Summary and Conclusions

Two-third of the world's population seeks health care from sources other than conventional system of medicine. While many of these individuals undoubtedly self-medicate, most of them take treatment from learned practitioners of indigenous systems of medicine, like Ayurveda, Homeopathy, Traditional Chinese Medicine, Traditional Hawaiian Medicine, Unani, etc in the countries depending on their origin traditionally. In much of the developing world, 70–95% of the population relies on these traditional medicines for primary care. The global market for traditional medicines was estimated at US$ 83 billion annually in 2008, with a rate of increase that has been exponential.

According to one of the definitions used by the Cochrane Collaboration, ***'complementary and alternative medicine'(CAM)*** is a broad domain of healing resources that encompasses all health systems, modalities, practices and their accompanying theories and beliefs, other than those intrinsic to the politically dominant health system of a particular society or culture in a given historical period. CAM has undergone a revival and has become quite popular in Asia, Europe, Australia, China and USA.

Patients are becoming growingly worried about the adverse effects and toxicity of many conventional drugs, in fact a major cause of death and hospitalization and the inability of conventional medicine to provide adequate clinical effectiveness for chronic diseases. Increasing numbers of patients, therefore, look for less-toxic alternatives, especially in the case of chronic illness. Current citizens' attitudes towards health include a preference for natural treatments and products over chemical drugs, a holistic view of health, a belief in individual responsibility for achieving health, less unquestioning acceptance of medical authority and anti-technology sentiments

The scientific community can no longer ignore the worldwide exponential surge in public enthusiasm for CAM therapies. Use of CAM, particularly biologically based CAM therapies (herbal), is common and more likely to be used by those with chronic diseases. This surge in interest relates to the chronicity of many illnesses, the information explosion on the internet, and a more active participation of individuals in their own health care.

TM/CAM practices have developed within different cultures in different regions. So there has been no parallel development of standards and methods, either national or international, for evaluating them. Evaluation of TM/CAM products is also problematic. This is especially true of herbal medicines. Research into TM/CAM has been inadequate, resulting in paucity of data and

inadequate development of methodology. This in turn has slowed development of regulation and legislation for TM/CAM. National surveillance systems to monitor and evaluate adverse events are also rare. So although many TM/CAM therapies have promising potential, and are increasingly used, many of them are untested and their use not monitored.

As a result, knowledge of their potential side-effects is limited. This makes identification of the safest and most effective therapies and promotion of their rational use more difficult.

It is common practice in contemporary medicine to follow stringently the scientific method in the process of validating the efficacy and the effectiveness of new or improved modes of treatment intervention. It follows that the complementary or alternative interventions succinctly outlined above, as well as those not cited, must be validated by stringent research before they can be reliably integrated into conventional medicine.

The field of evidence-based research will need to refine and finalize its tools and protocols. The critical process of evidence-based research in CAM rests on the reliability of the process of evaluation of the research methodology, design, and data analysis.

The plant used in CAM may become item of interest, when a new chemical compound is isolated from it, which serve either as active moiety or form a lead to further synthesize a new compound, for the treatment of a disease. Approaches like high-throughput screening, generation of phytochemical profiles, development of quality controls and standardization parameters for raw materials and finished products, and clinical trials, certainly help to derive rationale of their usage in different diseases. Thus, medicinal plants have played a major role in the development of modern medicine and continue to be widely used in their original form.

The present study is based on development of the evaluation parameters for medicinal plants used in CAM (*Ayurveda)* and which are used traditionally, but have not been scientifically explored for their therapeutic claims. The study, therefore, is designed to evaluate two plants mentioned in CAM for ROS generated disorders, using modern scientific methodologies, so as to justify their traditional role.

The following plants are selected for the study-

A. Leaves and roots of *Granthiparni- Leonotis nepetaefolia* (Labiatae)

B. Whole plant of *Changeri- Oxalis corniculata* (Oxalidaceae)

Various herbs have also been identified as possessing anti-inflammatory and antioxidative properties, and some of these are currently being used to treat inflammatory disorders and disorders caused by reactive oxygen species (ROS). The scope of ROS-mediated diseases is believed to be broad, and herbs that scavenge reactive oxidant moiety before they damage tissue may prevent or slow each of these processes. However, there is limited scientific data to support such a conclusion for the above herbs, and the majority of the reports proclaiming benefits of these herbs are based on testimonials, case reports, and unsubstantiated claims.

Leonotis nepetaefolia (LN) is also known *Granthiparni* or *Granthika* in Ayurveda. Roots of this plant have been used in *Brahat Guduchi Taila* and *Mritsanjivani Sura*, Ayurvedic formulations and these are used in indications of *swasa* (Asthma and bronchitis), *Kandu* (Fever) and *Visa* (poisonous conditions). *L. nepetaefolia* (*shandileer*) leaves were used by African and tropical countries for the treatment of asthma and respiratory disorder. *Oxalis corniculata* (*Changeri*-OC) is also known as wood sorrel, yellow Indian sorrel, used in Ayurvedic formulation (*changeri ghrita*) in conditions of increased *kapha*, *vata*, *hrid* roga and piles. It is also known to cure dysentery, skin diseases and the leaves are commonly chewed to treat diarrhoea and mouth ulcers.

