DISCUSSION

The thesis covers development of a Siddha formulation (Chooranam) from widely used Indian Medicinal herbs and its evaluation for anti asthmatic activity using animal models. Poly herbal formulation has unique importance in the treatment of certain diseases such as Cancer, Diabetes and Asthma.

In my present work we selected the four Indigenous herbs *Adhatoda vasica* (AV) (Leaf), *Solanum xanthocarpum* (SX) (Whole plant), *Tylophora asthamtica* (TA) (Leaf) and *Ocimum tenuiflorum* (OT) (Leaf) with the support literature background. The selected plants have folklore uses in any one of respiratory problems such as Cough, Wheezing, Expectorant or Bronchial inflammation. They are also used by Siddhars since time immemorial for various bronchial problems.

In addition, the four above said herbs were found to contain bioactive compounds, such as flavonoids, alkaloids, terpenoids and tannin. So, if they give in combined form in the form of poly herbal dose “Chooranam” it has more synergistic property and provide a more beneficial effect than single herbs on its extract or formulation. The objective of the research work was to prepare polyherbal preparation with multi directing mechanisms targeting on Asthma.

With this aim the present study focused to prepare poly herbal formulation ie, Chooranam from selected plants, they were authenticated by bonafide botanist. Its identity was done by powder microscopy analysis.

**POWDER MICROSCOPY STUDY**

The plant drugs are generally used in the powdered form in Siddha formulation, where the macro and morphology is generally not applicable. So, the identity of the plant through the powder microscopy study is essential. The powdered crude drug can be identified based on the presence or absence of different cell types. In powdered microscopy of all selected plants the following characters were observed.
Microscopical studies of *Adathoda vasica* have brought to light certain essential features, which are of diagnostic values. Small pieces of epidermal peeling appeared in surface view with epidermal cells, stomata, trichome and cystolith. (Figure 11.1,2). Long cylindrical calcium carbonate crystals were often seen dense, large cavities on the surface of epidermal cell. The cystolith is 210µm long and 15µm thick (Figure 11.4). The trichome is 270 µm long and 10 µm thick (Figure 11.6). Microscopical studies of *Ocimun tenuiflorum* have the non glandular trichomes were abundant in the powder. They are multicellular, uniseriate and unbranched.. The basal cell of the trichomes is often dilated (Figure 12.4,5,6) The trichomes are 250-450 µm long and 20µm wide (Figure 12.7). Microscopical studies of *Solanum xanthocarpum* revealed to have the fragments of adaxial epidermis were also equally common in the powder. The epidermal cells have less waxy, thick anticlinal walls. Stomata are sparsely seen. The guard cells are narrowly elliptical; the stomatal pore is not visible. The guard cells are 12x30µm in size (Figure. 13.3). Ovate, simple starch grains are abundant in the powder. They are concentric with central hilum. The grains are 40 x 80µm in size (Figure 13.6). Microscopical studies of *Tylophora asthamatica* showed the presence of Isodiametric flat thick sclerenchyma elements called “stone cells” or brachysclereids are very common in the powder. They vary in size;they measure 50-90µm in diameter. The sclereids have very thick lignified walls with narrow canal like simple pits and wide cell lumen. The canal –like pits are either simple or branched (Figure 14.1). Narrow wide, rectangular parenchyma cells, either solitary or in small bundles are often seen in the powder. On the lateral walls are seen circular or angular wide and prominent simple pits. The cells are 50-150µm long and 10-20µm wide (Figure 14.2).

The characters were compared with the literature and the plant identity was confirmed.

The powder microscopy of chooranam was also found to contain thin pieces of epidermal peelings were frequently seen with densely stomataferous. The stomata are diacytic type (Figure 15.1,2). The body of the gland is circular multicellular and measures 30µm wide Star shaped clustered epidermal trichomes of, a central short stalk; from the tip of the stalk arise several horizontally spreading lateral unicellular, pointed trichomes were common in chooranam (Figure 15.7,8,9). Long, nonseptate, unbranched (non articulated and non anastomosing) latex producing canals called laticifers were seen in the
powder. The latcifer has thick cell walls (Figure 15.10,11). Long spindle shaped libriform fibres were frequently seen. The fibres are 480µm long (Figure 15.12,13.)

