1. Introduction

Inflammation is a dynamic and beneficial tissue response to protect the host body against invasion by pathogens consisting of a multifaceted network of intercellular cytokine signals. It is characteristically a defensive mechanism of the body in order to eliminate the spread of pathogenic agents and to remove necrosed cells and tissues (Withers, 1992). Cornelius Celsus in the first century characterized the inflammation by five cardinal signs such as redness, swelling, heat, pain and loss of function. It is divided into two types such as, Acute Inflammation and Chronic Inflammation. Acute inflammation is a physiological innate host response to injury aimed at removing pathological noxious substances and restoring homeostasis. Chronic inflammation occurs either after the acute inflammation that persists for the longer time duration or the stimulus that induces chronic inflammation persists for the longer time (Majno and Joris, 2004). Inflammation is a feature of many diseases, including pulmonary inflammatory diseases such as asthma, infectious diseases, allergic conditions such as atopic dermatitis and cancer. Inflammatory responses occur in three different phases, each of these is mediated by different mechanisms: An acute transient phase characterised by vasodilation and increased capillary permeability, a sub-acute phase, characterised by infiltration of leukocytes and phagocytic cells, a chronic proliferative phase, in which tissue degeneration and fibrosis occurs.

The following drugs are currently used for the treatment of various inflammatory conditions.

Ulcerative Colitis:
- Anti-inflammatory: Mesalazine, Prednisolone, Methylprednisolone, Butezonide
- Immunosuppressive:
  - Azathioprine, 6-mercaptopurine, Methotrexate, Cyclosporine, Tacrolimus; Biologics: Infliximab, Adalimumab, Certolizumab Pegol
  - Atopic Dermatitis
  - Rituximab, Omalizumab, Ligelizumab, Dupilumab, Pitrakinra, Mepolizumab
- Bronchial asthma
  - At present Glucocorticoids and B₂ Adrenoceptor agonists are the most effective drugs for the treatment of airway inflammation and obstruction with Theophylline, Leukotriene receptor antagonists and anticholinergic as second- or third-line therapy.

Rheumatoid arthritis
- Non-steroidal anti-inflammatory drugs (NSAIDs) are the first line treatment and disease-modifying anti-rheumatic drugs (DMARDs) are the second line treatment for arthritis.

Conventional DMARDs: Methotrexate, Leflunomide, Hydroxychloroquine, Sulfasalazine;
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Biologic DMARDs: Anti-TNF drugs, Rituximab, Abatacept, Anakinra, Tocilizumab (Sharma and Sharma, 2008).

Nowadays, the management of above described inflammatory diseases is very difficult because of their chronic and prolonged treatment that can results in development of drug resistance or side effects. All the above mentioned drugs are frequently associated with the variety of detrimental side effects such as; bone marrow depression, diabetes, metabolic disorders, gastro-intestinal irritation, ulcers, hypertension, glaucoma, weight gain and skin thinning etc (Wolfe and David, 1999; Schoepe et al, 2006).

Medicinal plants have been source of wide variety of biologically active compounds for many centuries and used extensively as crude material or as pure compounds for treating various disease conditions (Arif et al, 2009). The use of herbal medicines becoming popular due to toxicity and side-effects of allopathic medicines. Medicinal plants play an important role in the development of potent therapeutic agents. There are over 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative applications (Dasilva, 1999). India is the biggest repository of medicinal plants in the world may maintain an important position in the production of raw materials either directly for crude drugs or as the bioactive compounds in the formulation of pharmaceuticals and cosmetics etc (Tiwari, 2008). The compounds isolated from herbal medicine serve as a replacement for plants extracts that have been studied for the long time to establish a new safe and effective topical anti-inflammatory drug (Cordell and Colvard, 2005; Khanna et al, 2007; Lee et al, 2009). Hence, it is of supreme importance to search less toxic drugs from plant origin for their anti-inflammatory effects (Fabricant and Farnsworth, 2011).

