INTRODUCTION

1.1. Complementary and Alternative Medicine (CAM)

Traditional medicines/Complementary and Alternative Medicines (TM/CAM), including herbal medicines, have been, and continue to be, used in every country around the world in some capacity from time to time (Figure 1.1). 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 [1].

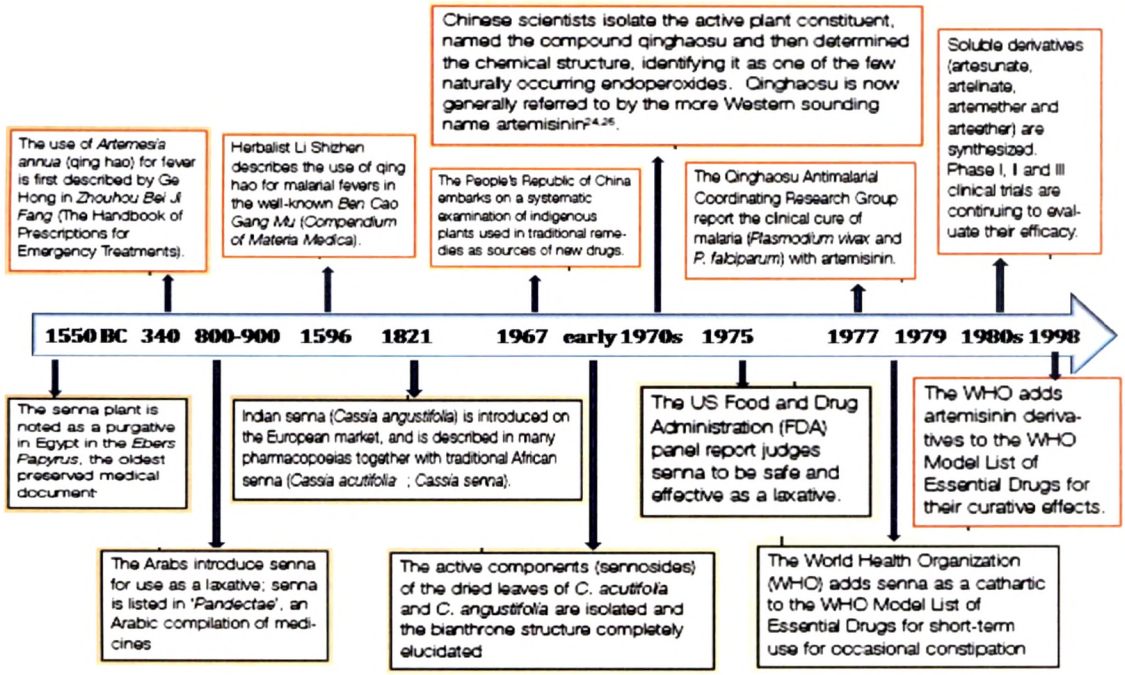

Figure 1.1: From ancient tradition to modern drug discovery: artimesinine, its derivatives and senna [2]

More and more individuals are looking outside the borders of conventional medicine for at least part of their health care needs [2]. 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 [3].

The term ''complementary and alternative medicine'' (CAM) was defined more than a decade ago as ''interventions neither taught widely in medical schools nor generally available in

mainstream hospitals.''[2] CAM is now considered a field of study, which should be defined by inclusion according to its unique characteristics.

CAM may be referred as: "additional to western medicine," "lies outside official health sector," "practices of unregistered (non- licensed) practitioners," "neither taught widely in global medical schools nor generally available in world's hospitals." CAM is a broad range of healing philosophies, approaches and therapies. The term "complementary" suggests in addition to conventional, "alternative," in lieu of conventional and "integrative," a holistic approach. "Conventional" is that which is widely accepted and practiced by the mainstream medical community. It is not necessarily more scientific or better studied. Scientific or not, patients are not getting something important from their physicians. Teenagers surveyed in a University of Bath study viewed scientists as: "dangerous cranks and geeks." The personal, "hands-on" approach is fading. CAM practitioners may be sensitive and empathetic to patients' non-scientific needs [4].

CAM approaches include: homeopathy, acupuncture, chiropractic, traditional chinese medicine (TCM), naturopathic, spiritual, energy healing, prayer, meditation, and others. These "disciplines" are not well defined and are generally based on shaky scientific data but studies do support benefit of some homeopathic remedies, acupuncture and herbal medicines. CAM approaches rarely employ "single bullet" therapy to treat disease. The patient is involved with healing. An herb may be given for synergistic benefit of all its components and other complementary therapies. The philosophies on how therapies work, likewise, vary. Homeopathic therapies are thought to work based on small, or even negligible, doses of toxins. "Energy" therapies are thought to work by mental or conscious energy (Reiki, Jorhei), mind-body effects, placebo effects, and no known mechanism. CAM may provide benefit simply by empowering the patient to actively pursue treatment. This optimism may provide a "placebo effect" [5].

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 [6].

In the 21st century, there is an ongoing effort to integrate complementary and alternative medicine into conventional medicine practice (Integrative Medicine). In 1998, the National Institutes of Health, recognizing the need to vigorously evaluate CAM therapies, created the National Center for Complementary and Alternative Medicine (NCCAM), which supports ongoing scientific research and educational programs [7, 8].

The National Center for Complementary and Alternative Medicine in the USA defines CAM as follows: CAM is a group of diverse medical and health care systems, practices, and products that are not presently considered to be part of conventional medicine. Alternative medicine often refers to the use of therapies as substitutes for biomedical treatment, and complementary medicine refers to the use of such therapies in conjunction with biomedicine. Integrative medicine combines treatments from conventional medicine and CAM for which there is some high-quality evidence of safety and effectiveness. It is also called integrated medicine.

The NCCAM's categorization of CAM is as follows [6]:

• **Whole Medical Systems**: Whole medical systems are built upon complete systems of theory and practice. Often, these systems have evolved apart from and earlier than the conventional medical approaches e.g. Ayurveda, Anthroposophic medicine, Homeopathy, Naturopathic medicine, Traditional Chinese medicine etc.

• **Mind Body Medicine**: Mind-body medicine uses a variety of techniques designed to enhance the mind's capacity to affect bodily function and symptoms. Some techniques that were considered CAM in the past have become mainstream (for example, patient support groups and cognitive-behavioral therapy). Other mind-body techniques are still considered CAM, including meditation, prayer, mental healing, and therapies that use creative outlets such as art, music, or dance.

• **Biologically-Based Practices**: Biologically based therapies in CAM use substances found in nature, such as herbs, foods, and vitamins. Some examples include dietary supplements, herbal products, and the use of other so-called natural but as yet scientifically unproven therapies (for example, using shark cartilage to treat cancer).

• **Manipulative and Body-Based Practices**: Manipulative and body-based practices in CAM are based on manipulation and/or movement of one or more parts of the body. Some examples include chiropractic or osteopathic manipulation and massage.

• **Energy Medicine**: Energy therapies involve the use of energy fields. They are of two types:

A) **Biofield therapies** are intended to affect energy fields that purportedly surround and penetrate the human body. The existence of such fields has not yet been scientifically proven. Some forms of energy therapy manipulate biofields by applying pressure and/or manipulating the body by placing the hands in, or through, these fields. Examples include Qigong, Reiki, and Therapeutic Touch.

B) **Bioelectromagnetic-based therapies** involve the unconventional use of electromagnetic fields, such as pulsed fields, magnetic fields, or alternating-current or direct-current fields.

Table 1.1: Commonly used TM/CAM therapies and therapeutic techniques [11]

	Ayurveda	Chinese medicine	Unani	Naturopathy	Osteopathy	Homeopathy	Chiropractic	Others
Herbal medicines	•	•	•	•	■	•	-	•
Acupuncture/ acupressure	-	•	-	-	■	-	-	■
Manual therapies	•	Tuina	•	■	•	-	•	Shiatsu
Spiritual therapies	•	•	•	•	-	-	-	Hypnosis, healing, meditation
Exercise	Yoga	Qigong		Relaxation	-	-	-	-

• commonly used therapeutic technique

■ sometimes used therapeutic technique

1.2. The upsurge of CAM

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 (Table 1.1) and more likely to be used by those with chronic diseases [9-11]. 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.

1.2.1. An alternative approach to health care in developed countries

In many developed countries popular use of CAM is fuelled by concern about the adverse effects of chemical drugs, questioning of the approaches and assumptions of allopathic medicine, and greater public access to health information [12]. At the same time, longer life expectancy has

brought with it increased risks of developing chronic, debilitating diseases such as heart disease, cancer, diabetes and mental disorders [13]. Approximately 30-50% of the European population use CAM as self-support and 10-20% of the European population has seen a CAM physician/practitioner within the previous year. The percentage of the population which has used CAM at least once is 48% in Australia, 70% in Canada, 42% in USA, 31% in Belgium and 49% in France [14].

1.2.2. Accessible and affordable in developing countries

In developing countries, broad use of CAM is often attributable to its accessibility and affordability. In Africa up to 90% of the population uses CAM to help meet their health care needs [14].

In Asia and Latin America, populations continue to use CAM as a result of historical circumstances and cultural beliefs. In China, Traditional Chinese Medicine (TCM) accounts for around 40% of all health care delivered. Millions of Indians use herbal drugs regularly, as spices, home-remedies, health foods as well as over-the-counter (OTC) as self-medication or also as drugs prescribed in the non-allopathic systems [3].