By comparisons with the individual herbal powder, it was matched with that, so the chooranam was found to contain all the herbal powders its identity was confirmed.

The organoleptic characters of the chooranam were shown in the table no. 3. Light brown smooth fine powder with astringent taste.

**PHYSIO-CHEMICAL ANALYSIS**

The ash content of the crude drug is generally taken to be the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it. But, it may also involve the inorganic matter added for the purpose of adulteration. Hence, its determination furnishes a basis by judging the identity and purity of the herbal drug in the powdered form. The acid insoluble ash is of more value to detect the earthy matter adhering to the drug. So, we can obtain the evidence of the presence of foreign matter, which likely to occur with root, rhizomes and pubescent leaves. In my study the values obtained are in the permissible limit and compared with Chooranam.

**EXTRACTIVE VALUES**

Extractive values of the crude drugs determine the amount of active constituents in a given amount of medicinal plant material when exhausted with solvents. It is employed for that material for which no chemical or biological assay method exist. As mentioned in different official books (Anonymous, 1996; Anonymous, 2006; Harborne JB,1973) the determination of water soluble and alcohol soluble extractive, is used as means of evaluating crude drugs which are not readily estimated by other means. The extraction of any crude with a particular solvent yields a solution containing different phyto constituents. The composition of these phyto constituents in that particular solvent depends upon the nature of the drug and solvent used. The use of single solvent can be the means of providing preliminary information on the quality of a particular drug sample. The water soluble extractive values play an important role for the evaluation of crude drugs. It can be used to indicate poor quality, adulteration with any unwanted material or incorrect processing of the crude drug during the drying, storage etc. The alcohol soluble extractive is also indicative for the same purpose as water soluble extractive values. In the present study the water soluble extractive was found to be more than alcohol soluble extractive.
extractive (Table no. 4) which indicates that the both chooranam and its individual powders were found to contain more water soluble phyto constituents. Such as glycoside, flavonoids, tannins, protein etc.

**LOSS ON DRYING**

The loss on drying is the loss of weight in percentage w/ w resulting from water and volatile matter of any kind that can driven off under specified conditions. It is determined at 105°C for the presence of excess of moisture which is conductive to the promotion of mould and bacterial growth and subsequently to deterioration and spoilage of drug (Table no.4).

**PARTICLE SIZE**

The prepared Chooranam was passed through th specified mesh given in table no. 5. With remaining residual content 0.136 gm. So, it was negligible and completely passed through the sieve. So, it indicates that the chooranam was a fine powder.

**PHYTOCHEMICAL TEST**

The preliminary phytochemical screening was carried to reveal the presence of bioactive compounds. The table no. 6 showed that the presence of alkaloids, glycosides, phenolic compounds (Flavonoid, Tannin) terpenoids, protein in the herbal powder and its formulation.

**HPLC**

Chooranam and its herbal ingredients were subjected to reverse phore chromatography as described in the materials and methods section. As given in figures 17 to 22 most of the major peaks of chooranam appeared in time between 3.223; 1.880; 2.190; 39.50; 40.4.

**HPTLC**

Figures 23 to 27 and tables 7 to 9 summarize the Rf value and colour of the spot visible in TLC profile of HPTLC of chooranam in the solvent system. The best resolution of compounds was observed in aqueous extract of chooranam showed 23 peaks and spots shown in TLC plate. Visualization was attempted by spraying vanillin sulphuric acid reagent, which was shown in TLC finger print of chooranam (Figure no. 23) revealed the
different chemical constituents in chooranam. Figures 24, 25 and 26 showed the
densitometer fingerprint of chooranam. Figure 27 showed the densitometer fingerprint
of individual herbal powders.

