ABSTRACT
The thought of our body as a choir, with every body function being a different voice: if one of these voices is out of tune, the whole choir is affected. The immune system is the conductor of our body which helps to bring out the best in every function, and get them all to work together for the best results. If the immune system is compromised, one suffer from extreme fatigue, loses appetite, the body hurts by getting ill often and this could be life threatening. Among the various factors of immune system, macrophages are the first line of defense and constitutes important participant in the bi-directional interaction between innate and specific immunity. Also macrophages are amenable to activation by Biological Response Modifiers (BRM) of different origin. It has been found that certain Biological Response Modifiers impart their function with a distinct duality. Also they are capable of acting as immunopotentiator. BRMs also termed as adaptogens which acts as metabolic regulators and increases the ability of macrophages to adapt to environmental factors and to avoid damage from such factors. (Samuelsson et. al. 2009, Panossian et. al. 1999)

Natural products, including plants, animals and minerals have been the basis of treatment of human diseases. History of medicine dates back practically to the existence of human civilization. The current accepted modern medicine or Allopathy has gradually developed over the years by scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies. The history of medicine includes many ludicrous therapies. Nevertheless, ancient wisdom has been the basis of modern medicine and will remain as one important source of future medicine and therapeutics. The future of natural products drug discovery will be more holistic, personalized and involve wise use of ancient and modern therapeutic skills in a complementary manner so that maximum benefits can be accrued to the patients and the community. The Greek physician Galen (AD 129–200) devised the first pharmacopoeia describing the appearance, properties and use of many plants of his time. The foundations of the modern pharmaceutical industry were laid when techniques were developed to produce synthetic replacements for many of the medicines that had been derived from the forest.

The health promotive, disease preventive and rejuvenation approach available in the Indian systems of medicine like ‘Ayurveda’ is gaining greater attention and popularity
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in many regions of the world. A majority of the present day diseases are reported to be due to the shift in the balance of the pro-oxidant and the antioxidant homeostatic phenomenon in the body. Pro-oxidant conditions dominate either due to the increased generation of the free radicals caused by excessive oxidative stress of the present day life, or due to the poor scavenging/quenching in the body caused by depletion of the dietary antioxidants (Schulz et al., 2000; Dringen, 2000). The disease preventive and health promotive approach of ‘Ayurveda’, which takes into consideration the whole body, mind and spirit while dealing with the maintenance of health, promotion of health and treating ailments is holistic and finds increasing acceptability in many regions of the world. Traditionally, Rasayana drugs are used against a plethora of seemingly diverse disorders with no pathophysiological connections according to modern medicine. In fact this study of BRMs is focused on experimental basis than the experiential basis. Though, this group of plants generally possesses strong antioxidant and pro-oxidant activity, only a few have been investigated in detail. Therefore, there is a need of discovery of new therapeutic drugs.

Ayurvedic pharmacology classifies medicinal plants into different groups according to their actions. One of these is the ‘Rasayana’ group. The word ‘Rasayana’ literally means the path that ‘Rasa’ takes (‘Rasa’: plasma; Ayana: path). It is believed, in Ayurveda that the qualities of the ‘Rasadhatu’ influence the health of other dhatus (tissues) of the body. Hence any medicine that improves the quality of ‘Rasa’ (‘Rasayana’) should strengthen or promote the health of all tissues of the body. These Rasayana plants are said to possess the following properties: they prevent ageing, re-establish youth, strengthen life, brain power and prevent diseases (Govindrajan et al., 2005), all of which imply that they increase the resistance of the body against any onslaught.

Macrophages are quiescent cells which get activated when stimulated. Different types of agents such as antibiotics, antimetabolites and cytokines may exert an immunomodulating action that is expressed in the augmentation and/or inhibition of different immune responses (Pai et al., 1997). One of the most promising recent alternatives to classical antibiotic treatment is the use of immunomodulators for enhancing host defense responses (Tzianabos, 2000).