1.3. Challenges in developing CAM as therapeutic system

Complementary and alternative medicine (CAM) use is on the rise. Countries face major challenges in the development and implementation of the regulation of TM/CAM and herbal medicines. These challenges are related to doctor-patient communication, lack of knowledge about TM/CAM within national drug regulatory authorities, regulatory status, assessment of safety and efficacy, quality control, safety monitoring, drug/herb and herb/herb interactions [15-17].

1.3.1. Doctor-Patient communication

More individuals now visit non-standard practitioners rather than primary care physicians. Unconventional medicine approaches are now being used by over 1/3rd of Americans [18]. The popularity of CAM has affected doctor-patient communication [19]. Robinson and McGrail [20], in a review of qualitative and quantitative studies, determined that up to 77% of patients do not disclose their CAM use to medical practitioners. The main reasons given for non-disclosure were concerns about a negative response from practitioners, the belief that practitioners do not need to know about their CAM use, and that practitioners did not ask.

1.3.2. Lack of knowledge about herbal medicines within national drug authorities. The general lack of knowledge about herbal medicines within national drug authorities and the lack of appropriate evaluation methods are factors that delay the creation or updating of national policies, laws and regulations for traditional medicines, contemporary/alternative medicines and herbal medicines [17].

1.3.3. Challenges related to the regulatory status of herbal medicines: A single medicinal plant may be defined as a food, a functional food, a dietary supplement or a herbal medicine in different countries, depending on the regulations applying to foods and medicines in each country. This makes it difficult to define the concept of herbal medicines for the purposes of national drug regulation, and also confuses patients and consumers [17].

1.3.4. Challenges related to the assessment of safety and efficacy: Requirements and methods for research and evaluation of the safety and efficacy of herbal medicines are more complex than those for conventional pharmaceuticals. A single medicinal plant may contain hundreds of natural constituents, and a mixed herbal medicinal product may contain several times that number. Such an analysis may actually be impossible in practice, particularly in the case of mixed herbal medicines [17].

1.3.5. Challenges related to quality control of herbal medicines: The safety and efficacy of herbal medicines is closely correlated with the quality of the source materials used in their production [17]. Good Manufacturing Practice (GMP) specifies many requirements for quality control of starting materials, including correct identification of species of medicinal plants, special storage and special sanitation and cleaning methods for various materials.

1.3.6. Challenges related to safety monitoring of herbal medicines: Adverse events arising from consumption of herbal medicines may be due to any one of a number of factors. These include the use of the wrong species of plant by mistake, adulteration of herbal products with other, undeclared medicines, contamination with toxic or hazardous substances, overdosage, misuse of herbal medicines by either healthcare providers or consumers and use of herbal medicines concomitantly with other medicines [17].

1.3.7. Drug/Herb and Herb/Herb Interactions

Any information available regarding herbs and supplements is likely to be questionable, unreliable, incorrect, inaccurate or unavailable. Supplemental therapies can: (1) Conflict with prescribed drugs (Ma Huang and beta-blockers), (2) Have synergistic effect, (gingko and

warfarin), (3) Have additive effect (St. John's Wort and serotonin reuptake inhibitors), (4) Change metabolism of drugs (St. John's Wort/cyclosporine), or be totally ineffective. Concerns include: abandonment of standard approaches, herb-drug interactions, and lack of herbal standardization for content. There is no industry policy. Other issues include potential for toxic adulterants (heavy metals in Chinese herbs), direct/indirect dose-dependent, toxic effects (chaparral, pennyroyal, oleander), and interference with drug absorption (aloe vera) and metabolism (grapefruit pulp/felodipine) [21].

"Dietary supplements" were defined in 1994 with the passage by the United States Congress of the Dietary Supplement Health and Education Act (DSHEA, Public Law 103-417, October 25, 1994) which established dietary supplement standards. This act ultimately opened the door to continued use of unproven supplements. The DSHEA defined "dietary supplement" as (1) A product (other than tobacco) intended to supplement the diet that contains at least one of the following: a vitamin, mineral, amino acid or herb (or other botanical); (2) A dietary substance for use to supplement the diet by increasing the total dietary intake; (3) A concentrate, metabolite, constituent, extract, or combination of any ingredient described above. Dietary supplements are available widely through health food stores, grocery stores, and pharmacies [22].

St. John's Wort can influence drug metabolism by its effects on P-glycoprotein MDRL expression and CYP3A2 activity. Therefore, there is a possibility for an interaction with cyclosporine interaction and HIV protease inhibitors [23]. Licorice, similar to aldosterone, can lower serum potassium. A metabolite of licorice, 3-monoglucuronyl-glycyrrhetinic acid, a potent inhibitor of 11-beta hydroxysteroid dehydrogenase, prevents hydrocortisone breakdown and acts as a mineralocorticoid. Licorice also can increase interferon levels and can act as a monoamine oxidase inhibitor. Licorice has been associated with torsades de pointes [24].

There are multiple components in ginkgo bilobydes (flavonoids, terpenoids, and organic acids) each with different potential affect. Gingkolyde C inhibits platelet activating factor. It may act as an antioxidant (prevents cell damage, lipid peroxidation) and a vasodilator [25, 26]. Until more data is available, it should be avoided with warfarin and nonsteroidal anti-inflammatory drugs. Supplements have the potential for a hypertensive effect, (Kola Nut, yohimbine, ephedra [i.e., Ma Huang], licorice, goldenseal, and ginseng) or a sympathetic or inotropic effect (Ma Huang, carnitine, coenzyme Q10, hawthorn berry, guanara, motherwort [negative inotrope], Foti, panax ginseng) [27].

1.4. Framework for action

1.4.1. Evidence-based Research in CAM in the Next Decades

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 next decades will witness an increasing number of evidence-based research directed at establishing the best available evidence in CAM [28, 29]. Evidence-based medicine was first conceived by Archibald Cochrane as a new perspective on medical intervention that must not to be confused with medicine based on research evidence. Evidence-based research is a research movement in the medical sciences that is based upon the application of the scientific method for the conscientious, explicit and judicious use of current best evidence, evaluated by a systematic process, in making decisions about the care of individual patients. By contrast, medicine based on the evidence is the traditional approach to medical treatment, which rests on long-established existing medical traditions, supplemented by individual pieces of research, which may or may not have undergone adequate or sufficient scientific scrutiny [30]. Figure 1.2 shows that evidence exists for herbal products but too often evaluation is inadequate.

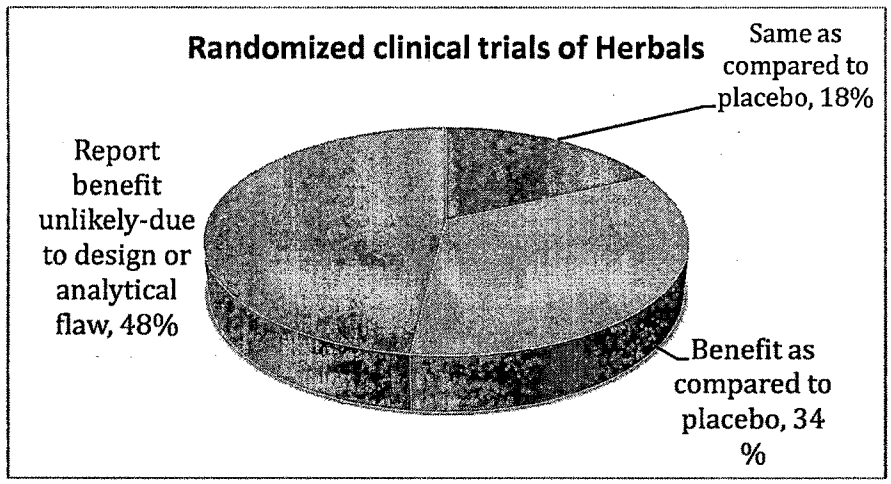

Figure 1.2: Evidence exists for some herbal medicines- but too often evaluation is inadequate. % of randomized clinical trials (RCTs) showing benefit of herbal medicines (based on 50 RCTs and 10 herbal medicines for 18 therapeutic indications) [31]

Clinical and translational CAM research in the 21st century will rely upon an evidence-based model of systematic evaluation of the research evidence. It can be proposed that progress in evidence-based CAM will actualize along several dimensions as shown in figure 1.3. For example, 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 stringency of the tools utilized to evaluate the scientific literature determines the validity of this systematic evaluation. The process of dissemination of evidence-based evaluative outcomes of CAM research will also need to be improved. The integration of CAM into conventional medicine depends as much on the fundamental research that demonstrates its clinical effectiveness as on the practical, contextual and intelligible nature of its dissemination. The integration of CAM in every-day medical decision-making and treatment will require a concerted effort to expand and to deepen education about CAM as well as about the systematic and critical process of evidence-based research [32].

1.4.2. Translational Research in TM/CAM

The American Physiological Society (APS) has defined translational research as 'the transfer of knowledge gained from basic research to new and improved methods of preventing, diagnosing, or treating disease, as well as the transfer of clinical insights into hypotheses that can be tested and validated in the basic research laboratory.' It is a two way process of iterative learning from experimentation [33].