The present study, therefore, designed to evaluate these two plants for pharmacognostical parameters, chemical investigation and therapeutic claims. Both the above cited plants were commonly used in ROS generated disorders viz. asthma & bronchitis and heart diseases. The study was planned to establish their pharmacognostical standard, extraction and fractionation, chemical profiling thereof, isolation of compound from bio-active fraction and assessment of therapeutic claims. Plants were collected, authenticated and subjected for evaluation of quality control parameters as per WHO guidelines for plant materials. Extracts and fractions were prepared by standard methods, evaluated for the presence of various phytoconstituents by chemical tests and TLC. Chemical fingerprinting, identification and quantification of marker compound were also performed by using sophisticated technique like HPTLC and HPLC. Evaluation of biological activity of fraction/extracts lead to isolation of bioactive chemical entities that were characterized by spectral analysis correlated with previously reported literature. Biological evaluation of bioactive fractions was carried out on various *in-vitro, ex-vivo and in-vivo* models of ROS generated inflammation and ischaemic heart disease.

Summary and Conclusions

Authenticated plant materials were subjected to detailed systematic pharmacognostical and phytochemical evaluation as means of standardization of selected plants. LN is deciduous, stout, erect, woody and 2-3 meters in length. Leaves are cauline, ramal, opposite, charactaceous and membranous. Leaves are 3-8 cm long with dentate margin, trichomes on lamina and acute apex. Because of its leaf shape the plant is known as *Lion's ear*. Root system is well developed with numerous thin, hairy lateral roots arise from main primary root (0.5-1 cm in diameter). Roots are grayish yellow in colour with few longitudinal furrows. Microscopy of TS shows mesophyll is not differentiated into palisade and spongy parenchyma, but, mostly consists of spongy parenchyma which is thin walled and loosely arranged. In the midrib region there are conjoint, colleteral, lignified vascular bundles, leaf also showed the presence of non- lignified, multicellular, trichomes on both the surfaces. Mature root shows a thin bark and a very wide xylem, cork exfoliating, generally detached. Pholem fibres are generally not observed. Secondary xylem forms major part of root consisting of vessels, xylem fibres and xylem parenchyma. Xylem fibre in powder microscopy is seen as elongated lignified, pointed ends with moderately wide lumen. In quantitative microscopy of LN, the stomatal index for upper and lower surfaces was found to be 7.8 to 9.0 to 10.6 and 9.7 to 10.3 to 11.8 respectively. Vein islet number and vein- let termination number are 9 to 12 (Average 10. 5) and 11 to 16 (Average 13) respectively. OC is a small procumbent herb also known as yellow Indian wood sorrel, with stems rooting and pubescent with appressed hairs, leaves palmately 3-foliolate. Flowers are axillary, sub-umbellate and yellow in colour. Transverse section (T.S.) of root shows 3-4 layers of cork, consisting thin-walled rectangular cells, brownish in appearance. Few starch grains simple, round to oval measuring 3-11μ in dia., are present scattered throughout the region. T.S. of stem showed single layered epidermis, composed of rectangular to oval cells, some of which are elongated to become unicellular covering trichomes. Leaf TS showed petiole with single layered epidermis of rectangular or circular, thin-walled cells. Cortex is 3-4 layered consisting thin-walled, circular, oval or polygonal parenchymatous cells. Powder is greenish-brown in color. Fragments of trichomes (unicellular covering and warty), lignified vascular bundles and starch grains were observed.

Proximate analysis and estimation of secondary metabolites in extracts serve as means of physicochemical evaluation of plant and plant material. The total moisture (LN) is found to be 7.8% and 5.1 % w/w for leaf and root respectively. The total ash for leaf and root is found to be

5.88% w/w and 4.08% w/w respectively. Alcohol and water extractive values were found to be 29. 2 %w/w and 27.92 %w/w for LN leaf, and 21.83% w/w and 28.39% w/w for LN root respectively. Elemental analysis of leaf and root indicates presence trace elements of magnesium (LNL-504. 58 ppm, LNR- 576.05 ppm), manganese (LNL-34.72 ppm, 96.55 ppm) and zinc (53.33 ppm and 60 ppm). Major secondary metabolite like phenolic and flavonoids were estimated by reported methods. Higher amounts of flavonoids (31.23% w/w of total aqueous extract) were observed in total aqueous extract in LNL. Proximate analysis of OC indicated that the moisture content (6.94%) of the plant drug is not too high, thus it could discourage bacterial, fungi or yeast growth. The ash value determinations are equally important. The total ash (21.98%) is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica. Higher amount of total ash indicates presence of inorganic salts. Alcohol and water extractive values were found to be 17.71% and 24.64 % respectively. Elemental analysis of leaf and root indicates presence trace elements of magnesium (490.28 ppm), manganese (68.23 ppm) and zinc (65. 23 ppm). Secondary metabolite like phenolic and flavonoids were estimated by reported methods. Higher amounts of flavonoids (32.3% w/w of total aqueous extract) were observed in total aqueous extract.

