INTRODUCTION
1. INTRODUCTION

Vaccination is regarded as one of the most cost-effective preventative healthcare measures. The vaccines for polio, tuberculosis, tetanus, diphtheria, pertussis, influenza, measles, mumps, rubella and hepatitis prevents at least three million deaths per year worldwide accompanied by a significant reduction in health care costs. Traditionally, vaccines comprise either live-attenuated, replicating pathogens or non-replicating, inactivated pathogens or their subunits. Live vaccines, still used to immunize against measles or rubella, are safe for the majority of recipients. Although cost-effective and rather easy to manufacture, live vaccines may cause disease when given to a recipient with an unrecognized immunodeficiency. Inactivated vaccines consist of killed pathogens or isolated non-replicating subunits. They are safe for immunocompromised individuals, but they often show limited immunogenicity. The vast majority of current vaccines act by inducing antibodies. However, many new vaccine targets against difficult pathogens such as HIV, malaria, and TB, the induction of cell-mediated immunity is likely to be necessary in addition to humoral responses. Therefore, the majority of these vaccines require association with additional substances -adjuvants- in order to enhance the potency or stimulate the appropriate and effective immune responses with less antigen doses and fewer administration. Adjuvants (from Latin verb *adjuvare* meaning “to help”) can be defined as a group of structurally heterogeneous compounds able to enhance or modulate the intrinsic immunogenicity of an antigen.

Combined use of vaccine and immunomodulators is considered as one of innovative approaches in adjuvant discovery and development. A wide array of compounds such as mineral salts, oil based adjuvants, cytokines, saponins, carbohydrate adjuvants has been studied with antigens over the past several years. Despite assessments of a large numbers of adjuvants, with few exceptions aluminium salts (alum) remains the only worldwide approved vaccine adjuvant for human use. However, majority of these fail for human use due to limitations of their own inherent reactogenicity and generation of desired immune response. Further, cytokines are included in the modern classification of adjuvants. However, the practical application of
cytokines as an adjuvant limited by requirement of multiple doses, toxicity and immunogenicity of heterologous cytokines\(^\text{14}\). Alum has a good record of safety and has been considered the adjuvant of choice for vaccination against infections that can be prevented by Th-2 type of immune responses (humoral immunity), and as such has been widely and successfully used in many licensed vaccines such as diphtheria, pertussis, tetanus (DPT); alone or in combinations and poliomyelitis group of vaccines\(^\text{15,16,17}\). However it has limitations particularly regarding generation of strong cell-mediated (Th-1) or cytotoxic immune responses, which are essential to combat life-threatening diseases such as HIV, malaria, and tuberculosis. Further, there is considerable interest in use of combination vaccines such as DTP-Hib, DTP-Hib-IPV, DTP-HBV-IPV-Hib etc. Adjuvants in combination vaccines can be used to reduce the immunization dose and number of injections, thereby decreasing undesired side effects. Therefore, limitations of existing immunomodulators with respect to cost, safety profile and efficacy has prompted investigators to work on safer and effective immunomodulators as vaccine adjuvants\(^\text{18}\).

An exciting area of research that has emerged is the ability of plant-derived immunomodulators to elicit vaccine specific faster and stronger immune responses. Traditional Systems of Medicine (TSM) such as Indian Ayurveda, Chinese, Korean, etc are increasing being recognized as bioprospecting tools for the adjuvant discovery and development. Ayurveda mentions a special therapeutic class known as ‘Rasayana’ which enlists herbal drugs for increasing body resistance to external threats of infection and aging. Several evidence-based studies on such botanicals and their preparations have shown to exert strong modulatory properties like increase in the cytokine expression\(^\text{19,20}\); enhanced activation of CD4 and CD8 T cells, enhanced NK cell activity\(^\text{21}\), which is one of emerging targets for adjuvant discovery. Botanicals with adjuvant activity such as Withania somnifera, Asparagus racemosus, Panax ginseng, Actinidia eriantha, Echinacea species, Curcuma longa, Astragalus, Angelica are outcome of such approaches. Therefore, approaches towards medicinal use of botanicals in adjuvant discovery and development are becoming important with renewed interest in natural products and ethnopharmacology in contrast to random screening approach, which will be more cost and time effective\(^\text{22,23}\).
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The research described in this thesis is aimed at studying immunomodulatory potential of Ayurveda based rasayana botanical *Tinospora cordifolia* (TC) in purview of Th1/Th2 immunity using flow cytometry and newer immuno-pharmacological models (diphtheria toxoid immunity). The safety and quality issues are addressed as per international regulatory guidance on medicinal plants. In this research, medicinal plants from traditional/ethnopharmacological source have been studied in a modern context using state of the art technologies such as flow cytometry, MS and immunological assays, thereby creating an evidence base for further/newer application in immune therapies particularly in the area of immuno-adjuvants.