**HEAVY METAL ANALYSIS**

All the heavy metals under studies were found within the permissible limit as per
WHO (World Health Organization) (Anonymous, 1998) and FDAC (Food and Drug
Administration) i.e for Mercury (1 ppm), Lead (10ppm), Arsenic (10 ppm) and Cadmium
(0.30 ppm)

**MICROBIAL ANALYSIS**

In the presented study, herbal drugs and its chooranam were tested for microbial
limit test, all Show the total aerobic viable count, with in the limits prescribed by WHO
for them. However none of them could pass the limit test for Total yeast and mould,
Salmonella spp, S.aureus, Pseudomonas aeuginoa, Coliforms.

**PESTICIDE RESIDUE AND AFLATOXIN ANALYSIS**

WHO and FAO (Food and Agricultural organization) set the limit of pesticides. In
the present study herbal powders and its chooranam were tested for pesticidal residue
(Chlorpyriphos, DDT, Endosulfan, Malathion, Parathion) and Aflatoxins were not
founded in this sample.

**IN-VITRO ANTIOXIDANT STUDIES**

We investigated the possible anti oxidant properties of individual herbal powder
and its chooranam by Nitric oxide radical scavenging and DPPH radical scavenging
methods, because such action may contribute to explain that the therapeutic effect in
asthmatic conditions. Besides the herbal powders were found to contain phenolic
compounds. Hence in our present study, we aim to evaluate the anti oxidant activity of
individual powders and compared with its chooranam.

**Nitric oxide radical scavenging method**

Nitric oxide is a free radical produced in mammalian cells involved in the
regulation of various physiological processes (Lata et al.,2009). However excess
production of nitric oxide is associated with several diseases (Gibahander,.2002). In the
The present study, the nitrite produced by sodium nitro prusside was reduced by both the chooranam and its herbal powders. This may be due to the antioxidant principle in the sample which competes with oxygen to react with nitric oxide, thereby inhibiting the generation of nitrite. This may be due to the antioxidant principle in the sample which compete with oxygen to react with nitric oxide, thereby inhibiting the generation of nitrite. In the present study the chooranam and its herbal powders showed better activity in competing with oxygen to react with nitric oxide thus the inhibition of generation anions. The IC$_{50}$ values of Adhatoda vasica, Ocimum tenuiflorum, Solanum xanthocarpum, Tylophora asthamtica, Siddha formulation and Vitamin C were found to be 250 µg/ml, 157 µg/ml, 128 µg/ml, 143 µg/ml, 92 µg/ml and 15 µg/ml respectively.

**DPPH radical scavenging method**

1-diphenyl – 2- picric hydrazyl (DPPH) radical was widely used as the model system to investigate the scavenging activities as several natural compounds such as phenolic and anthocyanins and in crude mixtures of plants. DPPH was used to determine the proton radical scavenging action of drugs, because it posses a proton free radical and show the characteristic absorbance at 517 nm. From the present results, it may be concluded that chooranam reduces the radical to corresponding hydrazine when it reacts with hydrogen donors in antioxidant principles. The IC$_{50}$ values of Adhatoda vasica, Ocimum tenuiflorum, Solanum xanthocarpum, Tylophora asthamtica, Siddha formulation and Vitamin C were found to be 138.5 µg/ml, 150 µg/ml, 80 µg/ml, 30 µg/ml, 20 µg/ml and 17.5 µg/ml respectively.

**SAFETY PROFILE STUDY**

**Acute Toxicity Study**

The toxic effect of the formulation was evaluated as per the OECD guidelines 425 and 407 respectively by acute and subacute method. All the results pooled from these studies were summarized in table 16, showing for each test group the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test or killed for humane reasons, time of death of individual animals, a description and the time course of toxic effects and reversibility, and necropsy findings. Mortality in the acute oral toxicity test was not seen in the limit test up to dose 4000 mg/kg body weight. So one fifth, one-tenth of the upper bound dose was considered as
test dose for present pharmacological studies. No other toxic symptoms were observed in any of the test dose treated animals.

For the usage of all the animals used in the present investigations were approved by the Ethical Committee and the number is XII/CPCSEA/IAEC/VELS/PCOL/08.08.2010.