Phytoconstituents of varying polarity were extracted by successive extraction process and qualitative phytochemical screening showed the presence of various types of phytoconstituents like alkaloids, volatile oils, phytosterols, fats and oils, flavonoids, amino acids, carbohydrates etc in LNL and phytosterols, terpenes, fats and oils, carbohydrates in LNR. Phytochemical studies of successive extracts shows presence of fats and fixed oils, carbohydrate and glycoside, saponins, flavanoids, proteins, amino acids and phytosterols in OC. TLC studies confirmed the presence of above constituents in successive extracts.

HPTLC finger printing of alcoholic extract serves a rapid and authentic means of standardization of plant extracts. It has been done in three mobile phases having varied polarity. Methanol extract of LNL was developed in mobile phase petroleum ether: ethyl acetate (3:1), ethyl acetate: formic acid: methanol (4:0.25:0.5), toluene: ethyl acetate: methanol: ammonia (1:1.5:2:0.25) mobile phases were found most suitable for separation of non polar, medium polar and polar compounds. Methanol extract of LNR was developed in mobile phase Hexane: ethyl acetate (3:1), Benzene: diethyl ether (2:3) and ethyl acetate: methanol: formic acid: water (2:1:0.25:0.5)

mobile phases were found most suitable for separation of non polar, medium polar and polar compounds. Hexane: Ethyl acetate (3:1), Ethyl acetate: chloroform: methanol (4:0.5:0.5), Toluene: ethyl acetate: formic acid: water (20:100:10:10) mobile phases were found most suitable for separation of non polar, medium polar and polar compounds from extracts of *O. corniculata*. Developed plates were scanned in CAMAG scanner at absorption maxima of 254 nm and 366 nm. Plates were than sprayed with anisaldehyde sulphuric reagent, heated in an oven at 105°C for 10 min. and scanned at 540 nm for presence of various phytoconstituents

Fractionation of plan material by using two immiscible solvents provides separation of compounds by their partition coefficient. Total methanol and total water extracts were prepared for the study. Sub-fractions of methanol extracts were prepared by following scheme. Non-polar compounds like terpene and sterols were separately studied by preparing terpene rich extract in dichloromethane and unsaponifiable fractions of both the plants. Total flavonoids fraction and alkaloidal fraction were prepared for LNL.

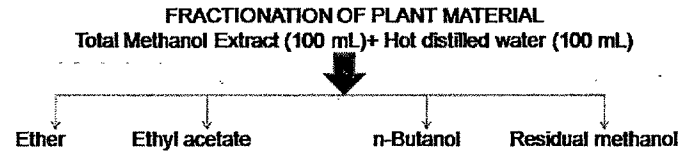

Figure 5.1: Fractionation of plant material

All the fractions and sub-fractions were analysed by HPTLC for compounds of varied polarity. LNLAL-01 (an alkaloid- leonurine) was isolated from alkaloid fraction of LNL and purified by preparative TLC and recrystallization. LN-02 was isolated from terpene rich fraction of LNL and unsaponifiable fraction of LNR and identified as β-sitosterol by co-TLC and spectral data with standard. OC-01 was isolated from flavonoids rich fraction of *O. corniculata* and identified as swertisin by comparison of spectral studied with previously cited literature.

LKLAL-01was quantified by validated HPTLC and HPLC methods. Mobile phase chloroform: ethyl acetate: methanol (3.5:1.0:0.5 v/v) was used for HPTLC and alkaloid was separated at Rf 0.52. Peak was detected by UV analysis at 290 nm. Regression equation was found to be Y= 2006.48 + 3.228*X with correlation coefficient 0.9927. Method was found to be robust and accurate. The content of LNLAL-01 in the *L. nepetaefolia* leaves by HPTLC method was found to be 0.082% w/w. LNLAL-01 was estimated by HPLC in alkaloid fraction of LNL at 290 nm

wavelength with flow rate of 0.8 mL/min. The retention time (R_t) of isolated alkaloid was found to be 3.447 min. in mobile phase consisting acetonitrile-water (3.5:6.5, v/v) with 1% diethylamine. LNLAL-01 was quantified in alkaloid fraction by using regression equation y = 1.918x + 2.639 with correlation coefficient R^2 = 0.994. The content of LNLAL-01 in the *L. nepetaefolia* leaves by HPLC method was found to be 0.076% w/w.