1.1. BACKGROUND

1.1.1. Immunomodulation

Immunity is a complex mechanism with multiple functions. Immunomodulators are defined as substance biological or synthetic which can stimulate, suppress or modulate any of the components of the immune system. As their function indicates, generally it can be classified into immunostimulating and immunosuppressive agents and both need to be tackled in order to regulate the normal immunological functioning. Hence both agents have their own standing and search for better agents exerting these activities is becoming the field of major interest all over. Apart from specific stimulative or suppressive activity certain agents have been shown to possess activity to enhance the efficacy of vaccines hence are termed as immunoadjuvants and since specific immunoadjuvants are used with specific vaccines, therefore could be considered as immunostimulants. In clinical medicine, both aspects of immunomodulation viz immunostimulation and immunosuppression are equally important and Immunosuppression has attained a status of clinical science and many biological targets have been identified. In contrast, the clinical potential of immunostimulation is yet to be realized. However, with increased understanding of intricate mechanisms of innate and adaptive immunity, there is a renewed interest in immunostimulating therapies.
1.1.2. Newer Targets for Immunostimulants

Several targets have been proposed for immunostimulating therapy. Among them, TLR receptors and modulation of Th1/Th2 immunity remain as emergent ones.

Toll-like receptors: (TLRs) are receiving considerable attention as potential regulators of the immune response through their ability to recognize pathogen-associated molecular patterns (PAMPs). They constitute the first line of defence against many pathogens and play a crucial role in the function of the innate immune system. Agonists of TLRs act directly on DCs, inducing the up-regulation of cytokines, MHC class II and costimulatory molecules, and promote DC migration to the T-cell area of the lymph node (LN). They are receiving attention in diseases such as sepsis, autoimmune disorders, atherosclerosis, cancer, asthma and allergy. Various TLR agonists have been identified and are also under evaluation as vaccine adjuvants. Microbial sources remain important sources for discovery of newer and safer TLR agonists.

Cytokine mediated: The regulation of complex interactions among immune cells under homeostatic and diseased conditions is provided by differential distribution and regulated expression of cytokines and their receptors. Although a variety of cells secrete cytokines, T-helper cells (Th) are major prolific producers of cytokines. There are evidences to show that Th cytokines mediate or restore immune homeostasis by regulating functions such as T cell subset differentiation, hematopoiesis, lymphocyte recruitment, inflammation, angiogenesis and repair processes. These attributes have made Th cytokines as logical targets for immunomodulation. Various agents including peptides, botanicals and synthetic agents represent effective sources for adjuvant development.

1.1.3. Th1/Th2 immunity and Immunomodulation

One of the emerging hypotheses based on Th cytokines balance and is currently under investigation. This theory is based on concept of polarization of T helper (Th) immune responses in two regulatory subsets known as Th1 and Th2 following disease or antigen stimulation. [These subsets can be distinguished by in vivo and in vitro by the
patterns of cytokines production are capable of driving immune response preferably towards cellular immunity (Th1) or antibody production (Th2)]. As shown in figure 1, naïve CD4+ T-helper cells (Tho) can differentiate in presence of the antigen towards either Th1 or Th2 cells, depending on the cytokine environment.