**Sub-Acute Toxicity**

**Clinical signs:** Animals were not shown any significant toxic clinical signs during the dosing period of 28 days.

**Mortality:** All animals from control and all the treated dose groups survived throughout the dosing period of 28 days and it was found one animal dead after 19 days of treatment in high dose. (Table. No.16)

**Body weight:** Results of body weight determination of animals of control and test dose groups exhibited comparable body weight gain throughout the dosing period of 28 days. (Table.No.17)

**Food consumption:** During dosing period, the quantity of food consumed by animals from different dose groups was found to be comparable and normal with that of control animals. (Table.No.18)

**Ophthalmoscopy:** Ophthalmoscopic examination of animals in control and test product–treated groups did not reveal any major and remarkable abnormality.

**Functional Observations:** These tests conducted on the experimental animals at termination and recorded did not reveal any abnormalities.

**Haematological investigations:** The results of haematological investigations (Table.No.20) conducted on day 28, revealed no significant changes in the values of different parameters investigated when compared with those of respective controls; however, the increase or decrease in the values obtained was within normal biological and laboratory limits or the effect was not dose dependent. No major changes in the values observed.
Biochemical Investigations: Results of Biochemical investigations conducted on days 28 and recorded in (Table. Nos. 21,22 and 23) it revealed the no significant changes in the values of different parameters studied when compared with those of respective controls; however, the values obtained were within normal biological and laboratory limits.

Decreased ALP levels showed in animals in 200-mg/kg dose group (P<0.05), LDL levels were elevated in animals of 200mg/kg dose group (P<0.05). Protein levels decreased in animals of 200 and 400mg/kg group (P<0.01). Urea and Creatinine levels were decreased in animals of 200mg/kg group (P<0.05). All other parameters were found to be near normal.

Urine analysis: Urine analysis data (Table. No. 24) of control group and treated group of animals determined in week 4 and animals in week 6 did not reveal any abnormalities.

Organ Weight: Group Mean Relative Organ Weights (%of body weight) are recorded in (Table.No.25). Comparison of organ weights of treated animals with respective control animals on day 29 was found to be comparable.

Necropsy: Gross pathological examination of animal organs in control as well as the high dose treated groups did not reveal any abnormalities shown in Figure. 30 and have wide safety margin.

ANTIASTHMATIC STUDY

Bronchial asthma is one of the most complex disorders of the airway characterized by reversible airway obstruction inflammation, bronchial hyper-responsiveness and excessive mucous production. It is known that asthma can be triggered by various infections, dust, cold air, exercise, emotion, perfumes, chemical, environmental tobacco smoke and histamine. In asthma some pathophysiological changes to the airways, thickening of the airways walls, which have been produced reduction of airflow and the development of hyper responsiveness in airway system. Asthma is a chronic condition. These symptoms may be due to liberation of endogenous and intrinsic mediators like histamine, leukotrienes (LTs), bradykinin, prostaglandins (PGs), nitric oxide, platelet
activating factors (PAF), chemokines and endothelin from mast cells during the allergic reactions and inflammation of the air passages in the lungs.

Large numbers of medicinal plant preparations have been reported to possess bronchodilatory effects; some of these include Adhatoda vasica (Amin & Mehta, 1959), Benincasa hispida (Kumar & Ramu, 2002), Albizzia lebbeck (Tripathi & Das, 1977), Cissampelos sympodialis (Thomas et al., 1997), and Sarcostemma brevistigma (Saraf & Patwardhan, 1988). Phytoconstituents like alkaloids and flavonoids are attributed to possess bronchodilatory activity (Amin & Mehta, 1959; Saraf & Patwardhan, 1988).

**Effect of Siddha formulations on histamine and acetylcholine aerosol induced bronchospasm in guinea pigs**

Histamine and acetylcholine antagonists can be conveniently recognized and assayed by their ability to protect guinea pigs against lethal effects of bronchospasm induced by histamine and acetylcholine, respectively (Broadbent & Bain, 1964). The results of the present study showed that prior treatment of Siddha formulation protected the animals to a significant extent from the development of asphyxia produced by both the spasmogens. This is indicative of antihistaminic and anticholinergic activities of the Siddha formulation. Interestingly, the effect was significantly higher suggesting the synergistic action of chooranam.