LN-02 (β-sitosterol) was quantified by validated HPTLC methods. Mobile phase toluene: chloroform: methanol (4:4:1 v/v) was used for HPTLC and alkaloid was separated at Rf 0.42. Peak was detected by derivatization with anisaldehyde sulphuric acid followed by heating at 110°C for 5 min. and detected at 540nm. Regression equation was found to be Y= 23725.79+ 5.807*X with correlation coefficient 0.9950. Method was found to be robust and accurate. The content of β-sitosterol in the LNL and LNR by HPTLC method was found to be 0.083% w/w and 0.0646 % w/w respectively.

OC-01was quantified by validated HPTLC and HPLC methods. Mobile phase ethyl acetate: methanol: water (4:0.5:0.5 v/v) was used for HPTLC and OC-01 was separated at Rf 0.48. Peak was detected by UV analysis at 338 nm. Regression equation was found to be Y= 3359.43 + 42.25*X with correlation coefficient 0.9984. Method was found to be robust and accurate. The content of OC-01 in the *O. corniculata* by HPTLC method was found to be 0.241% w/w. OC-01 was also estimated by HPLC in flavonoids fraction of OC at 338 nm wavelength with flow rate of 0.8 mL/min. The retention time (R_t) of isolated flavonoid was found to be 4.68 min. in mobile phase consisting acetonitrile: water (1:3 v/v). OC-01 was quantified in flavonoids fraction by using regression equation y = 5.696x + 10.95 with correlation coefficient R^2 = 0.997. The content of OC-01 in the *O. corniculata* by HPLC method was found to be 0.187% w/w.

Biological screening of medicinal plant is of vital importance, to provide a scientific basis to their continued usage leading to justification to their therapeutic claims. Animal studies also lead to identification of new chemical entity or a lead for more potent synthetic drugs. Natural products have been the single most productive source of leads for the development of drugs. Over a 100 new products are in clinical development, particularly as anti-cancer agents and antiinfectives. Various screening approaches are being developed to improve the ease with which natural products can be used in drug discovery campaigns, and data mining and virtual screening techniques are also being applied to databases of natural products.

Various herbs have been identified as possessing antioxidative and anti-inflammatory properties, and some of these are currently being used to treat disorders caused by reactive oxygen species (ROS). The scope of ROS-mediated diseases is believed to be broad, and herbs that scavenge reactive oxidant chemicals before they damage tissue may prevent or slow each of these processes.

The selected plants were screened by *in-vitro, ex-vivo* and *in-vivo* methods. All the prepared extracts, fractions, subfractions and isolates were screened for *in-vitro* activities and components that found promisingly active were further evaluated by *in-vivo* methods.

Acute toxicity studies were performed following OECD guidelines (2001) (OECD 423, Acute Toxic Class Method) for all the tested substances at oral dose of 2000 mg/kg and 3000 mg/kg of extracts and 500 mg/kg and 1000 mg/kg of fractions. There were no signs of any toxicity found in animals, after the administration of the test doses, for 24 hrs.

All fractions were studied for their *in-vitro* antioxidant potential at concentrations of 10, 20, 40, 80, 100 µg/mL. Three methods were selected viz. DPPH radical scavenging assay, $FeCl_3$ reducing power and phosphomolybdenum method. Ascorbic acid and Butylated Hydroxy Toluene (BHT) were selected as standard. *L. nepetaefolia* constituents show antioxidant property in all three methods. IC_{50} (DPPH assay) of terpene rich fractions of LNL (TER LNL-32.42 µg/mL, UNS of LNL- 43.29 µg/mL), flavonoids rich fraction of LNL, nBuOH of TMLNL-35.78 µg/mL, and total methanol extract of LNR (TMLNR- 25.34 µg/mL) was comparable to standards. The results of $FeCl_3$ reducing power assay show following fractions have IC_{50}; TMLNL- 35.89 µg/mL, FFLNL- 34.74 µg/mL, TER LNR- 38.22 µg/mL and UNS of LNR- 46.27 µg/mL. Terpene and flavonoids present in fractions of *L. nepetaefolia* found to reducing molybdenum, IC_{50} of fractions, TER LNL-23.50 µg/mL, nBuOH of TMLNL- 14.01 µg/mL, FFLNL- 22.51 µg/mL, TMLNR-22.48 µg/mL and EA of TMLNR-13.86 µg/mL. The results clearly indicate that antioxidant compounds present in the plant and fraction rich of terpene, sterols and flavonoids show potent antioxidant activity. LN, contains labdanoid diterpenoids, labdanic acid;a coumarin, the terpenic alcohols; nepetaefolinol and diterpenoids, may be responsible for its antioxidant activity.