The plant steroids have many medicinal activities like antibacterial, hepatoprotective, cytotoxic, anti-tumor, immunosuppressive, plant growth hormone regulator, anti-helminthic and cardiotonic activity. Glucocorticoids are the steroidal agents used to treat the variety of inflammatory disorders but, on long term usage it shows severe side effects. In order to overcome these undesirable side-effects, the research work is going to identify novel bioactive phytoconstituents with therapeutic potential with no or minimum side effects. The various plant steroids have structural similarity with glucocorticoids such as Hecogenin, Diosgenin, Solasodine, Glycyrrhizin, Boswellic acid, Guggulsterone, Sarsasapogenin or Withaferin-A present in plants such as Boswellia serrata, Trigonella foenum graecum, Smilax officinalis, Solanum xanthocarpum, Commiphora mukul, Withania somnifera, Glycyrrhiza glabra,
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proved to be anti-inflammatory agents by recent clinical and pre-clinical studies. Therefore, it is essential to search these plants to detect the lead anti-inflammatory activity molecule by using various inflammatory models.

**Boswellia serrata**

*Boswellia serrata* contains boswellic acid as one of chemical constituent. Boswellic acid is a pentacyclic triterpene molecule having steroid like structure. *Boswellia serrata* has been proved to be a potent anti-inflammatory drug in *in-vivo* animal models as well as in clinical studies (Dahmen et al, 2001; Chrubasik et al, 2007). Boswellic acid is reported to decreases the 5-hydroxyeicosatetraenoic acid and leukotriene B\textsubscript{4} levels (pro-inflammatory 5-lipoxygenase products), that was the active chemotactic factors causing increased vascular permeability (Ammon et al, 1991). Boswellic acid decreases polymorphonuclear leukocyte infiltration and migration, decreases the primary antibody synthesis and inhibition of classical complement pathway (Sharma et al, 1988; Sharma et al, 1989; Shah et al, 2009).

**Trigonella foenum graecum**

Fenugreek (*Trigonella foenum graecum*) contains diosgenin, a steroidal saponin. Fenugreek has been reported to suppress inflammation and used for the treatment of rheumatoid arthritis (Shishodia and Aggarwal, 2006; Liagre et al, 2004). The leaf extract of fenugreek reported to possess a potent anti-inflammatory activity in formalin induced paw inflammation in rat (Ahmadiania et al, 2001). The chemokines, produces recruitment of lymphocytes leading to tissue damage that are expressed during the inflammatory process are inhibited by steroidal glycoside from *Trigonella foenum graecum* (Liagre et al, 2004).

**Smilax officinalis**


**Solanum xanthocarpum**

*Solanum xanthocarpum*, contains steroidal glycoalkaloids like Solasodine belonging to family Solanaceae. *Solanum xanthocarpum* was traditionally used for the treatment of bronchial
asthma in asthmatic patients (Govindana et al, 2004). Solanum species contains 16-dehydropregnenolone acetate, which is a starting material for synthesis of oral contraceptive and anti-inflammatory steroidal drugs. The steroidal alkaloid fraction of *Solanum xanthocarpum* have been reported to possess immune-suppressive and membrane stabilizing effect on mast cells degranulation model (Chitravanshi et al, 1990). Solasodine (SS), Saponin isolated from Solanum xanthocarpum was found to produce anti-allergic activity (Gupta, 1994). It has been used as anti-allergic agent (Gupta, 1994), immuno-suppressive agent, hepatoprotective and membrane stabilizing agent (Chitravanshi et al, 1990). It is used as starting material for the synthesis of oral contraceptive, steroidal hormone (Solouki et al, 2011).

**Commiphora mukul**

*Commiphora mukul* (Guggul) contains steroidal compounds called guggulsterones, which ranges from E to Z. These are an active principle of the plant and accounts for their anti-arthritis activity (Gebhard et al, 2009; Duwiejua et al, 1993; Satyavati, 1993). *Commiphora mukul* possesses anti-inflammatory property and its steroidal fraction considered as an active principle for this activity. HPLC analysis showed that steroidal fraction mainly contains guggulsterone Z that is responsible for anti-inflammatory activity (Sosa et al, 1993).