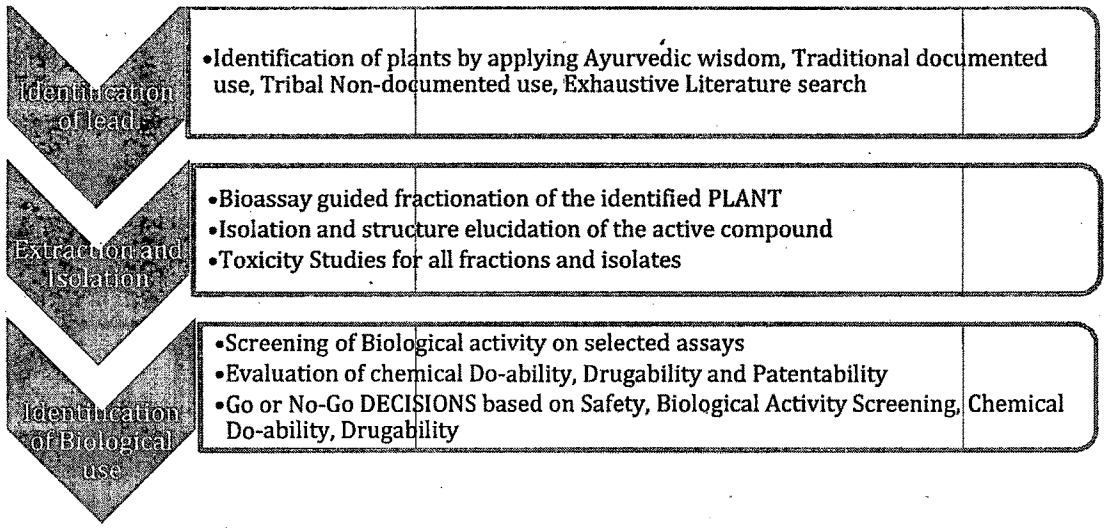

Figure 1.3: The Approach: Research design for the evaluation of natural product

Translational research can enhance many aspects of the pharmaceutical business. The efficient use of predictive technology and new techniques could ensure the timely removal of poor compounds and facilitate the identification and acceleration of good compounds that fulfils a medical need, as well as those that are based on a better clinical profile that will deliver the label the clinician and patient needs. In this way, translational research will bring increased confidence in the rationale supporting the mechanistic approaches at a much earlier point in the research and development process. Increased confidence in the mechanism of an unprecedented approach, coupled with an earlier ability to determine whether it can be differentiated from existing therapies, together with the ability to construct pharmacokinetic-pharmacodynamic models, will allow more precise dose selection for Phase IIb. It is reasonable to assume that these strategies will improve drug candidate survival and overall productivity of the drug development process.

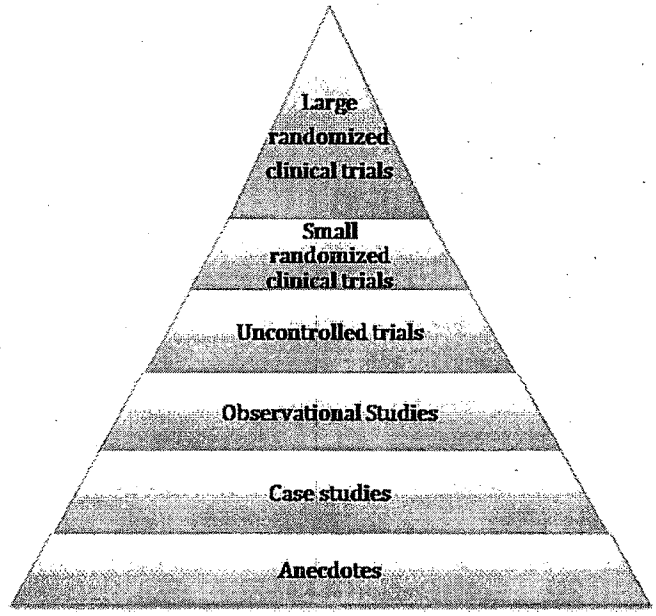

Figure 1.4: A hierarchy of evidence-Translational research approach

The integration of this bi-directional thought process can be extremely powerful. An example from bench to bedside includes xenograft testing for oncology; and in bedside to bench clinical experience an observation that has been translated back into a legitimate drug target and discovery effort, is the case of sildenafil citrate. So, effective translational research in drug development implies a required two-way dialogue and interaction between scientists in preclinical drug discovery and clinical developers, who understand the disease and its symptomatology, to take novel drugs and biologicals to volunteer and patient trials.

With the application of translational research to exploratory drug development, it is often necessary to implement different early phase clinical trial designs to validate new biological end-points [34].

To maximize the potential of CAM as a source of health care, other issues should also be settled. They relate to: policy; safety, efficacy and quality; access; and rational use.

1.4.3. Regulatory Policy: basis of sound action in CAM

Relatively few countries have developed a policy on TM/CAM e.g. only 25 of WHO's 191 Member States.

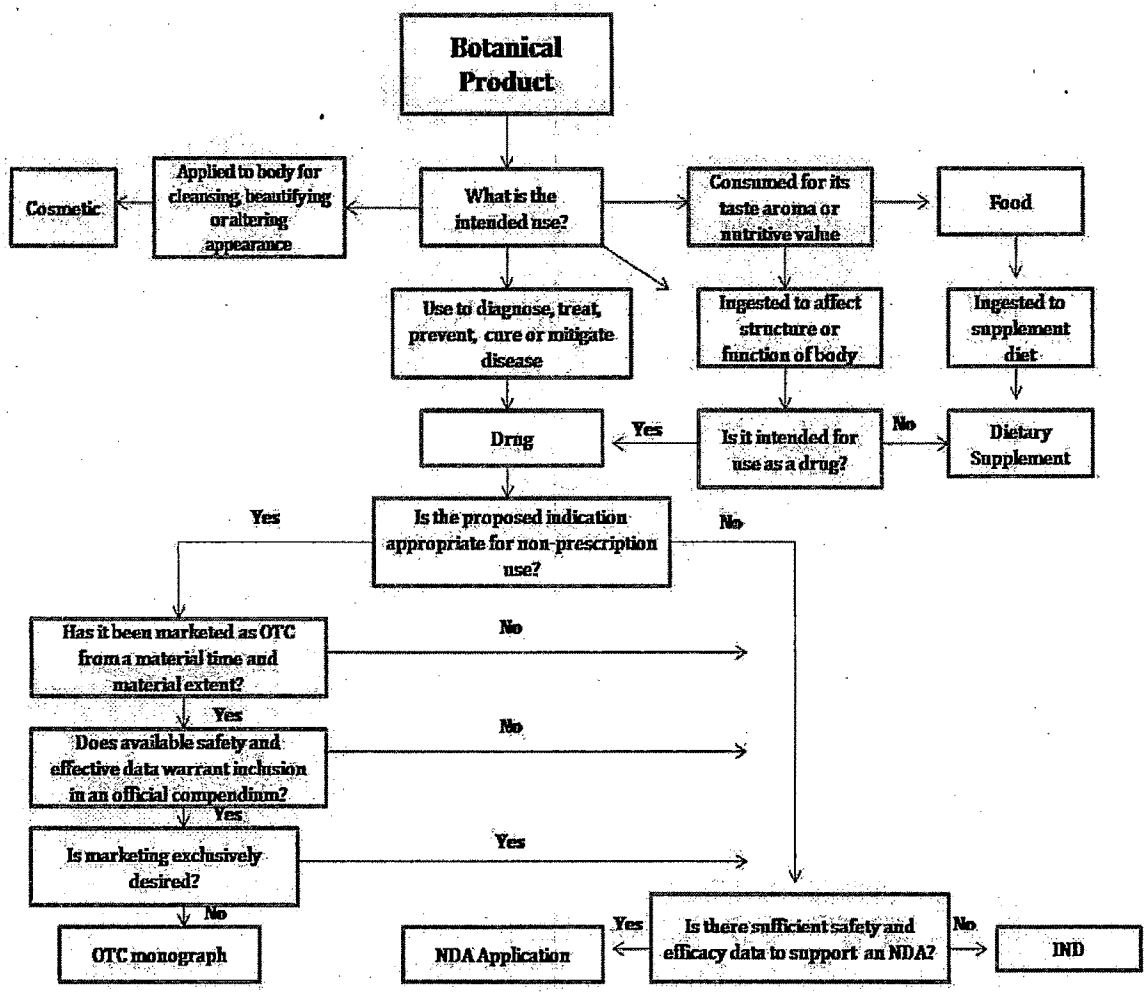

Figure 1.5: Regulatory approaches for a botanical product. OTC-over the counter, IND- investigational new drug, NDA-new drug application [35]

Yet such a policy provides a sound basis for defining the role of TM/CAM in national health care delivery, ensuring that the necessary regulatory and legal mechanisms are created for

promoting and maintaining good practice, that access is equitable, and that the authenticity, safety and efficacy of therapies are assured. It can also help to ensure sufficient provision of financial resources for research, education and training [36].

In fact, many developed countries are now seeing that CAM issues concerning safety and quality, licensing of providers and standards of training, and priorities for research, can best be settled within a national policy framework (Figure 1.5). The need for a national policy is most urgent, however, in those developing countries where TM has not yet been integrated into the national health care system, even though much of their population depends on TM for health care. An increased number of national policies would have the added benefit of facilitating work on global issues such as development and implementation of internationally accepted norms and standards for research into safety and efficacy of TM/CAM, sustainable use of medicinal plants, and protection and equitable use of the knowledge of indigenous and traditional medicine [37].

1.4.4. Safety, efficacy and quality aspects of TM/CAM 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, the effectiveness and quality of which can be influenced by numerous factors. Unsurprisingly, 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 [38].

Table 1.2: Key needs in ensuring the safety, efficacy and quality of TM/CAM

At national level	• National regulation and registration of herbal medicines. • Safety monitoring for herbal medicines and other TM/CAM. • Support for clinical research into use of TM/CAM for treating country's common health problems. • National standards, technical guidelines and methodology, for evaluating safety, efficacy and quality of TM/CAM. • National pharmacopoeia and monographs of medicinal plants.
At global level	• Access to existing knowledge of TM/CAM through exchange of accurate information and networking. • Shared results of research into use of TM/CAM for treating common diseases and health conditions. • Evidence-base on safety, efficacy and quality of TM/CAM products and therapies.