Summary and Conclusions

O.corniculata constituents show antioxidant property in all three methods. IC_{50} (DPPH assay) of extracts varied from 28.89 µg/mL to 76.13 µg/mL, giving significantly less potent activity than standard used. The results of $FeCl_3$ reducing power assay show following fractions have IC_{50} TAOC- 36.25 µg/mL, OCFF- 38.73 µg/mL and TMOC- 41.74 µg/mL. In phosphomolybdenum method, IC_{50} of fractions OCFF-38.48 µg/mL, TAOC-42.38 µg/mL, EAFTMOC-47.17 µg/mL and TMOC-51.33 µg/mL found to reduce molybdenum. *O. corniculata* extracts/fractions contain variety of antioxidant compounds like ascorbic acid, β-carotene and flavonoids which could be responsible for its potent antioxidant activity. All the fractions, found with potent antioxidant activity, were separated and screened on HPTLC and derivatized with DPPH to understand the pattern of constituents present in them.

The herbal medications of Labiatae family are famous for their cytotoxic effects. Mentha family decoction or infusions have been used for the treatment of various cancers. Methanol extract of aerial parts of the plant LN has in-vitro antioxidant activity and significant activity observed in brine shrimp lethality test. The effects of *L. nepetaefolia* extracts/fractions and isolates on the proliferation of non-small cell lung cancer A549 were determined using the MTT assay. Methanol extract of leaf and non polar fraction show significant cell growth inhibition on A549 cell lines. Fractions that significantly inhibited cell growth were unsaponifiable fraction of LNL 22.17±0.8478, TMLNL 25.67±4.3769, ether fraction of TMLNL 36.91±6.7938, flavonoid fraction of TMLNL 38.725±9.8358, petroleum ether LNR 23.12±7.9903, terpene LNR 33.263±7.6254 and ether TMLNR 23.57±1.4156. Flavonoid rich fraction of TMLNL was found to inhibit growth of cancerous cells by 38%. It appears that the plant extracts/fractions could induce ROS in the induction of apoptosis and fractions were selectively toxic against the cancer cell lines tested, motivating further work to determine the underlying mechanism(s), signal transduction pathways, leading to growth inhibition induced by phytoconstituents.

It has been found that the 5-lipoxygenase pathway has been the major focus of study due to the pronounced pro-inflammatory role of the leukotrienes. The leukotrienes were well known to medicine as the slow reacting substance of anaphylaxis (SRS-A). LOXs are potential target for the rational drug design and discovery of mechanism–based inhibitors for the treatment of bronchial asthma, inflammation, cancer and autoimmune diseases. Many constituents of *L. nepetaefolia* show significant activity against lipoxygenase. There are relatively very few reports on the anti-inflammatory activity of *L. nepetaefolia*. Baicalein and Indomethacin were used as

standards. Fractions and sub-fractions viz. TMLNL, TWLNL, UNSLNL, ETTMLNL, EATMLNL, TWLNR and ETTMLNR achieved more than 90% inhibition in the assay. IC_{50} values ranged from LNLAL-01-11.91±2.052 to FFLNL-85.64±4.532. Compound LNLAL-01 an alkaloid, was found to be most active against the enzyme. It was observed that both root and leaf parts are active against the enzyme and non-polar fractions shows significantly better activity in LOX assay.

Various inflammatory mediators, including reactive oxygen species (ROS), are released from activated leukocytes (neutrophils, eosinophils, macrophages) and play a central role in the pathogenesis of asthma. Evidence for increased oxidative stress in asthma is further provided by the finding of a decreased antioxidant capacity in plasma and BAL fluid of asthmatic patients. ROS can initiate and also perpetuate inflammatory cascades and cause subsequent tissue damage. The anti-inflammatory effects of the test samples, that were found active in lipoxygenase activity, were assessed *in vitro* using the modified cell based assay of Tan and Berridge (2000) [30] based on reduction of the highly water-soluble tetrazolium salt WST-1 in the presence of activated neutrophils. The analysis revealed that the effect of the terpene fraction exceeded that of the more polar n-butanol, methanol and water extracts. TER LNR, ET of TMLNL and TMLNR showed the highest inhibitory effects at 400 µg/mL viz. 69.54%, 64.89% and 60.28% with IC_{50} values 241.91±16.669 µg/mL, 245.36±18.2046 µg/mL and 279.01±12.8694 µg/mL, respectively. Furthermore, it is interesting to note that the significant antioxidant effect of non-polar sub-fractions is in correlation with the observed cytotoxic activity and antiinflammatory activity of this fraction.