**Glycyrrhiza glabra**

Glycyrrhetinic acid is isolated from *Glycyrrhiza glabra* which is a pentacyclic triterpenoid derivative of the beta-amyrin type. Glycyrrhetinic acid reported to show the promising anti-inflammatory action, inhibit the release of histamine, serotonin, and bradykinin and lowers vascular permeability (Akamatsu et al, 1991; Abe et al, 1980). It is also reported to inhibit formalin-induced edema formation, granuloma weight and exudate amount (Nasyrov and Lazareva, 1980). *Glycyrrhetinic acid* reported to gives protection against lung inflammatory diseases by creating anti-inflammatory chemokines, IL-8 and eotaxin 1 from lung fibroblasts. It has an anti-inflammatory or allergic action by the suppression of platelet activating factor production (Ichikawa et al, 1989). It is reported to have allergic action by the suppression of PAF production (Ichikawa et al, 1989) and lowers vascular permeability (Abe et al, 2003).

**Withania sominifera**

*Withania sominifera* commonly known as Ashwagandha contain pharmacologically active compounds Withanolide (steroid lactone) (Kazutoshi et al, 1999; Bhakare and Khotpal, 1993).
In the Indian System of Medicine, *Withania somnifera* having numerous application for the treatment of inflammation (Mathur et al, 2006). Withaferin-A (WF) is a steroidal lactone isolated from the *Withania somnifera* (Indian Ginseng and Ashwagandha) widely used in traditional Indian medicine as anti-inflammatory agent (Mishra et al, 2000). The methanolic fractions of *Withania somnifera* aerial parts showed significant anti-inflammatory activity; probably the activity was attributed due to the presence of biologically active steroids withaferin A (Al-Hindawi et al, 1992; Sabina et al, 2008).

**Agave sisalana**

Hecogenin (HG) is a steroidal sapogenin present in the leaves of *Agave genus* species such as *Agave sisalana, Agave cantala, Agave aurea* and various agave species (Paik et al, 2005; Murthy et al, 2001). The extracts obtained from above plants have been used for their cardioactive, larvicidal, hypotensive and fungicidal activity (Achenbach et al, 1994; Gondim, 2006). The triterpene saponins exhibited suppressive effect on ethanol induced gastric mucosal lesions in rats and ethanol and indomethacin induced gastric damage in rats (Morikawa et al, 2006; Ramasubramancaraja and Babu, 2011). The HG was found to present an anti-proliferative activity in human osteosarcoma cells (Corbiere et al, 2003). The anti-cancerous activity of HG was also conducted and found to present an anti-proliferative activity in human osteosarcoma cells (Corbiere et al, 2003). The documented reports of Cerqueira et al (2012) shown the effect of HG on oxidative stress, lipid peroxidation and myeloperoxidase, a biomarker of inflammation, as well as its gastric ulcer protective effect was confirmed with histological and COX-2 immunohistochemistry studies (Cerqueira et al, 2012). Hecogenin acetate at two different concentrations 75 and 100 μM induced G0/G1 phase arrest (74% and 84.3% respectively) in a human lung cancer cell line by modulating reactive species production and inducing cell cycle arrest (Gasparotto et al, 2014). Steroidal saponins isolated from agave species, exhibit anti-inflammatory activity in acetic acid induced membrane permeability model (Da Silva et al, 2002; Da Silva and Parente, 2005). Furthermore, Mana et al, found that *Agave* species leaves contains compounds having antitumor activity (Mana et al, 2010). This anti-inflammatory activity was mainly attributed due to the presence of steroidal saponins and terpenes and was found due to its ability to block the inflammatory cascade associated with arachidonic acid by inhibiting the enzymes cyclooxygenase 1 and 2 (Da Silva et al, 2002; 2005; Augustin et al, 2011).