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. If TM/CAM is to be promoted as a source of health care, efforts to promote its rational use and identification of the safest and most effective therapies will be crucial [39].

Also, if access is to be increased substantially, the natural resource base upon which certain products and therapies depends must be protected. Raw materials for herbal medicines, for instance, are sometimes over-harvested from wild plant populations [40].

Another major challenge concerns intellectual property and patent rights. The economic benefits that can accrue from large-scale application of TM knowledge can be substantial.

Questions about how best these benefits can be shared between innovators and the holders of TM knowledge have not yet been resolved though.

Table 1.3: Framework for inclusion of TM/CAM as therapeutic system

Policy	Integrate TM/CAM with national health care systems, as appropriate, by developing and implementing national TM/CAM policies and programmes.
Safety, efficacy and quality	Promote the safety, efficacy and quality of TM/CAM by expanding the knowledgebase on TM/CAM, and by providing guidance on regulatory and quality assurance standards.
Access	Increase the availability and affordability of TM/CAM, as appropriate, with an emphasis on access for poor populations.
Rational use	Promote therapeutically sound use of appropriate TM/CAM by providers and consumers. Implementation of the strategy will initially focus on the first two objectives. Achieving the safety, efficacy and quality objective will provide the necessary foundation for achieving the access and rational use objectives.

1.4.5. Rational use: ensuring appropriateness and cost-effectiveness

Rational use of TM/CAM has many aspects, including qualification and licensing of providers, proper use of products of assured quality. Proper use of products of assured quality could also do much to reduce risks associated with TM/CAM products such as herbal medicines. However, regulation and registration of herbal medicines are not well developed in most countries, and the quality of herbal products sold is generally not guaranteed [41].

More work is also needed to raise awareness of when use of TM/CAM is appropriate (and cost-effective) and when it is not advised, and why care should be taken when using TM/CAM products.

The opportunities presented by safe and effective CAM therapeutics are many. Good controlled data are hard to find. The motivation for research from supplement manufacturers is virtually absent. Herbs are non-proprietary and there is little desire on their part for further study.

Among our responsibilities as health care providers are to promote safe and effective therapies, educate the patient, communicate and manage illnesses, protect against toxic therapies, and permit safe therapies for chronic diseases. Patients are taking their health into their own hands. Until carefully controlled trials support, or refute, the use of such supplements and other approaches, those of us prescribing standard approaches cannot be fully capable of providing sound advice to our patients.

Physicians must be aware of CAM practices so that they can best counsel their patients in an atmosphere of open communication. Rather than dismissing a patient's highly motivated intentions toward health conscious behaviors, it behooves the physician to understand the range of CAM treatments and when they might be safely integrated with conventional medicine.

It should be noted that medical doctors increase their attempts to ask their patients about their use of CAM.

Despite the lack of scientific rigor in previous studies of CAM therapies, the NCCAM, a part of the NIH, is now actively coordinating clinical trials, advancing scientific research, and training researchers to study CAM. Ultimately it will be the fusion of the best medical practices from those which are rigorously studied in clinical trials that will provide the most favorable clinical outcomes in medicine.

1.5. Antioxidants in Herbals

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 chemicals before they damage tissue may prevent or slow each of these processes. However, there is limited scientific data to support such a conclusion [42], and the majority of the reports proclaiming benefits of herbs are based on testimonials, case reports, and unsubstantiated claims. There are, however, some reports of clinical trials where herbs have been effective in treating inflammatory and ROS mediated disorders. The present study was planned on the usage of plant of CAM in ROS-mediated inflammatory disorder (Asthma) and ischaemic heart disease.

1.5.1. Inflammation and mechanism of damage by ROS

Inflammation is the initial response of the body to tissue damage caused by mechanical, chemical, or microbial stimuli. Cytokines are the physiological messengers of the inflammatory response and some of the principal molecules involved are tumor necrosis factor-alpha (TNF-α), interleukins (IL-1 and IL-6), interferons, and colony stimulating factors (CSFs) [43]. The main cells involved in the inflammatory response are monocytes/macrophages, polymorphonuclear leucocytes (PMNs), and endothelial cells. When these cells become activated, they aggregate and infiltrate tissue where they undergo a respiratory burst, increasing their oxygen use and production of cytokines, ROS, and other mediators of inflammation. ROS can initiate and also perpetuate inflammatory cascades and cause subsequent tissue damage.

1.5.2. Role of inflammatory mediators and ROS in the pathogenesis of asthma

Asthma is an inflammatory disease of the lungs characterized by reversible (in most cases) airway obstruction. Asthma has many prominent features including the infiltration of eosinophils, neutrophil accumulation in the airway, and the shedding of epithelial cells in the airway [44]. Various inflammatory mediators, including reactive oxygen species, are released from activated leukocytes (neutrophils, eosinophils, macrophages) and play a central role in the pathogenesis of asthma.

1.5.3. Role of ROS in the pathogenesis of atherosclerosis and myocardial infarction

Oxidative modification of low-density lipoprotein (LDL) appears to play a key role in atherogenesis [45]. Infiltration of LDL and monocytes into the arterial intima play a primary role in the development of atherosclerosis. Monocytes transform into macrophages, and when LDL becomes oxidized, it is more rapidly taken up into macrophages to form foam cells and fatty streaks which eventually lead to fibrous plaque formation and ischaemic diseases [46]. Oxidized LDL has been shown to be chemotactic for circulating monocytes while inhibiting the mobility of resident macrophages [47].

1.6. Asthma

Asthma, one of the most important chronic diseases, remains a genuine medical mystery. With every new piece of the puzzle, the notion of asthma as one unifying disease concept is disappearing further into the realm of historical oversimplification. From time to time it was felt to abandon asthma as a disease concept. This concept is now more justified than ever. Asthma is at best a syndrome with different risk factors, different prognoses, and different responses to

treatment. Without better understanding of the underlying differences, targeted treatment efforts with improved outcomes will be incomplete and prevention will remain elusive [48].

Treatment ideally achieves a steady state of no asthma related symptoms and no exacerbations with minimum continuous or intermittent treatment. In practice, although this is achievable in most patients with mild asthma, many patients with more severe disease will not be optimally controlled. Treatment success varies from patient to patient, can change rapidly with exposure to environmental triggers, and depends highly on the correct use of guidelines by primary-care physicians and adherence to treatment by patients. Whether busy primary-care physicians will have the time and expertise for such an approach is questionable. Perhaps the future lies in highly personalised treatment and monitoring, aided by computer-based algorithms. Progress in understanding asthma and its underlying mechanisms is slow; treatment can be difficult and response unpredictable; and prevention or cure is still a pipedream [49].

1.6.1. Pathophysiology

Asthma is a common chronic disorder of the airways that involves a complex interaction of airflow obstruction, bronchial hyperresponsiveness and an underlying inflammation. It is characterized by a specific pattern of inflammation in the airway mucosa, and involves the infiltration of eosinophils, increased numbers of Th_2 cells relative to Th_1 cells, and increased numbers of activated mast cells [50-52]. In addition, there are characteristic structural changes to the airways (termed remodelling), some of which might even precede the development of the disease. These changes include subepithelial fibrosis (basement membrane thickening), airway smooth muscle hypertrophy and hyperplasia, angiogenesis and increased mucus secretory cells (goblet-cell hyperplasia and submucosal-gland hyperplasia) [53, 54]. Neural mechanisms are also important in asthma, such as the sensitization of sensory nerve endings in the airways and reflex effects on airway tone. Asthma is a highly complex disease (Figure 1.6) that involves many inflammatory cells, mediators and inflammatory proteins, and therefore treatments that target a single cell or mediator are unlikely to be effective.

Airflow limitation in asthma is recurrent and caused by a variety of changes in the airway. These include [57-59]:

1.6.1.1. Bronchoconstriction: In asthma, the dominant physiological event leading to clinical symptoms is airway narrowing and a subsequent interference with airflow. In acute

exacerbations of asthma, bronchial smooth muscle contraction (bronchoconstriction) occurs quickly to narrow the airways in response to exposure to a variety of stimuli including allergens or irritants. Allergen-induced acute bronchoconstriction results from an IgE-dependent release of mediators from mast cells that includes histamine, tryptase, leukotrienes, and prostaglandins that directly contract airway smooth muscle [55]. Aspirin and other nonsteroidal anti-inflammatory drugs can also cause acute airflow obstruction in some patients, and evidence indicates that this non-IgE-dependent response also involves mediator release from airway cells [56].