![A simplified model to show Th1-Th2 paradigm. It hypothesizes that under the influence of cytokines naïve T cells differentiate into one of two phenotypes, Th1 or Th2. These subsets counteract each other and have characteristic activities. Adapted from Expert reviews in molecular medicine, 2000.](image)

Figure 1: A simplified model to show Th1-Th2 paradigm. It hypothesizes that under the influence of cytokines naïve T cells differentiate into one of two phenotypes, Th1 or Th2. These subsets counteract each other and have characteristic activities. Adapted from Expert reviews in molecular medicine, 2000.

The differences in the cytokines secreted by Th1 and Th2 cells determine the different biological actions of these subsets. These subsets are characterized by following functional differences:

- Th1 subset is polarized by presence of signature cytokines such as IFN-gamma, IL-12 and is responsible for cell-mediated functions (delayed type hypersensitivity, activation of Tc cells) and for the production of IgG antibodies. This subset is also associated with promotion of excessive inflammation and tissue injury.
- Th2 subset is dominated by secretion of cytokines like IL-4, IL-5, IL-10 and is responsible for eosinophil activation, differentiation, help to B cells and
promotion of IgM, IgE and non-complement activating IgG isotypes. This subset also supports allergic reactions.

According to Th1-Th2 balance concept progression of disease depends on the balance between Th1 and Th2 cytokines and ideal immune therapy can restore this balance to achieve homeostasis. Several studies in both mice and humans now document that \textit{in vivo} outcome of many diseases can be critically influenced by relative levels of Th1 or Th2 like activity\textsuperscript{36, 37, 38}. An excessive Th1 immune response can eventually lead to autoimmunity, the breakdown of material of the individuals own body, e.g. insulin dependent diabetes mellitus (IDDM), multiple sclerosis (MC), Crohn's disease, etc\textsuperscript{39,40,41}. On the other hand, an excessive Th2 response, leads to sensitivity towards foreign components e.g. allergies and related diseases such as atopic dermatitis, asthma, occupational asthma, food allergy and other forms of hypersensitivity like systemic anaphylaxis and acute urticaria\textsuperscript{42}. Further, imbalance of Th1 and Th2 cytokines is also found under immunosenescence, in response to various immune therapies, toxic components, radiation, etc\textsuperscript{43}. Currently, much of the literature supports the view that Th1–Th2 is essential to immune homeostasis and many of the Th-cell directed therapies have provided modest clinical benefits\textsuperscript{44, 45, 46}.

1.1.4. Modulators of Th1/Th2 immunity: potential for adjuvant activity

The Th1/Th2 response is recognized as a contributor to imbalances in immune function. Th1 cells are involved with cell-mediated or innate immunity and deals with intracellular pathogens like viruses, yeast, and intracellular bacteria. On the other end of the spectrum is the Th2 subset, which is involved in Humoral or antibody-mediated immunity and has implication for allergies, asthma, and extracellular infectious microbes. Therapeutic strategies aimed at rectification of Th1 and Th2 imbalance are emerging as major area of immunopharmacology. Sources for development of Th1/Th2 modulatory agents can be classified as endogenous and exogenous and derived immunomodulators are known as endogenous and exogenous agents\textsuperscript{47}.

\textbf{Endogenous:} (Includes cytokines, growth factors, antagonists of cytokines, soluble receptors, receptor antagonists, etc. They have shown considerable efficacy in
management of infectious diseases, asthma, inflammation and cancer. The clinical success of this approach till date is suboptimal. Treatment with recombinant cytokines has yielded a number of adverse effects, such as transient lymphopenia induced by interferon (IFN), Interleukin (IL) - 2, and Tumor necrosis factor (TNF). Monocytopenia has been reported with the use of IFN-gamma and TNF, while IL-2, IFN-[alpha], and TNF induce neutrophilia. These side effects are attributed to phenomenon of pleiotropism observed in activities of cytokines and disruption of in vivo immune homeostasis by artificial introduction of cytokines. Further, cytokine therapy has limitations in cost, manufacture and oral administration.