Glucocorticoids (GC) are universally accepted agents for the treatment of anti-inflammatory and immunosuppressive disorders. They are used in the treatment of rheumatic diseases and
various inflammatory diseases such as allergy, asthma and sepsis. They bind with GC receptor (GR) and form GC–GR complex with the receptor and exert their actions. On activation the GC–GR complex up-regulates the expression of nucleus anti-inflammatory proteins called as transactivation and down-regulates the expression of cytoplasmic pro-inflammatory proteins called as transrepression (Smoak and Cidlowski, 2004). It has been observed that transactivation mechanisms are notorious for side effects and transrepressive mechanisms are identified for beneficial anti-inflammatory effects of GC therapy. GC hampers the function of numerous inflammatory mediators such as cytokines, chemokines, adhesion molecules, arachidonic acid metabolites, release of platelet-activating factor (PAF), inflammatory peptides and enzyme modulation involved in the process of inflammation. The GC resistance is a serious therapeutic problem and limits the therapeutic response of GC in chronic inflammatory patients. It has been observed that the GC resistance can be attributed to cellular microenvironment changes, as a consequence of chronic inflammation. Various other factors responsible for resistance have been identified, including alterations in both GR-dependent and GR independent signaling pathways of cytokine action, hypoxia, oxidative stress, allergen exposure and serum derived factors (Nissen and Yamamoto, 2000).

Glucocorticoids at the high concentrations interact with DNA recognition sites to activate transcription through increased histone acetylation of anti-inflammatory genes and transcription of several genes linked to glucocorticoid side effects (trans-activation). Glucocorticoids also have post-transcriptional effects and decrease stability of some pro-inflammatory mRNAs (Barnes et al, 2004). The decreased glucocorticoid responsiveness is found in patients with severe asthma and asthmatics who smoke, as well as in COPD patients (Hew et al, 2006). Several molecular mechanisms of glucocorticoid resistance have now been identified which involve phosphorylation and other post-translational modifications of GR. Another mechanism of GC resistance are reduced histone deacetylase-2 (HDAC2) expression, excessive activation of transcription factor activator protein 1, cytokine activation of mitogen-activated protein (MAP) kinase pathways, increased P-glycoprotein-mediated drug efflux and increased levels of the macrophage migration inhibitory factor (Barnes and Adcock, 2009). Some of the above listed resistance mechanisms may be partially reversible such as, elevated HDAC2 expression can be reduced by the administration of theophylline. Besides that, several p38 MAP kinase inhibitors are currently in development that might impact on steroid resistance (To et al, 2010; Barnes and Adcock, 2009).
Therefore GCs remain a helpful adjunctive treatment for the treatment of Rheumatoid arthritis, other systemic inflammatory and autoimmune disorders. Many physicians and health care providers have had a problem with the long term GC treatment because of their well-known adverse effects associated with high doses. All these detrimental side effects of GCs eliminate the extended use of GCs therapy in clinical practice. However, these adverse events with GCs are dose related and long term use of low dose GCs may still be a feasible therapeutic alternative for some patients.

Fluticasone (FC) propionate is a topically active corticosteroid molecule (Phillipps, 1990). It is greatly lipophilic in nature (3-fold more lipophilic than beclomethasone dipropionate and 300-fold more lipophilic than Budesonide). It is more potent in vitro than beclomethasone, dexamethasone and budesonide in inhibiting anti-CD3-induced human T-lymphocyte proliferation, growing levels of secretory leucocyte protease inhibitor and attenuating tumour necrosis factor-α induced expression of endothelial cell adhesion molecule in bronchial epithelial cells. It is also more potent and longer-acting than other corticosteroids in inhibiting interleukin-5 induced blood eosinophilia, oedema formation, and IL-5 stimulated eosinophil lung tissue accumulation. Therefore, FC has increased intrinsic glucocorticoid potency and elevated topical anti-inflammatory activity (Johnson, 1990). FC has been shown to attenuate pulmonary inflammation in laboratory animals (Lapae Silva et al, 1994) and human beings (Booth et al, 1995), and inhibit chemotaxis of neutrophil (Llewellyn et al, 1994), endothelial cell adhesion molecule expression (Johnson, 1995), and cytokine production (Masuyama et al, 1994). But, FC is associated with the variety of detrimental side effects such as; diabetes, metabolic disorders, gastro-intestinal irritation, ulcers, hypertension, glaucoma and bone marrow depression (Wolfe and David, 1999; Schoepe et al, 2006). Hotchkiss et al. (1988) have studied the effect of FC on Ozone-induced rhinitis and mucous cell metaplasia in rat nasal airway epithelium on intranasal instillation of FC at a dose of 50 μg/nasal passage by decreasing neutrophilic inflammation and expression of cytokines which modulate airway mucin expression (e.g., TNF-α, IL-6) (Hotchkiss et al, 1988). The dose of HG (50 μg/animal) and in combination HG + FC (25 μg/animal each) was selected on the basis of pilot study. Initially the dose finding study was performed by selecting various doses of HG such as 50, 75 and 100 μg/animal and all doses exhibits significant anti-inflammatory activity in mice. Hence, smallest dose of drug was selected for further anti-inflammatory study in various animal models (50 μg/animal).
The main aim of this research work was to reduce or minimize the side effects of glucocorticoids (Fluticasone) by combining with plant steroid (Hecogenin) at low dose in various anti-inflammatory animals models such as Croton oil induced ear edema, Cotton pellet induced granuloma, TNBS induced colitis, DNFB induced dermatitis, OVA induced asthma and CFA induced arthritis. So, this low dose combination will be used as a better therapeutic approach for the treatment of various inflammatory disorders such as asthma, dermatitis, colitis and rheumatoid arthritis.