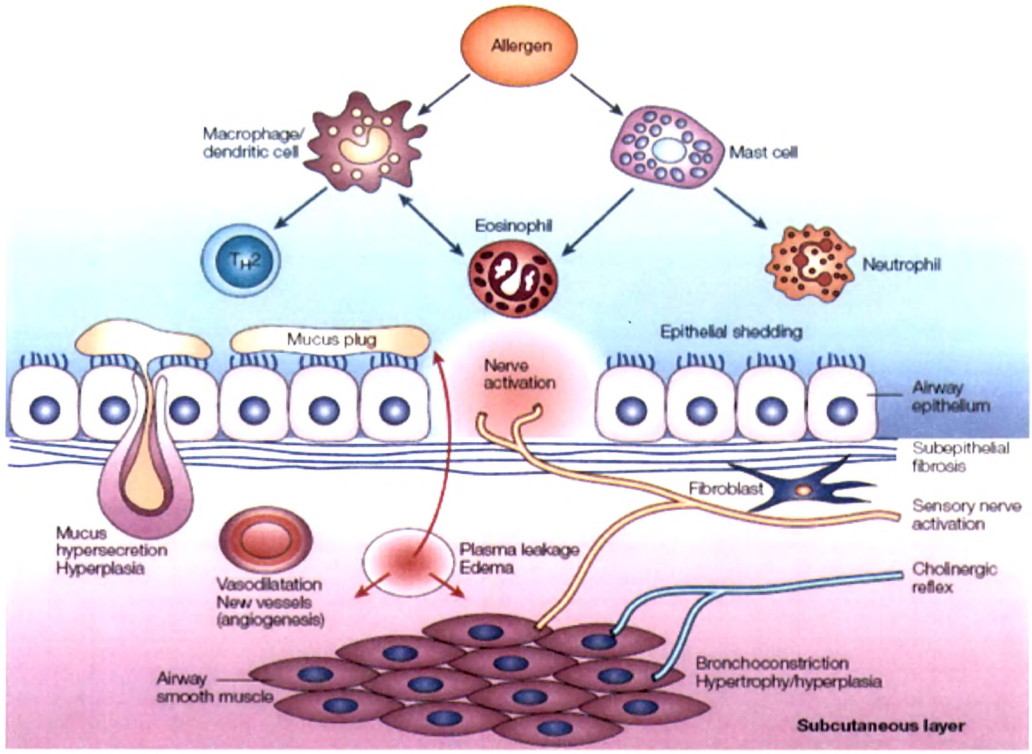

Figure 1.6: The pathophysiology of asthma. Several inflammatory cells are recruited and/or activated in the airways, releasing a variety of inflammatory mediators that have acute effects on the airway (such as bronchoconstriction, plasma leakage, vasodilatation, mucus secretion, sensory nerve activation and cholinergic reflex-induced bronchoconstriction), together with structural changes (remodelling) that include subepithelial fibrosis, increased numbers of blood vessels and mucus-secreting cells, and increased thickness of airway smooth muscle as a result of hyperplasia and hypertrophy [57].

In addition, other stimuli (including exercise, cold air, and irritants) can cause acute airflow obstruction. The mechanisms regulating the airway response to these factors are less well defined, but the intensity of the response appears related to underlying airway inflammation.

Stress may also play a role in precipitating asthma exacerbations. The mechanisms involved have yet to be established and may include enhanced generation of pro-inflammatory cytokines.

1.6.1.2. Airway edema: As the disease becomes more persistent and inflammation more progressive, other factors further limit airflow. These include edema, inflammation, mucus hypersecretion and the formation of inspissated mucus plugs, as well as structural changes including hypertrophy and hyperplasia of the airway smooth muscle. These latter changes may not respond to usual treatment.

1.6.1.3. Airway hyperresponsiveness: Airway hyperresponsiveness, an exaggerated bronchoconstrictor response to a wide variety of stimuli is a major, but not necessarily unique, feature of asthma. The degree to which airway hyperresponsiveness can be defined by contractile responses to challenges with methacholine correlates with the clinical severity of asthma. The mechanisms influencing airway hyperresponsiveness are multiple and include inflammation, dysfunctional neuroregulation, and structural changes; inflammation appears to be a major factor in determining the degree of airway hyperresponsiveness. Treatment directed toward reducing inflammation can reduce airway hyperresponsiveness and improve asthma control.

1.6.1.4. Airway remodeling: In some persons who have asthma, airflow limitation may be only partially reversible. Permanent structural changes can occur in the airway; these are associated with a progressive loss of lung function that is not prevented by or fully reversible by current therapy. Airway remodeling involves an activation of many of the structural cells, with consequent permanent changes in the airway that increase airflow obstruction and airway responsiveness and render the patient less responsive to therapy [60]. These structural changes can include thickening of the sub-basement membrane, subepithelial fibrosis, airway smooth muscle hypertrophy and hyperplasia, blood vessel proliferation and dilation, and mucous gland hyperplasia and hypersecretion. Regulation of the repair and remodeling process is not well established, but both the process of repair and its regulation are likely to be key events in explaining the persistent nature of the disease and limitations to a therapeutic response.

1.6.2. Pathophysiologic mechanisms in the development of airway inflammation

Inflammation has a central role in the pathophysiology of asthma. As noted in the definition of asthma, airway inflammation involves an interaction of many cell types and multiple mediators with the airways that eventually results in the characteristic pathophysiological features of the disease: bronchial inflammation and airflow limitation that result in recurrent episodes of cough,

wheeze, and shortness of breath. The processes by which these interactive events occur and lead to clinical asthma are still under investigation. Moreover, although distinct phenotypes of asthma exist (e.g., intermittent, persistent, exercise-associated, aspirin-sensitive, or severe asthma), airway inflammation remains a consistent pattern. The pattern of airway inflammation in asthma, however, does not necessarily vary depending upon disease severity, persistence, and duration of disease. The cellular profile and the response of the structural cells in asthma are quite consistent.

1.6.3. Inflammatory Cells involved in Asthma

1.6.3.1. Lymphocytes: An increased understanding of the development and regulation of airway inflammation in asthma followed the discovery and description of subpopulations of lymphocytes, T helper 1 cells and T helper 2 cells (Th_1 and Th_2), with distinct inflammatory mediator profiles and effects on airway function. After the discovery of these distinct lymphocyte subpopulations in animal models of allergic inflammation, evidence emerged that, in human asthma, a shift, or predilection, toward the Th_2-cytokine profile resulted in the eosinophilic inflammation characteristic of asthma [61]. In addition, generation of Th_2 cytokines (e.g., interleukin-4 (IL-4), IL-5, and IL-13) could also explain the overproduction of IgE, presence of eosinophils, and development of airway hyperresponsiveness. There also may be a reduction in a subgroup of lymphocytes, regulatory T cells, which normally inhibit Th2 cells, as well as an increase in natural killer (NK) cells that release large amounts of Th1 and Th2 cytokines [62, 63]. T lymphocytes, along with other airway resident cells, also can determine the development and degree of airway remodeling. Although it is an oversimplification of a complex process to describe asthma as a Th2 disease, recognizing the importance of *n* families of cytokines and chemokines has advanced our understanding of the development of airway inflammation [64, 65].

1.6.3.2. Mast cells: Activation of mucosal mast cells releases bronchoconstrictor mediators (histamine, cysteinyl-leukotrienes, prostaglandin D2) [66, 67, 68]. Although allergen activation occurs through high-affinity IgE receptors and is likely the most relevant reaction, sensitized mast cells also may be activated by osmotic stimuli to account for exercise-induced bronchospasm (EIB). Increased numbers of mast cells in airway smooth muscle may be linked to airway hyperresponsiveness [69].

1.6.3.3. Eosinophils: Increased numbers of eosinophils exist in the airways of most, but not all, persons who have asthma [70, 71, 72]. These cells contain inflammatory enzymes, generate leukotrienes, and express a wide variety of pro-inflammatory cytokines. Increases in eosinophils often correlate with greater asthma severity. In addition, numerous studies show that treating asthma with corticosteroids reduces circulating and airway eosinophils in parallel with clinical improvement [73].

1.6.3.4. Neutrophils: Neutrophils are increased in the airways and sputum of persons who have severe asthma, during acute exacerbations, and in the presence of smoking. Their pathophysiological role remains uncertain; they may be a determinant of a lack of response to corticosteroid treatment [74]. The regulation of neutrophil recruitment, activation, and alteration in lung function is still under study, but leukotriene B4 may contribute to these processes [75, 76, 77].

1.6.3.5. Dendritic cells: These cells function as key antigen-presenting cells that interact with allergens from the airway surface and then migrate to regional lymph nodes to interact with regulatory cells and ultimately to stimulate Th_2 cell production from naïve T cells [78].

1.6.3.6. Macrophages: Macrophages are the most numerous cells in the airways and also can be activated by allergens through low-affinity IgE receptors to release inflammatory mediators and cytokines that amplify the inflammatory response [79].

1.6.3.7. Resident cells of the airway: Airway smooth muscle are not only a target of the asthma response (by undergoing contraction to produce airflow obstruction) but also contribute to it (via the production of its own family of pro-inflammatory mediators). As a consequence of airway inflammation and the generation of growth factors, the airway smooth muscle cell can undergo proliferation, activation, contraction, and hypertrophy-events that can influence airway dysfunction of asthma.

1.6.3.8. Epithelial cells: Airway epithelium is another airway lining cell critically involved in asthma [80]. The generation of inflammatory mediators, recruitment and activation of inflammatory cells, and infection by respiratory viruses can cause epithelial cells to produce more inflammatory mediators or to injure the epithelium itself. The repair process, following injury to the epithelium, may be abnormal in asthma, thus furthering the obstructive lesions that occur in asthma.

1.6.4. Inflammatory Mediators

1.6.4.1. Chemokines: These are important in recruitment of inflammatory cells into the airways and are mainly expressed in airway epithelial cells [65]. Eotaxin is relatively selective for eosinophils, whereas thymus and activation-regulated chemokines (TARCs) and macrophage-derived chemokines (MDCs) recruit Th_2 cells. There is an increasing appreciation for the role this family of mediators has in orchestrating injury, repair, and many aspects of asthma.