Exogenous: Another therapeutic approach is to look for immunostimulating and adjuvant activities in exogenous molecules i.e. from natural, semi-synthetic or synthetic sources. Some of representative examples from exogenous sources are Bacterial (MPL), Synthetic (levamisole, imiquimod, resiquimod), fungal (glucans), higher plants (flavanoids, terpenoids, sterols, polysaccharides and polypeptides), minerals (selenium, propagermanium, ammonium trichloro (dioxyethylene-O-O)-tellur-ate) and nucleic acid derivatives (CpG nucleotides, bropirimine and inosine-5-methyl monophosphate). Exogenous agents have distinct advantages in contrast to peptides is their ease of manufacture, characterization (simpler analytical controls), stability (longer shelf lifes), possibility of analogue research (for improved benefit: risk ratio) precise dose fractionation (predictable pharmacokinetics and pharmacodynamics), and most important possibility of oral administration. Further, the success of exogenous sources is known in immunosuppressive therapy where with the exception of monoclonal antibody against the CD-3 marker of lymphocytes (OKT-3), majority of the agents used in clinical practice such as cyclosporine, tacrolimus, leflunomide or brequinar sodium, sirolimus etc are derived from exogenous sources.

1.1.5. Th1/Th2 modulatory agents and protective immunity: Newer applications in vaccine delivery and efficacy

Antigen recognition through T cell receptors results in production of cytokines that amplify immune responses necessary for elimination of antigen. Protective or
immunopathologic outcome is dependent on production of appropriate set of cytokines in response to infectious agents. Th1/Th2 dichotomy, has proven useful in the analysis of immune responses to infections. For example in infection with *M. tuberculosis*, a Th1 response has been shown protective. IFN-gamma, a Th1 cytokine is essential in tuberculosis immunity that is responsible for macrophage activation. Mice that lack IFN-gamma or IFN-gamma receptors are highly susceptible to mycobacteria that show its importance in control of tuberculosis. Similarly Th2 response or IL-4 is important for helminth infection. An ideal vaccine formulation should elicit the appropriate set of cytokines to elicit safer protective immune response. Conventional vaccines have been designed essentially with the aim to induce antibodies (whether it be neutralizing or bactericidal) against molecules expressed at the surface of viruses or bacteria. In recent years, there is interest in newer vaccine formulations and improving conventional ones that elicit balanced humoral and cellular immune responses. In addition to approaches based on the identification of immunogens containing T cell epitopes, one of the emerging strategies is use of Th1/Th2 immunomodulatory agents in vaccine formulations to improve their efficacy. Alum is the only adjuvant that is approved for human use. It is now established that alum is not always the appropriate choice as an adjuvant due to its limitations in engaging T cell immunity. Th1/Th2 modulatory agents from endogenous and exogenous sources including QS-21, cytokines, CpG oligonucleotides, MPL, etc are the outcome of such newer approaches in adjuvant discovery. There is a current need for non-peptide compounds that have advantages of inducing cellular and humoral immunity, safety, tolerability and ease of manufacture. Various botanical and microbial immunomodulators are currently under screening as sources for bioprospecting of such agents.

1.1.6. Botanicals, Ethanopharmacology and Th1/Th2 modulatory agents

As discussed in preceding sections, there is a current need for Th1/Th2 modulatory agents. For such bioprospecting, diversity offered by natural products especially microbial and botanical sources will be important owing to their chemical complexity. The potential of microbial diversity has is already established and exploited in field of
immunostimulants. However, the potential of botanicals towards modulation of cytokine network is still unexplored. Thus with the promise of newer diversity and recent success of triptolide, QS-21, etc. there is a resurgent interest in the botanicals towards development Th1/Th2 modulatory agents.