Mast cells are the predominant inflammatory cells in the smooth muscle layer of bronchial biopsies and that their number is elevated in patients with asthma compared with those with eosinophilic bronchitis or healthy controls. In a mouse model of chronic asthma, mast cells can substantially influence features of chronic allergic inflammation and tissue remodelling. Thus, mast cells have the potential to drive important features of allergic inflammation independently of IgE. Mast cells may be instrumental in orchestrating TReg-cell-mediated peripheral tolerance is unprecedented. *Ex-vivo* studies carried out on mast cells degranulation induced by compound 48/80 for the active fractions. Various fractions (200 mg/kg and 400 mg/kg) and isolates (20 mg/kg) were given p.o. for 7 days prior to the experiment to the treatment groups. Almost all the fractions at dose of 400 mg/kg tested had significant inhibitory effect on mast cell degranulation

produced by compound 48/80. The percentage of disrupted cells were found to be in groups as TMLNL (400 mg/kg) 29.98±2.711, TWLNL (400 mg/kg) 29.03±2.022 ET TMLNL (400 mg/kg) 24.53±2.006 nBuOH TMLNL (400 mg/kg) 21.46±1.362, TMLNR (400 mg/kg) 18.56±1.305 TERLNR (400 mg/kg) 26.13±0.7077 LN-01(20 mg/kg) 72.17±3.82 LN-02 (20 mg/kg) 58.92±7.483. Methanol extract of root is found to be more potent in inhibition of degranulation of mast cells.

Physical and chemical stressors such as trauma, allergen, polluted air exposure, radiation etc. has been reported to concurrently produce immunodeficiency and oxidative stress. This was studied on milk induced leucocytes and eosinophil count. Various fractions (200 mg/kg and 400 mg/kg) and isolates (20 mg/kg) were given p.o. for 7 days prior to the experiment to the treatment groups. After parental administration of milk, there is increase in total leukocytes count, and this stressful condition can be made normalized by administration of an antistress or drugs having antioxidant ability. Furthermore, leukocytes during asthmatic inflammation release the inflammatory mediators like cytokines, histamine and major basic protein, which promote the ongoing inflammation. Amongst mice pretreated with various extracts of *L. nepetaefolia* ether sub-fraction of total methanol extract of leaves and roots (400 mg/kg) showed significant reduction in leukocyte count induced by milk, whereas other extracts had non significant reduction in leukocyte count. Amongst mice pretreated with various extracts/isolates of *L. nepetaefolia*, ether sub-fraction of total methanol extract of leaves, terpene fraction and methanol extract of roots at the dose of 400 mg/kg showed significant reduction ($p<0.001$) in eosinophil count induced by milk.

The carrageenan-induced paw oedema is commonly used as an *in-vivo* experimental model of acute inflammation. In the present study, an attempt has been made to evaluate the anti-inflammatory activity of *L. nepetaefolia* by use of the carrageenan-induced paw oedema model. The acute hind paw edema was produced by injecting 0.1 ml of carrageenan (freshly prepared as 1% suspension) locally into the plantar aponeurosis of the right hind paw of rats. Test extracts/fractions were administered for 7 days and 1 h prior to the injection of carrageenan. The results indicate ether sub-fraction of TMLNL (200 mg/kg) shows significant ($p<0.05$) inhibition 16.37% at 1 h and 39.31% at 4 h. Total methanol extract of root (200 mg/kg) produced significant ($p<0.05$) inhibition of 40.45% at 4 h. Maximum inhibition was observed with terpene rich fraction of root (200 mg/kg) 37.87% at 3 h and 59.78% at 4 h. Terpene rich fraction of root

(400 mg/kg) produced significant ($p<0.05$) 24.84% inhibition at 2 h and 45.28% inhibition of oedema at 4 h. This indicates non polar phytoconstituents of the plant have actions on early phase mainly by inhibiting histamine, which is abundantly present in the pro-inflammatory cells like neutrophils and mast cells.

Histamine is a potent bronchoconstrictor stimulant, which stimulates histamine H_1 receptors on airway smooth muscle, leading to direct bronchoconstriction. Fractions/extracts that were found most active were evaluated by *in-vivo* methods of histamine induced bronchospasm in guinea pigs. Experimental bronchial asthma was induced in guinea-pigs by exposing them to 0.25% histamine hydrochloride under constant pressure. Guinea-pigs were administered with the test extracts (100 and 200 mg/kg alkaloid fraction of LNL, terpene fraction of LNR in 10% tween80 in distilled water, p.o.) once daily for 7 days. On day 7, 2 h after the administration of last dose of test extract, the onset of convulsions was recorded and compared with day 1. Terpene fraction of LNR (200 mg/kg) significantly delayed (36.59%, $p<0.01$) the onset of convulsions in guinea pigs caused by acute bronchospasm induced by histamine aerosols. It was more effective with pretreatment of fractions for 7 days in this model. These observations substantiate its protective effect against bronchoconstriction. Thus it can be concluded that these studies corroborate the folklore reports of the preventive effects of *L. nepetaefolia* in bronchial asthma, bronchitis and other respiratory disorders.