1.6.4.2. Cytokines: These direct and modify the inflammatory response in asthma and likely determine its severity. Th_2-derived cytokines include IL-5, which is needed for eosinophil differentiation and survival, and IL-4 which is important for Th_2 cell differentiation and with IL-13 is important for IgE formation. Key cytokines include IL-1β and tumor necrosis factor-α (TNF-α), which amplify the inflammatory response, and granulocyte-macrophage colony-stimulating factor (GM-CSF), which prolongs eosinophil survival in airways. Recent studies of treatments directed toward single cytokines (e.g., monoclonal antibodies against IL-5 or soluble IL-4 receptor) have not shown benefits in improving asthma outcomes.

1.6.4.3. Cysteinyl-leukotrienes: CLs are potent bronchoconstrictors derived mainly from mast cells. They are the only mediator whose inhibition has been specifically associated with an improvement in lung function and asthma symptoms [81, 82]. Recent studies have also shown leukotriene B4 can contribute to the inflammatory process by recruitment of neutrophils [83].

1.6.4.4. Nitric oxide: NO is produced predominantly from the action of inducible NO synthase in airway epithelial cells; it is a potent vasodilator [84, 85]. Measurements of fractional exhaled NO (FeNO) may be useful for monitoring response to asthma treatment because of the purported association between FeNO and the presence of inflammation in asthma [86].

1.6.4.5. Immunoglobulin E: IgE is the antibody responsible for activation of allergic reactions and is important to the pathogenesis of allergic diseases and the development and persistence of inflammation. IgE attaches to cell surfaces via a specific high-affinity receptor. The mast cell has large numbers of IgE receptors; these, when activated by interaction with antigen, release a wide variety of mediators to initiate acute bronchospasm and also to release pro-inflammatory cytokines to perpetuate underlying airway inflammation [87, 88]. Other cells, basophils, dendritic cells, and lymphocytes also have high-affinity IgE receptors. The development of monoclonal antibodies against IgE has shown that the reduction of IgE is effective in asthma treatment [89, 90]. These clinical observations further support the importance of IgE to asthma.

1.6.5. Implications of Inflammation for Therapy

Recent scientific investigations have focused on translating the increased understanding of the inflammatory processes in asthma into therapies targeted at interrupting these processes [64]. Some investigations have yielded promising results, such as the development leukotriene modifiers and anti-IgE monoclonal antibody therapy. Other studies, such as those directed at IL-4 or IL-5 cytokines, underscore the relevance of multiple factors regulating inflammation in asthma and the redundancy of these processes. All of these clinical studies also indicate that phenotypes of asthma exist, and these phenotypes may have very specific patterns of inflammation that require different treatment approaches. Current studies are investigating novel therapies targeted at the cytokines, chemokines, and inflammatory cells farther upstream in the inflammatory process. For example, drugs designed to inhibit the Th_2 inflammatory pathway may cause a broad spectrum of effects such as airway hyperresponsiveness and mucus hypersecretion. Further research into the mechanisms responsible for the varying asthma phenotypes and appropriately targeted therapy may enable improved control for all manifestations of asthma, and, perhaps, prevention of disease progression.

1.6.6. Pharmacotherapy Management of Asthma

Drugs involved are bronchodilators with β_2-agonist, corticosteroids, antileukotrienes and anti immunoglobulins (Table 1.4) Drugs should be given by inhalation when possible so that the same beneficial effect can be achieved with a much smaller dose, thus causing lower systemic drug concentrations and fewer systemic adverse effects [89].

Table 1.4: Current therapies used in asthma

Bronchodilators	Anti-inflammatory therapies
Inhaled short-acting β_2-agonists: salbutamol and terbutaline	Inhaled corticosteroids: budesonide, fluticasone propionate, beclomethasone dipropionate and mometasone
Inhaled long-acting β_2-agonists: salmeterol and formoterol	Antileukotrienes: montelukast, pranlukast and zafirlukast
Inhaled anticholinergics: ipratropium bromide and tiotropium bromide	Cromones: sodium cromoglycate and nedocromil sodium
Theophylline: slow-release theophylline and aminophylline	Anti-immunoglobulin E: omalizumab

Over the past two decades many groups have tried to develop new types of drug for asthma, but only the leukotriene modifiers are on the market. Leukotriene modifiers consist of the

lipoxygenase inhibitors such as zileuton, and the leukotriene antagonists such as montelukast and zafirlukast. The drugs are given orally and a single drug can therefore treat both rhinitis and asthma. Both types of drug are effective in patients with mild or moderate asthma. Leukotriene antagonists cause some bronchodilatation within an hour of administration and results of long term studies have shown a reduction in symptoms and exacerbations. Patients have benefited, however, from several studies [90] designed to assess the role and merits of the drugs presently available. Most asthma guidelines include a stepwise approach to asthma treatment, which ranges from β_2-agonists alone for very mild intermittent asthma to oral corticosteroids for severe chronic asthma (Figure 1.7). Treatment should be determined by symptoms, exacerbations, and lung function since the weight of evidence suggests that none of the drugs in use changes the natural history of asthma. There is some controversy about inhaled corticosteroids, which are very effective at suppressing inflammation in asthma. Symptoms and airway obstruction have usually recurred, however, when the drugs are discontinued [91].

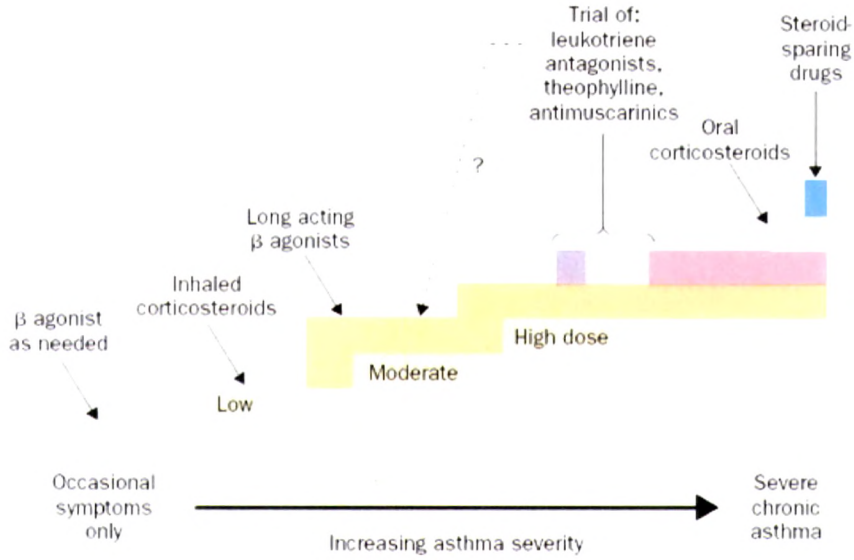

Figure 1.7: Treatment hierarchy used to add further drugs as asthma becomes increasingly severe [91]

1.6.7. Limitations of current treatment

A patient might have poorly controlled asthma due to poor management, severe asthma, or both. Poor compliance with treatment, especially inhaled corticosteroids, continues to be an important cause of poor asthma control and much effort is needed to ensure that patients understand why prophylactic treatment needs to be taken regularly and the dangers of poor compliance. Particular

care is needed for patients who are more likely to be non-compliant such as adolescents, the poor and socially deprived [92] and patients with psychosocial problems.

Long-term high doses of inhaled corticosteroids can cause systemic adverse effects, including reduced bone mineral density, [93] which is likely to predispose patients to osteoporotic fractures as they get older, and an increase in cataracts [94] and glaucoma [95].

Oral corticosteroids cause much morbidity and patients on long-term oral steroids need careful assessment to be sure that such treatment is necessary. For those who require prednisolone, prophylaxis against osteoporosis needs to be considered, ideally with a measure of bone mineral density [96].

Churg-Strauss syndrome has occurred in association with use of the leukotriene antagonists, but whether it is due to a direct drug effect or unmasking of the syndrome as inhaled or oral corticosteroids are reduced is still uncertain [97].

Recent studies of treatments directed toward single cytokines (e.g., monoclonal antibodies against IL-5 or soluble IL-4 receptor) have not shown benefits in improving asthma outcomes [98].

1.6.8. Herbal intervention

A survey by the National Asthma Campaign found that 60% of people with moderate asthma and 70% with severe asthma have used complementary and alternative medicine (CAM) to treat their condition. Herbal medicine is the third most popular choice of both adults (11%) and children (6%) suffering from asthma [99]. The historical importance of herbal medicine in the treatment of asthma is indisputable. Four of the five classes of drugs currently used to treat asthma-namely, β_2-agonists, anticholinergics, methylxanthines and cromones, have origins in herbal treatments going back at least 5000 years [100]. There is a large archive of information on herbal medicine from many cultures for the treatment of asthma.