There are two major ways of bioprospecting natural products for investigation. First, the classical method that relies on phytochemical factors, serendipity and random screening approaches. Second, uses traditional knowledge and practices as drug discovery engine. This is also known as ethnopharmacology approach, which is time and cost effective and may lead to better success than routine random screening. Traditional Chinese medicine, Japanese Kampo, Indian Ayurveda and such are becoming important bioprospecting tools. Well standardized extracts from such traditional texts such as Astragalus, Triterpygium, Angelica sinesis, Uncaria tomentosa, Echniaacea purpurea, Glycrrhiza glabra, Picorrihiza kurroa, Actinidia eriantha, Sho-saiko-to have shown their efficacy towards modulation of cytokine network (Table 1, next page).

There is a considerable interest in classifying immunomodulatory botanicals extracts in terms of Th1/Th2 dichotomy as it helps:

- Prioritizing their target therapeutic areas,
- Development of newer Th1/Th2 modulatory agents.
- Development of safer synergistic combinations.
- And also results in better understanding of immunological mechanisms and traditional uses.

Further, such approaches are also helping in identifying newer immunological targets of botanicals such as DCs, NK cells and macrophage activation. The present study is an attempt to apply such an approach to Ayurveda based Rasayana botanical.
<table>
<thead>
<tr>
<th>Name of botanical</th>
<th>Traditional use as per texts</th>
<th>Th1/Th2 studies on extract/fraction</th>
<th>Isolated lead</th>
<th>Current therapeutic area</th>
<th>Ref</th>
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<tbody>
<tr>
<td>Actinidia eriantha</td>
<td>Antitumor and immunopotentiating activities</td>
<td>Mixed Th1/Th2 response.</td>
<td>Polysaccharide</td>
<td>Vaccines</td>
<td>71</td>
</tr>
<tr>
<td>Asparagus racemosus</td>
<td>Ulcer, dyspepsia and debility</td>
<td>Mixed Th1/Th2 activity with Th2 preferential activity</td>
<td>Aqueous extract of roots</td>
<td>Vaccines</td>
<td>72</td>
</tr>
<tr>
<td>Withania somnifera</td>
<td>Anti-angiogenic and anti-metastatic</td>
<td>Th1 response up-regulator</td>
<td>Leaf extract: WSL-1 and derived compound: WSL-2</td>
<td>Tuberculosis, AIDS &amp; cancer</td>
<td>73</td>
</tr>
<tr>
<td>Picrorhiza kurroa</td>
<td>Asthma, Hepatitis, GIT and urinary disorders.</td>
<td>Mixed Th1/Th2 up-regulation.</td>
<td>‘RLJ-NE-299A’ Mixture of glycosides.</td>
<td>Vaccines</td>
<td>74</td>
</tr>
<tr>
<td>Ganoderma lucidum</td>
<td>Removal of toxins and stomach disorders</td>
<td>Th1 response up-regulator</td>
<td>Glycoprotein with Th1 up-regulation</td>
<td>Vaccines</td>
<td>75</td>
</tr>
<tr>
<td>Boswellia serrata</td>
<td>Anti-arthritic, Anti-inflammatory, modulatory and Anticancer activity</td>
<td>Mixed Th1 and Th2 immune responses</td>
<td>Biopolymeric fraction: BOS 2000.</td>
<td>Vaccines</td>
<td>76</td>
</tr>
<tr>
<td>Panax ginseng</td>
<td>General immunostimulant</td>
<td>Mixed Th1/Th2 up-regulation</td>
<td>Protopanaxadiol and Protopanaxatriol saponins</td>
<td>Anti-cancer, Vaccines</td>
<td>77</td>
</tr>
<tr>
<td>Withania somnifera</td>
<td>Immuno-stimulants</td>
<td>Selective Th1 up-regulating effect</td>
<td>Aqueous extract of roots</td>
<td>Vaccines, Anti-cancer &amp; immunodeficiency diseases</td>
<td>78</td>
</tr>
<tr>
<td>Astragalus membranous</td>
<td>Asthma, ulcer and general immunostimulant</td>
<td>Reverses Th2 bias in biological system</td>
<td>Triterpene saponins and polysaccharides with IL-2 activation</td>
<td>Anti-inflammatory, anti-viral and immune reconstitution</td>
<td>79</td>
</tr>
</tbody>
</table>

