control, @- compared with vehicle control

DISCUSSION

Effect of test drug (somanathi tamra bhasma) on cell mediated immunity (CMI)

Analysis of the data shows that the vehicle control (1 % C.M.C solution) produced weak suppression of immunological oedema at 24th and 48th hour after injection of the paw oedema eliciting agent. In test drug (Somanathi Tamra Bhasma) administrated group remarkable and statistically extremely significant suppression of paw oedema was observed both at 24th and 48th post injection. This indicates the effect and the test drug formulation (Somanathi Tamra Bhasma) possess very good immunological oedema suppression effect which is of much higher magnitude. The immunological oedema represents expression of cell mediated immunity hence based on the results obtained it can be inferred that Somanathi Tamra Bhasma has CMI suppression effect. In

the antibody formation response to foreign body- the induction phase is important. During this phase T-lymphocytes interact with B-lymphocytes and other cells involved in the immune mechanism in a complex manner. Initially the antigen presenting cells like macrophages process the antigen and present it to non-differentiated T-cells colony along with histocompatibility complex molecules and co-stimulatory factors. This result in the generation of two sub-sets of T-cells called as T_H-1 and T_H-2 cells. The former controls and modulates cell-mediated immune reaction and the latter is involved in the modulation of anti-body formation. T_H-2 cells produce cytokines Interleukin-4(IL-4) and tissue growth factor – b (TGB-b) both of which stimulate B-lymphocytes to proliferate and differentiate into anti-body producing plasma cells. The test formulation may be acting by modulating the formation and release of the above

cytokins from the specific sub-types of T-lymphocytes – hence it would be interesting to study its effect on the formation of the above cytokines in suitable *in vitro* conditions. Cell mediated immunity is the responsible for delayed type hypersensitivity and certain T cells suppress antibody production. The test sample was evaluated to assess their effect on cell mediated immunity against an experimental model, which is supposed to represent cell mediated immunity. It involved (25 mg/ml) to produce immunological oedema; as mentioned above the Th-1 T-lymphocyte pathway controls cell mediated immunity. The first step in this reaction is the antigen processing and presentation 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 phagocytizing capacity and stimulate another sub set of T-lymphocyte known as CD⁸⁺, which mature into cytotoxic cells, which will neutralize 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. Taking into consideration the above background material – the following mechanism can be suggested for cell mediated immunity suppression observed with the test formulation may be due to:-

- Interference with induction stage.
- Interfering with 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

Effect of test drug (somanathi tamra bhasma) on humoral antibody formation

Haemagglutination antibody titer is a primary parameter for studying the humoral response. Antigen and antibody reaction results in agglutination. Antibody molecules secreted by plasma cells mediate the humoral immune response. The test formulation did not modulate anti-body formation against SRBC in sensitized animals. This indicates at the dose level employed Somanathi Tamra Bhasma has no modulatory effect on Th-2 mediated pathway of immune reaction. It is a common observation that only cytotoxic agents suppress both antibodies mediated and cell mediated immunity while specific immunomodulators generally suppress either of the above reactions. Thus it can be suggested that the test drug has CMI suppression effect while not affecting the antibody formation.

Haematological parameters

The effect of SRBC injection on WBC, RBC and platelet related parameters was noted down to ascertain the influence the effect of test drug on them. The RBC related parameters like Hb content, RBC- count, PCV and the RBC indices were not affected by RBC injection. The test drug also did not influence them significantly. SRBC being foreign body to rats it was expected that they may elevate WBC count, surprisingly a moderate fall in WBC

count was observed in SRBC control and it was reversed by test formulation to moderate extent. The exact reason is not known however, since the measurement of WBC parameters was done on 10th day – that is 7 days after SRBC injection the above activity might have been observed.

Bio-chemical parameters

Biochemical parameters; which are used as bio-markers for assessing organ functions; especially that of liver and kidney; were assessed. Among them after injection of SRBC significant elevation in SGOT was observed while SGPT activity remained un-affected. Significant decrease in serum urea and moderate decrease in serum creatinine was observed in test drug administered groups. SGOT activity gets elevated in myocardial, hepatic or general tissue injury. The observed elevation may be due to it. Since other parameters were not affected it is difficult to attribute the exact reason. Analysis of the ponderal parameters revealed comparatively decreased body weight gain in test drug administered group. This might be indicative of interference with the absorption and utilization of the nutrients. Since the effect was not significant statistically it may not have any clinical implications.

CONCLUSION

Effect of Somanathi Tamra Bhasma on cell mediated Immunity was evaluated by using Immunological paw oedema method in albino rats and effect of Somanathi Tamra Bhasma on humoral antibody formation was evaluated by noting Antibody titer and Biochemical, Haematological and Histopathological findings. Analysis of the results obtained from animal study clearly establishes that the test drug Somanathi Tamra bhasma has CMI suppression effect. This effect was produced without affecting anti-body formation indicating specific nature of the observed effect. It is suggested that this effect may be due to modulation of the Th-1 pathway of adaptive immune reaction. Haematological parameter assessment did not reveal any remarkable change. Among the biochemical parameters elevation of SGOT was observed after SRBC injection and was moderately reversed by the test formulation. Thus it can be suggested that this formulation can be employed in clinical conditions involving inappropriate increase in CMI.

Abbreviations

S.T.B. - Somanathi Tamra Bhasma
 CMI - Cell Mediated Immunity
 NS - Normal saline
 CMC - Carboxymethylcellulose
 TPA - Triple Antigen
 SRBC - Sheep Red Blood Cells
 HPR - Histo-pathological report
 MCV - Mean corpuscular volume
 MCH - Mean corpuscular haemoglobin content
 MCHC - Mean corpuscular haemoglobin concentration
 RBC - Red blood corpuscles
 RDW-CV - Red cell Distribution Width Coefficient of Variation
 RDW-SD - Red cell Distribution Width-Standard Deviation
 SGOT- Serum glutamic oxaloacetic transaminase
 SGPT- Serum glutamic pyruvic transaminase
 WBC-White blood corpuscles

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