Drug therapy is normally used to control symptoms (Figure 1.8). However the use of complementary or alternative medicine (CAM) is widespread. In a United Kingdom survey of National Asthma Campaign members, only 41% said that they had not used CAM and of those 41%, 67% said that they would consider using CAM for their asthma in the future. The most popular forms of CAM in the study population were breathing techniques, homeopathy and

herbalism [101]. A survey of CAM use in asthma or rhino sinusitis sufferers in the USA found that 42% of the study population had used some form of CAM for their condition in the 12 months prior to the study. Herbal treatments emerged as being the most commonly reported form of CAM being used [102]. Another US survey of CAM use found that allergies and lung

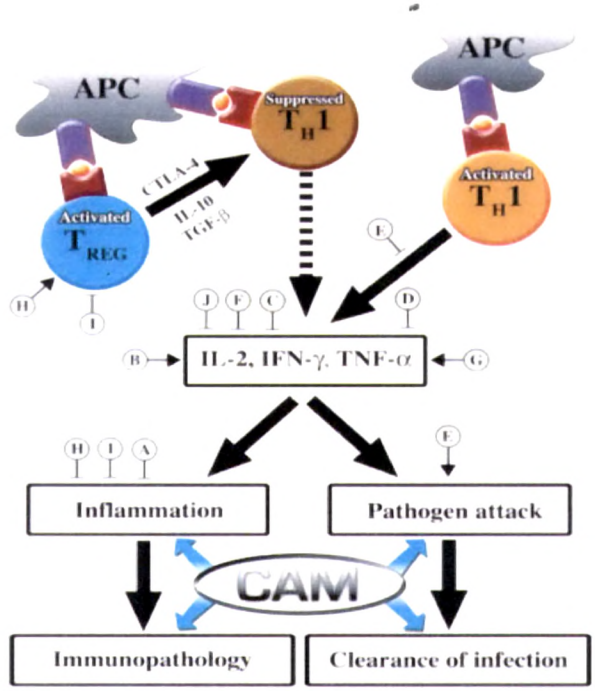

Figure 1.8: Modulation of Th_1 cell immune response by Treg cells and CAM. Sites of action throughout the Th_1 cell-mediated inflammatory pathway. A-Brazilian green propolis, B-Ganoderma lucidum, C- Piper linteus, D-Piper methysticum, E-Allium sativum, F-epigallocatechin gallate, G- KRN7000, H- probiotics, I- Staphylococcal superantigen B, J- Ergosterol peroxide [101].

problems as some of the most frequently reported medical conditions that CAM is used for, and the most popular forms of CAM for these conditions were herbs, relaxation and spiritual healing [103].

The reasons for people turning to CAM can be divided into positive and negative motivations [104, 105]. Positive motivations include perceived effectiveness and safety; 'spiritual' or holistic nature of the therapy; personal control over treatment; good relationship with the therapist; and accessibility. Negative reasons include dissatisfaction with conventional methods; rejection of the 'establishment'; and desperation. A study into the beliefs and motivations of CAM users in Canada supports this theory. It found the two main reasons people used CAM were that it allows

them to take a more active role in their health, and a feeling that conventional medicine was not effective for their health condition [106].

One of the positive motivations for using CAM is perceived safety. However there are risks with the use of herbal remedies including drug interactions, inconsistent dosing, contamination and natural toxicity [107]. Drug interactions could be a particular concern as a survey of herbal therapy users found that 81 % also used conventional medicines [108]. Barnes also found that herbal remedy users would be less likely to consult their general physicians for suspected adverse events to a herbal remedy than they would for a conventional over-the-counter medicine [109]. In fact, herbal therapy users tend to self-medicate or take the advice of a friend or relative [110, 111] so are unlikely to consult any practitioner at all on the use of herbal products.

Table 1.5: Herbs used to treat asthma by culture [112, 113]

Culture	Herbs used
CHINESE	Aconite; Artemesia; Asarum; Aster; Astragalus; Aurnantil; Bupleurum; Cinnabar; Cistanchis; Citrus reticulae; Coptis (goldenthread); Curculigo; Cornus; Cusctae; Dioscora (Chines yam); Epimedium; Fritillaria; Ginko bilboa; Ginseng; Gypsum; Juglandis; Kan lin (preparation); Licorice; Ligusticum chuan xiong; Longdan jichuan; Lumbricus spencer; Ma Huang (Epedra sinica); Magnolia; Minor Blue Dragon; Morus (mulberry) ; Peony; Perilla; Pinella; Prunus armeniacae (apricot/kernal); Psorale; Rehmannia; Scutellaria (skullcap); Tussilago (coltsfoot); Zingiber (ginger); Zizyphus (Chinese date)
JAPANESE (Kampo)	Hange-koboku-to; Moku-boi-tu; Saiboku-to; Shinpi-to; Sho-saiko-to; Sho-seiryu-to
INDIAN (Ayurvedic)	Ashatoda vasica (malabar nut); Coleus forskholii; Albizzia lekkek; Croton tiglium; Picrorrhiza kurroa; Tylophora indica/asthmatica (Indian ipecac)
LATIN AMERICAN	Allium cepa (onion); Aloe barbadensis; Desmodium (amor seco); Galphimia glauca
HAWAIIAN	Sophora chrysopylla; Aleurites moluccana (kukui, candlenut); Piper methysticum (kawa, kava); Solanum americum (popol, glossy nightshade)
WESTERN	Angelica; Belladonna (Deadly nightshade); Chinese skullcap; Coltsfoot; Coffee; Creosote; Garlic; Goldenseal; Henbane; Horseradish; Licorice; Ma Huang; Marijuana; Marshmallow; Mustard; Peppers (capsicums) ; Sarsparilla; Tea; Thyme; Wheatgrass

Whether herbal products are actually effective in the treatment of asthma is uncertain. A systematic review of herbs for asthma conducted in 2000 [111] found 17 randomized controlled trials: six assessing traditional Chinese herbs; eight assessing traditional Indian remedies; one assessing a Japanese herbal preparation; one assessing dried ivy-leaf extract, and one assessing use of marijuana. They found the methodological quality of the trials was poor and concluded that herbal products are of "uncertain value in the treatment of asthma". However, they also

concluded that were some "promising data". Given the high usage of herbal products among people with asthma, a new assessment of the current evidence is needed.

1.7. Cardiovascular Diseases

Cardiovascular disease (CVD) is known to be the major cause of mortality and morbidity worldwide accounting for 17 million deaths per annum, 30% of total deaths. Of these, 7.6 million are due to heart attacks and 5.7 million due to stroke [114]. Over 80% of CVD deaths occur in low and middle income countries [115]. It has been estimated that between 1990 and 2020, the increase in ischaemic heart disease alone will increase by 29% in men and 48% in women in developed countries and by 120% in women and 127% in men in developing countries [116]. In developing countries, it causes twice as many deaths as HIV, malaria and tuberculosis combined. CVD imposes high social costs, including impaired quality of life and reduced economic activity, and accounts for a large share of health service resources [117].

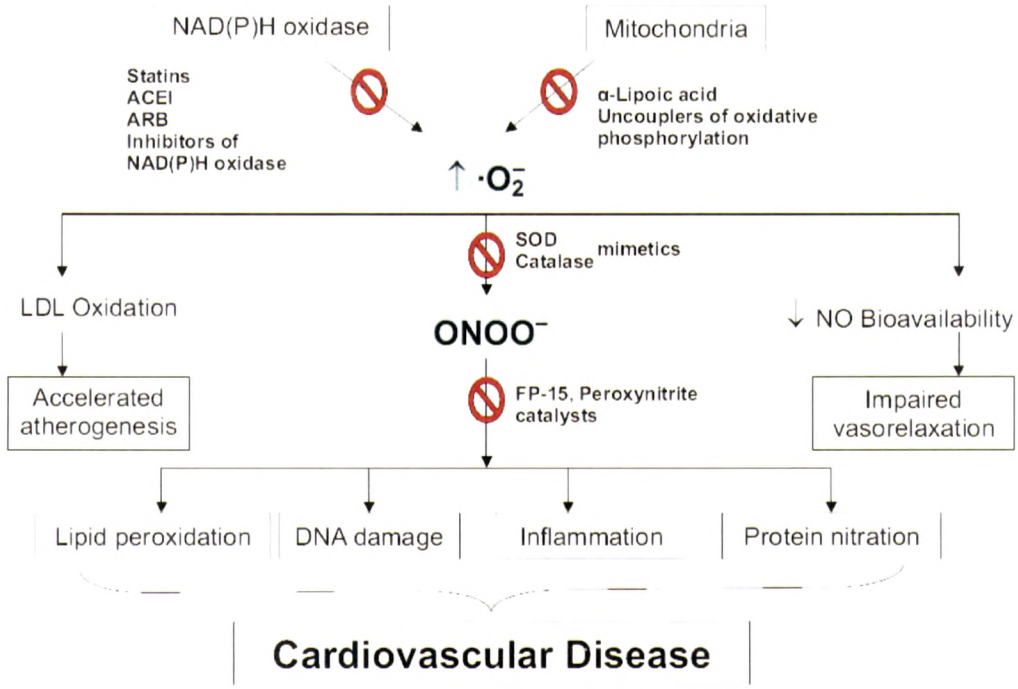

Figure 1.9: Reactive species in CVD pathophysiology. Increased levels of superoxide (O2-) cause LDL oxidation, lead to the formation of peroxynitrite ONOO– and decreased NO levels. ONOO– triggers CVD by lipid peroxidation, inflammation, protein nitration and DNA damage, resulting in a loss of vascular contractile function. Potential targets of intervention and clinical experimental therapeutics identified for each target are indicated. [118]

The individual risk for the development of CVD is dependent on nonmodifiable risk factors like gender, genetic predisposition, and age as well as modifiable factors such as nutrition and lifestyle habits [119]. It has been shown that dietary intake and food pattern can influence the development and progression of CVD [120]. Within this context, several epidemiological studies suggest that an increased intake of nutritional flavonoids is associated with a reduced risk for the development of CVD including coronary heart disease, stroke, hypertension, and vascular dementia [121, 122].

1.7.1. Pathology of ischaemic disease

Myocardial infarction (MI), the most dreaded sequel among ischemic heart disease, is invariably followed by several biochemical alterations such as, lipid per oxidation, free radical damage, hyperglycemia, hyperlipidemia etc., leading to qualitative and quantitative alterations [123].