In the present study, guinea pigs were used because of the extreme sensitivity of their airways to the primary mediators of bronchoconstriction, including histamine and leukotrienes, and their ability to be sensitized to foreign proteins. Moreover, the resemblance of pulmonary responses and anaphylactic sensitization to histamine challenge in both guinea pigs and humans made this species the model of choice. Guinea pig airways react to histamine, acetylcholine, leukotrienes, and other bronchoconstrictors in a manner similar to that seen in humans (Popa et al., 1973; Agrawal et al., 1991). Another similarity between the guinea pig model and asthmatic patients is that enhanced bronchoconstriction occurs in both species following sensitization, in response to β- adrenergic antagonists (Matsumoto et al., 1994). Thus, the guinea pig model resembles the human allergic pathology in several aspects, especially in terms of mediator release.
The role of histamine and acetylcholine in asthma is well established (Nelson, 2003). In the early stage of asthma, release of inflammatory mediators like histamine, tryptase, acetylcholine, leukotrienes, and prostaglandins are triggered by exposure to allergens, irritants, cold air or exercise (Bosquet et al., 2000). Some of these mediators directly cause acute bronchoconstriction. Spasmolytic drugs like β-adrenergic agonists, xanthine derivatives, and anticholinergics are used as quick relief medications in such acute asthmatic attacks (Horwitz & Busse, 1995). In the present study, we have used histamine and acetylcholine as spasmogens in the form of aerosols to cause immediate bronchoconstriction in guinea pigs. Mepyramine (8 mg/kg,.) and atropine sulphate (2 mg/kg,.) were used as reference standard against histamine and acetylcholine-induced bronchospasm respectively (Shah & Parmar, 2003).

The Siddha formulation have shown significant bronchoprotection against both the types of spasmogens as compared to control and also showed significantly protection from histamine-induced bronchoconstriction. Moreover, it showed anticholinergic action in the acetylcholine-induced bronchoconstriction. Histamine was released after degranulation of mast cell by an antigen exposure by antigenic stimulation causing smooth muscle contraction, increased vascular permeability and mucus formation. Histamine is one of the important mediators of allergy, inflammation and bronchoconstriction. Targeting histamine, either prevention of its release from mast cell or use of histaminic receptor antagonist becomes part of antihistaminic therapy in allergic diseases. In vivo study of Siddha Formulation have been also shown the significant increase in pre-convulsion time due to pre-treatment with Siddha Formulation at the dose of 200 and 400 mg/kg of body weight of guinea pigs, when the guinea pigs were exposed to histamine.

Since Siddha formulation showed significant inhibition of histamine-induced bronchospasm, this investigated to evaluate the Siddha formulation against another model for antihistaminic activity. Histamine is known to increase vascular permeability mainly by acting on postcapillary venules where the opening of endothelial gaps leads to increased extravasation of plasma proteins (Grega et al., 1981). In addition to its vascular effects, histamine excites small diameter afferent neurons and evokes the release of vasoactive mediators from local nerve endings (Saria et al., 1988). In the present study an attempt is made to further analyze the possible mode of action of Siddha formulation, for
their effect on mast cell degranulation.

**Mast Cell Degranulation**

The Siddha formulation, to the mast cell suspension significantly reduced degranulation of mast cells. Further, the degranulation induced by compound 48/80, a potent mast cell degranulator, was also prevented by Siddha formulation, in dose dependent manner. Histamine together with platelet activating factor (PAF) is contained in the granules of mast cells. Histamine is liberated in acute inflammation and is important during the early phase in initiating allergic responses. PAF causes vasodilatation, increased vascular permeability and is chemotactic to white blood cells. These mediators from mast cells can be released by injury, various histamine-releasing agents, interleukin-1, factors derived from neutrophils that release histamine, macrophages, and platelets. The protective effect of Siddha formulation against mast cell degranulation indicates that this action may be responsible for the observed anti-inflammatory effect. However, the effect of Siddha formulation on other inflammatory mediators cannot be excluded as mast cell degranulation is only one of the complex mechanisms in the pathogenesis of asthma.