Isoproterenol (ISO)-induced myocardial necrosis is a well established model of MI in rats. The activities and capacities of antioxidant systems of heart declined following ISO challenge leading to the gradual loss of prooxidant/ antioxidant balance which accumulates into oxidative damage of cardiac myocyte. As a result of this, cytosolic enzymes such as lactate dehydrogenase (LDH), transaminases (ALT, AST) and creatine phosphokinase (CPK) were released into blood stream and serve as the diagnostic markers of myocardial tissue damage. Drug from natural origin, such as naringin, silibinin and squalene on treatment evidenced by a decline in lactate dehydrogenase, glutamic oxalacetic transaminase and creatine kinase levels indicated their membrane stabilizing action. Therefore, the present study evaluated the role of *O. corniculata* extracts and OC-01 in combating ISO associated macromolecular damage in the myocardium of MI rats. Amongst various proposed mechanisms of ISO causing MI, generation of redundant free radicals is one of the main causative factors. Animals were treated with extracts/fractions (200 and 400 mg/kg/day) orally for a period of 21 days along with ISO (85mg/kg, s.c., at 24h interval) on 20th

and 21st day. Protective effect was evaluated by estimation of serum enzymes like lactate dehydrogenase (LDH), creatine phosphokinase (CPK), aspartate transaminase (AST) and alanine transaminase (ALT), malondialdehyde (MDA) and reduced glutathione (GSH). The activities of catalase and superoxide dismutase (SOD) were also assessed. Histological evaluation was performed on lower portion of the heart. Myocardial necrosis was performed by using triphenyltetrazoliumchloride (TTC) dye. In the rats pretreated with compound OC-01, ethyl acetate fraction of hydroalcoholic extract (EAFTMOC- 400 mg/kg), n-butanol fraction of hydroalcoholic extract (OCFF-400 mg/kg)) and total aqueous extract (TAOC-400 mg/kg) of *O. corniculata* fractions, a significant reduction ($p<0.01$) in the levels of serum transaminase enzymes (ALT, AST) and serum inflammatory markers (CK, LDH) compared with the ISO-administered rats (Group 2) was observed. Lipid peroxidation is an important pathogenic event in myocardial necrosis. Malondialdehyde (MDA) is a major lipid peroxidant end product; the increased level of MDA indicates activation of the lipid peroxidative process, resulting in irreversible damage to hearts of animals subjected to isoproterenol stress. In present study, n-butanol fraction and OC-01 significantly decreased MDA contents near to normal levels and also prevented the isoproterenol-induced lipid peroxidation. The activities of enzymes SOD, CAT and GSH were decreased significantly ($P<0.001$) in ISO treated rats when compared to those of control rats. The activities of antioxidant enzymes were maintained near to normal levels in animals pretreated with compound OC-01, ethyl acetate fraction of hydroalcoholic extract (EAFTMOC- 400 mg/kg), n-butanol fraction of hydroalcoholic extract (OCFF-400 mg/kg)) and total aqueous extract (TAOC-400 mg/kg) of *O. corniculata* as compared to ISO treated animals ($p<0.001$). In all the parameters studied, *O. corniculata* extract at a dose of 200 mg/kg showed a minor effect, whereas dose of 400 mg/kg showed a more significant effect ($p<0.001$). In histopathological examination animals pretreated with *O. corniculata* extract revealed much less intensity of thrombus formation, contraction band necrosis and inflammation. Histopathological findings and TTC stain study of the heart pretreated with OCFF and OC-01 present a well preserved normal morphology of cardiac muscle with no evidence of necrosis when compared to ISO-control heart. To conclude, the present results endorse our hypothesis that n-butanol fraction of *O. corniculata* has cardioprotective potential. OCFF pretreatment improved cardiac functions, the effect which can be attributed to presence of compounds like swertisin, carotenoids, flavone glycosides, vitamin C and many antioxidants that has ability of maintaining redox status which is

disturbed by ISO challenge, via restoration of endogenous antioxidants, controlling lipid peroxide formation and preserving activities of CK-MB, LDH enzymes. Preservation of histoarchitectural of myocyte by OCFF pretreatment reconfirms these effects.