Reactive oxygen species (ROS) are produced during the normal aerobic metabolism in biological systems by both endogenous and exogenous factors and become deleterious when not being eliminated by the endogenous systems. Antioxidants are used to prevent generation of ROS or to scavenge those formed. Deficiency of antioxidative defenses may lead to oxidative stress, which might be associated with a variety of disorders. In fact, oxidative stress results from an imbalance between the generation of reactive oxygen species and endogenous antioxidant systems [124].

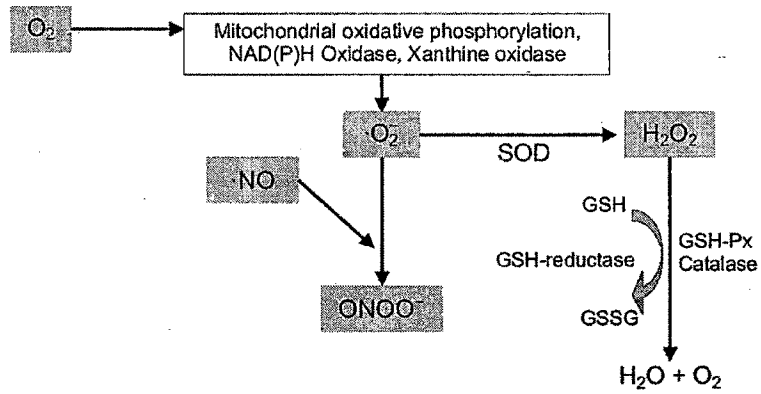

Figure 1.10: Generation of reactive species in CVD. Highlighted in gray are some of the most important ROS and RNS in vascular cells, Oxygen is converted to superoxide ·O2- via the activation of enzymatic and nonenzymatic pathways, which is then dismutated to H_2O_2 by SOD. H_2O_2 can be converted to H_2O by catalase or glutathione peroxidase (GSH-Px). Glutathione reductase regenerates glutathione (GSH). In addition, ·O2-reacts rapidly with ·NO to form ONOO– [135]

ROS are major sources of primary catalysts that initiate oxidation *in vivo* and *in vitro* and create oxidative stress which results in numerous diseases and disorders [125, 126] such as cancer

[127], cardiovascular disease [128], neural disorders [129], Alzheimer's disease [130] mild congnitive impairment [131], Parkinsons disease [132], alcohol induced liver disease [133], ulcerative colitis [134]. Oxygen derived free radicals such as superoxide anions, hydroxyl radicals and hydrogen peroxide are cytotoxic and give rise to tissue injuries [135]. Excessive amount of ROS is harmful because they initiate bimolecular oxidation which leads to cell death and creates oxidative stress. In addition, oxidative stress causes inadvertent enzyme activation and oxidative damage to cellular system [136]. Oxidative stress causes plaque formation leads to atherosclerosis and further ischaemic attacks.

Natural antioxidants present in our body are catalase, super oxide dismutase, glutathione peroxidase, while synthetic antioxidants like butylated hydroxy toluene and butylated hydroxy anisole are suspected to be carcinogenic and hence are no more in use. Therefore, search of new antioxidant molecule from natural sources is felt necessary [137].

1.7.2. Herbal intervention

Epidemiologic studies have demonstrated an association between increased intake of antioxidant vitamins such as vitamin E and vitamin C and reduced morbidity and mortality from coronary artery disease. This association has been explained on the basis of the "oxidative-modification hypothesis" of atherosclerosis, which proposes that atherogenesis is initiated by oxidation of the lipids in low-density lipoprotein (LDL), also termed lipid peroxidation. As a corollary to this hypothesis, antioxidants that inhibit lipid peroxidation in LDL should limit atherosclerosis and its clinical manifestations, such as myocardial infarction and stroke. There is a wealth of epidemiologic data linking the dietary and supplemental intake of antioxidant vitamins with a reduction in the clinical manifestations of atherosclerosis (Table 1.6) [138].

Many patients use herbal ingredients to treat chronic cardiovascular conditions and often combine herbal supplements with cardiovascular medications [139]. Patients may develop symptoms from a listed herbal ingredient, a contaminant or a drug–herb interaction.

Unfortunately, the research of adverse effects of herbal ingredients remains in its infancy. Herbal supplements commonly used to treat cardiovascular symptoms and conditions may cause a variety of adverse effects.

Garlic (*Allium sativum*) may have beneficial cardiovascular effects. A number of studies have demonstrated effects, such as lowering blood pressure, inhibiting platelet aggregation, enhancing

fibrinolytic activity, reducing serum cholesterol and triglyceride levels, and protecting the elastic properties of the aorta [140]. A placebo-controlled trial of 88 participants found garlic to be effective in decreasing total cholesterol in both healthy volunteers and patients with coronary heart disease [141]. A meta-analysis of five trials that studied the effect of garlic on cholesterol found that total cholesterol can be lowered about 9% in those people who took the equivalent of one half to one clove of garlic a day [142]. A more recent meta-analysis involving 13 trials concluded that garlic was better than placebo in decreasing total cholesterol but the effect was modest. It should be noted that the six trials within this meta-analysis considered to have the highest score for methodological quality did not show a significant difference between garlic versus placebo in decreasing total cholesterol [143].

Table 1.6: Example of commonly consumed herbal drugs for CVD and their adverse effect [144-146]

Herb	Condition	Major adverse effect
Aloe vera (*Aloe barbadensis*)	Hypercholesterolemia	Diarrhea, potassium depletion
Bitter orange (*Citrus aurantium*)	Obesity	Hypertension
Ephedra (*Ephedra sinica*)	Obesity	Stroke, myocardial infarction
Fenugreek (*Trigonella foenumgraecum*)	Hypercholesterolemia	Diarrhea, hypoglycaemia
Garlic (*Allium sativum*)	Hypercholesterolemia	Inhibition of platelet function
Ginkgo biloba	Claudication	Bleeding
Ginseng, Asian (*Panax ginseng*)	Diabetes	Insomnia
Glucomannan (*Amorphophallus konjac*)	Obesity	Esophageal or gastrointestinal obstruction
Green tea (*Camelia sinensis*)	Hypercholesterolemia	Arrhythmias, increased heart rate
Guar gum (*Cyamopsis tetragonolobus*)	Hypercholesterolemia	Esophageal or gastrointestinal obstruction
Guggul (*Commiphora mukul*)	Hypercholesterolemia	Headache, nausea
Horse chestnut (*Aesculus hippocastanum*)	Venous insufficiency	Antithrombotic effects
Maté (*Ilex paraguariensis*)	Obesity	Arrhythmias, increased heart rate
Policosanol	Hypercholesterolemia	Inhibition of platelet aggregation
Pomegranate (*Punica granatum*)	Hypertension	Angioedema
Psyllium (*Plantago ovata*)	Hypercholesterolemia	Esophageal or gastrointestinal obstruction
Red yeast rice (*Monascus purpureas*)	Hypercholesterolemia	Myopathy and rhabdomyolysis

Fuhrman et al. [147] studied the antiatherogenic properties of licorice root extract in 12 moderately hypercholesterolemic patients. Licorice consumption for 1 month was associated with a 19% reduction in plasma susceptibility to oxidation. Plasma LDL also showed increased resistance to three major atherogenic modifications: LDL oxidation (by 55%), aggregation (by 28%), and chondroitin sulfate binding ability (by 25%). Licorice consumption also reduced serum total cholesterol by 5%, LDL cholesterol levels by 9%, and VLDL levels by 14%. All of these parameters began to trend toward baseline levels after 1 month of placebo consumption.

Complementary and alternative medicine (CAM) covers a heterogeneous spectrum of ancient to new-age approaches that purport to prevent or treat disease. The predominant challenges for medicine today, however, come increasingly from chronic diseases that are prevalent among the

growing numbers of aging population. Chronic diseases often resist cure and may coexist with unrelieved pain. The growing popularity of CAM [148] is paralleled by an increasing amount of medical scientific information published in specialised journals on this subject. An increasing amount of medical scientific information is published in specialised journals on CAM.

In 2007, the CAM therapies most commonly used by U.S. adults in the past 12 months were nonvitamin, nonmineral, natural products (17.7%), deep breathing exercises (12.7%), meditation (9.4%), chiropractic or osteopathic manipulation (8.6%), massage (8.3%), and yoga (6.1%). In National Health Survey 2007 indicated that Americans spent $11.9 bn on CAM practitioner's visits and $ 21 bn spent on CAM products [149].

Herbal medicines can generate both benefit and harm. What does, however, seem to convince lay people easily is the widely held belief that 'natural' can be equated with safe. An example of this is a survey from Israel which demonstrated that 56% of the users of "natural drugs" believed that "they caused no side-effects" [150]. This belief is not just marginally misguided; Cuzzolin [151] shows that it is profoundly wrong. They confirm that serious adverse effects do happen and that these are more frequent when herbals are combined with conventional drugs. Indeed, herb-drug interactions are a serious issue and sadly one which we are only beginning to understand [152]. One U.S. survey showed that of the 22 most popular supplements, ten might have caused a total of 142 interactions in elderly users [153].

Herbal remedies can cause harm through a wide range of actions. It must be obvious that herbs can be toxic. The systematic evaluation of CAM therapies is essential. This could contribute to more efficient and equitable provision of health care services that takes into account the preferences and choices of patients and would move CAM research towards the model of whole systems research as advocated by Verhoef et al. [154].

The present study is based on development of the evaluation parameters for few medicinal plants used in CAM (*Ayurveda)* and in traditional medicines, but have not been scientifically explored for their therapeutic claims. The study, therefore, was planned 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-

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

II. Leaves of Changeri- *Oxalis corniculata* (Oxalidaceae)