**Anti- Inflammatory Activity**

**Carrageenan Induced Inflammation**

The present study reports the effect of Siddha formulation which produced the most potent anti-inflammatory property. The administration of carrageenan produced a significant edema in the rat paw, which was more intense in the control animals. The two main mediators of inflammation are prostaglandins and PAF in carrageenan- induced paw edema model. It has been established that the carrageenan- induced edema is expressed in two phases. When the first 3-h segment of the curve is analyzed, a biphasic response viewed. A rapid rise in foot volume occurs immediately after subplantar injection of carrageenan. Subsequently, a diminution of foot volume occurs at the end of 1 h, which has been designed as the first phase or early phase edema. A second period of edema formation begins to develop at a slow rate from the end of 1 h. Around 90 min, a strong acceleration of edema formation occurs which tapers off after 3 h. The edema volume present at 3 h minus the edema at 1 h represents the second phase or late phase edema volume.
The carrageenan-induced paw edema model in rats is known to be sensitive to cyclooxygenase (COX) inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents (Rao et al., 2005). The edema (inflammation) induced by carrageenan is shown to be mediated by histamine and 5-HT during first 1 h, after which increased vascular permeability is maintained by the release of kinins up to 1.30 h and from 1.30 to 3 h, the mediators appear to be prostaglandins, the release of which is closely associated with migration of leucocytes into the inflamed site (Di-Rosa et al., 1971). In the present study, Siddha formulations significantly reduced edema formation at 1, 2, and 3 h time points (both the phases of inflammation). The possible mechanism could be action on early phase mainly by inhibiting the mediator of inflammation, most probably by inhibiting the histamine and serotonin, which are present in the proinflammatory cells like neutrophils and mast cells. Siddha formulation diminished edema in both the phases of inflammation. These suggest that Siddha formulation possess antihistaminic activity and inhibition of prostaglandins where as, it is devoid of action on prostaglandin pathway but possess antihistaminic action. These data confirmed the findings from the earlier animal models of antihistaminic activity (histamine-aerosol in guinea pigs). It is well known fact that the ASA, standard anti-inflammatory drug, act by inhibiting the prostaglandins synthesis at late phase. In the present study, ASA inhibited only second phase of inflammation which is in accordance with earlier studies (Rao et al., 2005).

**FORMALDEHYDE-INDUCED RAT PAW EDEMA**

In order to further explore mechanism of Siddha formulation, formaldehyde-induced rat paw edema models were selected. It has been reported, carrageenan induces protein rich exudation containing large number of neutrophils (Kumar & Robbin, 1995). (Rowley & Benditt, 1959). Inflammation induced by formaldehyde is biphasic, an early neurogenic component is mediated by substance P and bradykinin followed by a tissue mediated response where histamine, 5-HT, prostaglandins, and bradykinin are known to be involved (Wheeler-Aceto & Cowan, 1991). In the formaldehyde-induced inflammation, Siddha formulation demonstrated significant anti-inflammatory activity that lasted up to 24 h in contrast to ASA, which was effective only at 1.5 h, suggesting its long duration of action. This indicated that Siddha formulation may have anti-inflammatory actions through inhibition of histamine, serotonin, and other inflammatory mediators.
Further, Siddha formulation were effective only on the second phase suggesting inhibitory action on histamine, serotonin, and other inflammatory mediators and not through substance P or bradykinin. This data are in accordance with the findings from the earlier animal models.

The Siddha formulation showed antiinflammatory activity, which was found to be statistically significant at 400mg/kg concentration in acute formaldehyde induced rat paw oedema model. However, this activity was less potent as compared to ASA. This activity appears to be significant in early phases of inflammation in which various biochemicals, viz. histamine, 5-HT, various kinins are involved. The results were significant when analysed statistically. According to this test, there was a significant difference between the drug treated groups and control at the level of P<0.05. To analyze the spectrum of antiinflammatory activity of Siddha formulation, Dunnet multiple range test was used. At 1 h, ASA exhibited good anti-inflammatory activity compared to Siddha formulation.