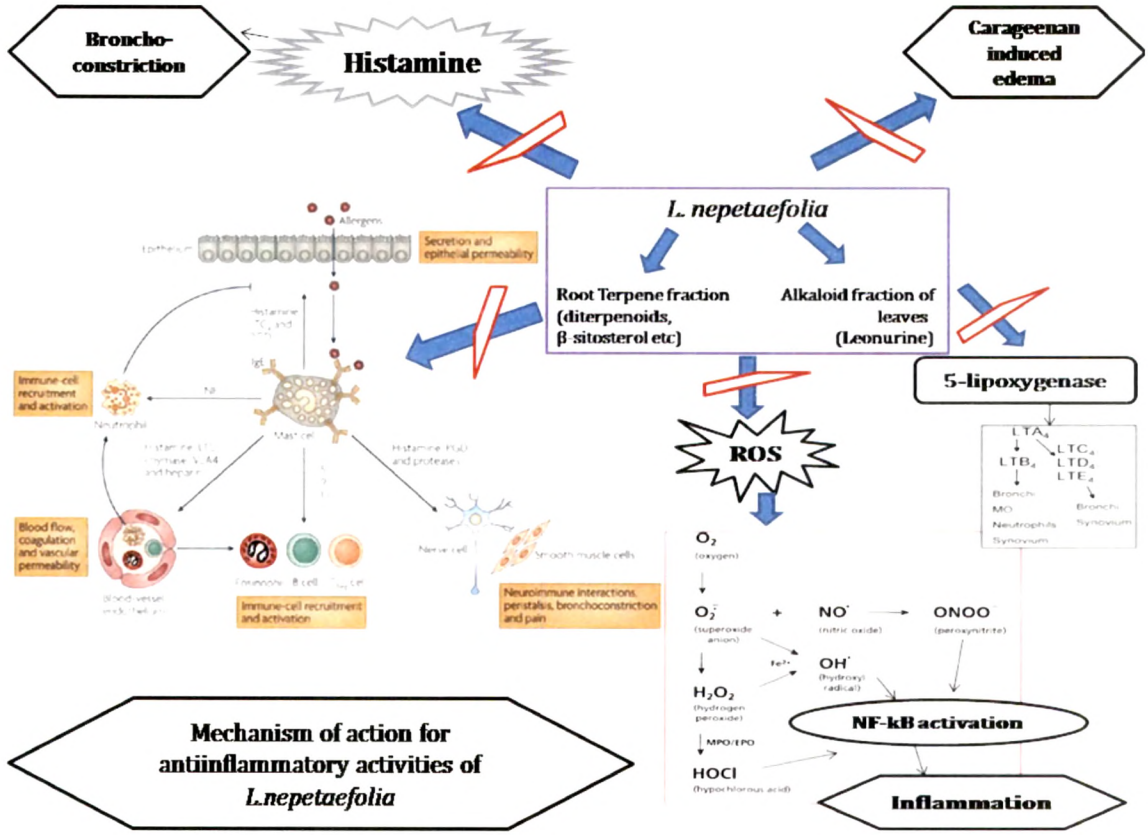

Figure 5.2: Antiinflammatory activity of *L. nepetaefolia;* ▱: indicates inhibition of activity; various pathways responsible for antiinflammatory activity of LN.

In conclusions, assessment of therapeutic claims of the selected plants led to the identification of chemical entity responsible for their medicinal value. The study also provides methods of analysis of phytoconstituents to standardize plant products with justification of their traditional use. Leonurine is found to be present in leaves of *L. nepetaefolia* with strong lipoxygenase inhibition activity (IC50 11.91±2.052 µg/mL), however, it was not found to be significantly effective in other *in-vitro* antioxidant assays, ROS inhibition assay (350.28±17.6707 µg/mL). The compound showed mild effect on inhibition of mast cell degranulation, inhibiting milk-

induced leucocytosis, eosinophil count and reduction in carrageenan induced paw edema. The terpene fraction of root of *L. nepetaefolia* was found to be most potent against all models of acute and chronic inflammation. The other compound, found to be present in leaf and root fractions, was β-sitosterol with mild effect as antiinflammatory agent. The present study indicates non polar phytoconstituents of the plant have actions on airway inflammation mainly by inhibiting histamine, which is abundantly present in the pro-inflammatory cells like neutrophils and mast cells. These observations substantiate its protective effect against bronchoconstriction. Thus it can be concluded that these studies corroborate the folklore reports of the preventive effects of *L. nepetaefolia* in bronchial asthma, bronchitis and other respiratory disorders.

Oxalis corniculata is known to contain ascorbic acid, carotene and oxalic acid. It has also been found fatally poisonous due to high amount of oxalis acid. Therefore, in our study we found plant rich in flavonoids and had potential antioxidant activity. Swertisin was identified and isolated from bioactive flavonoids rich fraction of *O. corniculata.* It was quantified in *O. corniculata* fractions and found to be 0.241% w/w by HPTLC and 0.187%w/w by HPLC. Various fractions were evaluated for its antioxidant activity and protective effect on heart. It was observed that flavonoids rich fraction and swertisin had significant protective effect in isoproterenol induced myocardial infarction. Thus it can be concluded that these studies corroborate the traditional reports of the preventive effects of *O. corniculata* in oxidative stress and ischaemic heart diseases.

The present study concludes that both the plants can be used in the treatment of ROS generated disorders which confirms their traditional claim. The phytochemical and analytical studies offer a better tool for quality control of crude plant material and its formulations. Both the plants offer clinical use in their respective indications and active constituents from this plants offer new lead for drug development.