Table 1: Th1 and Th2 immunity and botanicals with immuno-adjuvants potential.
1.2. THE PRESENT STUDY

1.2.1. Ayurveda, Rasayana and Immunomodulation

Ayurveda (Ayur: Life; Veda: Science) remains one of the most ancient medical systems widely practiced in the Indian subcontinent. Ayurveda mentions an entire section of materia medica termed as Rasayanas devoted to enhancement of body resistance. Rasayanas are single or multi-component herbal preparations used to rejuvenate or to prevent diseases and degenerative changes that lead to disease. This further promoted research on possible mode of action of Rasayana drugs. Several authors have supposed possible mechanisms as non-specific and specific immunomodulation, antioxidant, enhancing cellular detoxification mechanisms, cytoprotection and modulation of apoptosis pathways. Among mechanisms, the possible immunomodulatory or immune homeostatic effect of Rasayana drugs have been majorly studied. Several Rasayana drugs have been reported to possess pharmacological activities that are relevant to therapeutic areas like immunostimulants, asthma, arthritis, adaptogenic, stress, cancer, psoriasis, etc. Several immunomodulatory leads like glycyrrhizin, acemanan, picroside, diallyl disulphide, psoralen, baccosides, etc have come from biopropecting of Rasayana botanicals.

Modulation of Th1/Th2 immunity being central to immune homeostasis is an important target for such immunostimulants. However, very few systematic studies on Rasayana botanicals in terms of classifying their immunomodulatory effects in terms of Th1/Th2 dichotomy have been reported. Therefore in this study, we explore the potential of chosen Rasayana plant towards modulation of Th1/Th2 immunity. Further, towards newer applications, their potential in enhancing protective efficacy of vaccine was explored.

1.2.2. Test material under investigation

_Tinospora cordifolia_ (Willd.) Miers ex Hook. F. & Thoms is commonly known as Guduchi or Gulvel and belongs to the family Menispermaceae. Guduchi was chosen because it is one of top ten Rasayana drugs widely prescribed by Ayurvedic physicians.
for immune disorders globally. Secondly, it is used singly in Rasayana therapy. *Tinospora cordifolia* (TC) is mentioned in Indian Pharmacopoeia, Indian herbal pharmacopoeia and Ayurvedic pharmacopoeia of India and extract (TCE) prepared from TC stems are prescribed in Ayurveda to improve the immune system and the body resistance against several infections. Many studies have demonstrated anti-cancer and immunomodulatory activity of TC, resulting enhanced cellular immunity, higher antibody titer and increased phagocytic activity. Other pharmacological evidences hints that guduchi have anti-stress; anti-inflammatory, anti-diabetic, hepatoprotective, radioprotective and antidepressant actions. Furthermore, few *in vitro* and *in vivo* studies for the efficacy of TC and its derived constituents have been reported. However, the modulation of immune responses to confer T cell immunity and its immuno-adjuvant potential with vaccines still has not been reported. The present study is in attempt in this direction. Further, guduchi is among top ten herbs which has been short-listed by Government of India for evidence based research, commerce, and large scale cultivation. (http://www.planningcommission.nic.in/aboutus/taskforce/tsk_medi.pdf)

1.2.3. Study Objectives

The overall objective of the present study was to investigate the potential of selected Rasayana botanical to modulate Th1/Th2 immunity and further explore their application as an immuno-adjuvant for use in vaccine industry.

To realize this objective it was decided:

- To determine the quality of the test material and its chemical characterization with respect to marker content analysis.
- To carry out immuno-pharmacological evaluation of the extract with reference to Th1/Th2 immunity using flow cytometry.
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- To evaluate the comparative immuno-adjuvant potential of test extract and Quillaja saponin (QS) for their adjuvant potential in DPT vaccine with special reference to protective immunity against diphtheria.