Treatment with Siddha formulation significantly reduced the right paw edema in all animals of the experimental group in a dose-dependent manner, indicating its anti-inflammatory effect. Regarding the measurement of the joint, all doses of Siddha formulation decreased the joint size compared to control group (P<0.001). Comparison among the doses revealed that dose 200 and 400 did not differ from each other but differed statistically from dose 200mg / kg (P<0.001). It is showed that the acidic anti-inflammatory analgesics decreased pro-inflammatory prostaglandin concentrations by inhibiting cyclo-oxygenase.

**Chronic Inflammation Method - Cotton Pellet Granuloma Pouch**

In order to assess efficacy of Siddha formulation against proliferative phase of inflammation in which tissue degeneration and fibrosis occur, the widely used cotton pellet granuloma test was employed. During the repair process of inflammation, there is proliferation of macrophages, neutrophils, fibroblasts and multiplication of small blood vessels, which are the basic sources of forming a highly vascularised reddish mass termed granulation tissue (Swingle, 1974; Bhattacharya et al., 1992).

Efficacy of anti-inflammatory agents in chronic inflammatory states is indicated by their ability to inhibit the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation (Recio et al., 1995)
in the present study, Siddha formulation significantly reduced the granuloma formation. The effect was comparable with the standard drug prednisolone. This indicated that Siddha formulation can be good candidate for the chronic inflammatory diseases like asthma and also significantly inhibited the chronic inflammation.

From the results of the studies, it is obvious that Siddha Formulation possess antihistaminic, anticholinergic, and mast cell stabilizing action. Further, they had potent acute, sub-acute, and chronic anti-inflammatory activity, thereby indicating the possibility of developing the cheaper, safer and potent agent for the treatment of asthma. These findings scientifically validated and gave a pathway for the traditional use of these plants for treating inflammatory disorders like asthma in the folk medicine.

In drug discovery, most studies have examined on the antimicrobial potential of medicinal plants and other natural products (Wallace, 2004; Adonizio et al., 2006) measured as either killing or inhibiting the microbial growth, natural products including medicinal plants are still major sources of innovative therapeutic agents for the various conditions of human diseases (Erturk et al., 2006; Rangasamy et al., 2007). The population in rural developing countries relay heavily on traditional healers and medicinal plants as a basis to treat various maladies (Mantle, 2001) inspite of the availability of modern medicine (Kumar VP et al., 2006). The world health organization reported that 80% of the world populations rely mainly on traditional medicines (Tadeg et al., 2005). Herbal medicine of natives in every country forms a major part of the world heritage of the plant material (Mahasneh AM, 2002). Although active ingredients may occur in lower concentrations, plant extracts may be a better source of antimicrobials than synthetic drugs (Quave et al., 2008). The increased role of antibiotic resistant pathogenic microorganisms is greatly mediated by the increased frequency of mutations, misuse of antibiotics and other factors (Hentezer and Givskov, 2003). Evolving resistant microbial strains had compromised the use of newer generations of antibiotics (Kultur, 2007). Combating such situation had been so far dependent upon the traditional treatment of such microbial infections based on substances that kill or inhibit growth of causative pathogens.

Synergistic effects are often to bioactivity in plant extract and some activity is usually lost during purification (Cos et al., 2006). It is also believed that bacterial resistance to synergistic drug combinations present in plants may be slower than that for
single drug therapies. The traditional chemotherapeutic agents exhibit a broad range efficacy through toxicity or growth inhibition to target microorganisms (Hiller and Melzig, 2006). Due to the misuse of such agents in addition to selective pressure upon pathogens, an increased level of antibiotic resistance is on the rise (Lewis, 2001; Chomnawang et al., 2009). An alternative to the inhibition of bacterial growth would lie in an approach to prevent the pathogens from establishing a successful infection. This approach may be realized through developing new anti-pathogenic drugs.

Given the large number of organisms, including some plants that harbor or produce inhibitory metabolites, to control the activity of microbial pathogenic colonizers offers a continued challenge to search for new and novel antimicrobial substances (Wax et al., 2008; Gao et al., 2003; Poonguzhali et al., 2007).

In this microbial study the findings shows all the individual herbal plant powders are having more antibacterial activity than antifungal activity which is determined by zone of inhibition, MIC and MBC on different bacterial and fungal strains.