A number of natural products and synthetic immunopotentiators termed as Biological Response Modifiers (BRMs) are becoming increasingly popular for testing their potential for augmenting immune responses. Among the natural BRMs many herbs
and medicinal plants have long been known for their immunoaugmentary potential, however, only recently scientists have recognized them for their possible BRM actions. The herb *T. cordifolia* (guduchi) isolated from botanical sources have attracted a great deal of attention in the biomedical arena because of its broad spectrum of therapeutic properties and relatively low toxicity. While our understanding of the mechanism of action of these BRMs is still developing, it appears that the primary mechanism involves induction of the immune system. The basic mechanism of the immunostimulatory, antitumor, bactericidal and other therapeutic effects of BRMs is thought to occur via macrophage stimulation. We have focused this study on the role of the BRMs on macrophage functions. Macrophage activation with the BRM treatments can be measured by different markers; Analysis of the development of activation is facilitated when the operationally defined stages of activation is characterized using a library of markers for activation. **Morphological:** Increased adherence, spreading ruffled membrane, cytoplasmic granules and vacuoles. **Biochemical:** Increased or decreased lysosomal enzymes, increased adenyl cyclase, glucosamine uptake, glucose oxygenation, LDH, collagenase elastase, Plasminogen activation calcium influx, cGMP, prostaglandins, hydrogen peroxidase release. **Functional:** Increased phagocytosis, pinocytosis, bacterial activity, tumoricidal activity.

Macrophages play a pivotal role in tumor cell lysis and growth inhibition (Fidler *et al.* 1985, Fidler *et al.* 1988). They can be activated with a variety of agents (Adams and Hamilton, 1987). Activated macrophages are known to produce a number of cytostatic/cytotoxic mediators upon stimulation and thus play an important role in host defense (Singh and Sodhi 1998). In the present study we have used *Tinospora cordifolia* commonly known as guduchi, gallic acid (a derivative of *Embelica officinalis* or Amla), canova (a homeopathic medicament), spirulina (a neutraceutical), AOIM-Z (a complex product of ayurvedic medicines) as BRMs and cisplatin as an anticancerous drug to see its effect on macrophage activation. The direct drug treatment to J774A.1 cells showed activation as assessed by morphological, cytochemical, biochemical and functional assays. This study is an attempt to check the potential significance of these drugs used as immunomodulators for activation of macrophages.

In the present study, we have assessed the direct and indirect effects of BRMs on macrophage activation. This study was undertaken to investigate whether the BRM
treated macrophages are activated to produce the components involved in tumor killing. The effect of these BRMs on modulation of macrophage activity was checked by studying various parameters. The optimum drug dosages were standardized by viability (MTT) assay. Generation of nitrite, ROI, certain enzymes like lysozyme, NADH, NADPH, myeloperoxidase were studied. Phagocytosis and Pinocytosis assay were also carried out to check anti-infectious properties in drug mediated macrophage activation. In the functional assays micbicidal and tumoricidal activities were checked against (the bacterial strain *E. coli* and L929 fibroblast and Yac-1 lymphoma cell lines, respectively). The fibroblast cell line L929 was found to show enhanced cytolysis by the drug treated and activated macrophage cell supernatants, hence was checked for DNA damage if any by gel electrophoresis. The specific inhibitors (tyrosine-specific protein kinase and protein kinase C) treatment to macrophages along with the drugs showed the role of second messengers involved in signal transduction.

Enhanced nitrite, ROI, and TNF-α levels by macrophages were observed in drug mediated macrophage activation. The inhibition of kinase activation was most prominent in down regulation of the TNF-α mediated macrophage functions suggesting the involvement of second messengers in BRM mediated macrophage activation.

The investigations suggest that these components produced by BRM stimulated macrophages could be implicated as the additional effector molecules in the tumoricidal activity of macrophages. Since these components play such an important role in the process of macrophage activation, a thorough understanding of the various physiologically significant BRMs is of paramount importance.