1.2.4. Study approach

1.2.4.1. Plant material used

In this study, the aqueous extract of the selected plant- *Tinospora cordifolia* was used so as to remain as close as possible to the traditional methods used by Ayurveda practitioners.

1.2.4.2. Quality and safety of test material

Quality control and chemo-profiling of test material was done as per international regulatory guidance on medicinal plants. We have used validated and optimized HPLC-DAD method, for quantification of marker compounds and characterize test material. Since test material was intended as orally active Th1/Th2 modulatory agent, acute oral toxicity studies were carried out as per OECD guidelines to address its safety concerns.

1.2.4.3. Th1/Th2 immunity and test material

Flow cytometry was used to measure murine Th1 and Th2 subsets in peripheral blood. This approach is commonly used in clinical settings to study or characterize beneficial or toxic effects of immunomodulators on Th immunity. Flow cytometry based detection offer advantages of being sensitive, quantitative, multi-parametric and rules out variations due to heterogeneity of cells. Detection of Th subsets in healthy subjects is difficult and requires antigenic stimuli for clear classification of effects on Th1 and or Th2 subsets. T cell dependent antigens such as ovalbumin, sheep red blood cells (SRBC) or pro-inflammatory stimuli such as carragenan or LPS are commonly used for inducing polarization of Th1 and Th2 subsets in biological systems. We have used *in vivo* protocol of SRBC stimulation for studying effects of test material on Th1
and Th2 immunity. Levamisole, a clinically known orally active Th1/Th2 modulator and alum, an established adjuvant was used as positive control. As an initial step, towards assessment of Th1/Th2 immunity, effect of test material was established on lymphocyte expression in SRBC sensitized mice including CD3 (total T cells) and CD19 (total B cells). This was followed by examining effects of test material on T lymphocyte subsets: CD4 (T-helper cells) and CD8 (cytotoxic T cells). The trends obtained in above experiments were confirmed by examining effects of test material on CD4 positive intracellular cytokines (IFN-gamma, Th1), (IL-2, Th1 and T cell activation marker) and IL-4 (Th2). Finally, as a further proof of concept for trends observed in above experiment, we evaluated treatment effects on SRBC specific humoral (antibody titre) and cellular (DTH) immune responses using conventional immunopharmacological methods.

1.2.4.4. Applicability studies

We have used a guinea pig model and a host challenge procedure to assess comparative effect of test material and QS (Quillaja saponin) on protective immunity against diphtheria; DPT vaccine and lethal diphtheria toxin was used as antigen and challenge respectively. Effect of test material and QS on protective immunity was assessed using anti-diphtheria functional antibodies and challenge associated morbidity and mortality. Challenge associated morbidity was assessed using severity scores, weight loss, frequency of symptoms and degree of adrenal toxicity (histopathology and sera cortisol values). Effect of test material and QS on cytokines as a measure of Th1/Th2 immunity was established before as well as after diphtheria challenge. Studies related to selection of best dosage regimen and reproducibility of different test material and vaccine batches were also undertaken.
1.2.5. Summary

This study establishes potential of Ayurveda based Rasayana botanical- *Tinospora cordifolia* (TCE) towards modulation of Th1/Th2 immunity using flow cytometry and immunopharmacological models. TCE was found to have mixed effects on Th1 and Th2 immunity. Further, treatment of vaccinated animals with TCE or QS resulted in significantly higher diphtheria functional antibodies even with higher dilutions of vaccine, which is suggestive of adjuvant potential. In conclusion, this study suggests newer findings that will be helpful to better understand the immunopharmacological actions of test material. Such well-standardized mixture will be useful for bioprospecting of newer leads for conditions where Th1/Th2 modulation is required. As an outcome, currently this test material is part of Department of Science, Government of India and Industry sponsored project on discovery of newer vaccine adjuvants with Th1/Th2 modulatory activity.