Croton oil consists of a mixture of lipids that contains 12-O-tetracanoilphorbol-13-acetate and other phorbol esters as main irritant agents. The topical application of croton oil is associated with oxidative stress, inflammatory response, proliferation and activation of nuclear oncogenes (Garg et al, 2008). 12-O-tetracanoilphorbol-13-acetate is also stimulated protein kinase C that further activates other enzymatic cascades, such as mitogen activated protein kinases and phospholipase A2 with the release of platelet activation factor and arachidonic acid. The mediators involved in this inflammatory cascade endorse vasodilation, vascular permeability, the release of histamine and serotonin, leukocytes migration, synthesis of prostaglandins and leukotrienes. Corticosteroids, leukotriene B4 antagonists, cyclooxygenase inhibitors and 5-lipoxygenase inhibitors had previously shown the topical anti-inflammatory activity in croton oil induced skin inflammation (Murakawa et al, 2006).

The Cotton pellet induced granuloma model is famous for screening the chronic phase anti-inflammatory activity that is characterized by fibroblast proliferation, monocyte infiltration, exudation and angiogenesis (Majno, 1998; Vogel, 2002). The absorption of surrounding fluid by the cotton pellets increases the wet weight of the granuloma tissue (Raju et al, 2005; Zhu et al, 2011). In this study, HG and combination decreased the weight of dry granuloma tissue significantly as compared to DC group rats. This may be due to the ability of HG in reducing the number of fibroblasts, the synthesis of collagen, mucopolysaccharide and prevention of an angiogenesis process (Babu et al, 2009).

The Ulcerative colitis (UC) and Crohn's disease (CD) are two major types of Inflammatory Bowel Disease (IBD). These are characterized by unrelieved and relapsing inflammation of gastrointestinal tract (Cho, 2008). The pathogenesis of IBD is complex and the variety of factors responsible for IBD are participation of an environmental triggers, microbial agents, genetic predisposition and immune dysfunction (te Velde et al, 2006). TNBS induces diffuse colonic inflammation, characterized by increased leukocyte infiltration, edema and ulceration...
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(Isik et al, 2011). In fact, TNBS induced colitis is a hapten-induced colitis model in which Th1-mediated immune response involving various cytokines including TNF-α and IL-12 serving as effector cytokine which result in transmural infiltration and inflammation (Ikeda et al, 2008; Takagi et al, 2010). The characteristic features of TNBS induced colitic inflammation of the rat gut resemble to those of human IBD by numerous clinical and histopathological features (Ford et al, 2011).

Allergic dermatitis (AD) is a common skin disease characterized by edema, erythema, excoriation and scaling of skin (Leung and Bieber, 2003; Yang, 2012). The effective treatment of AD is prevention of allergenic agent. Topical steroidal therapy is very successful for the treatment of AD but, because of its potential adverse effects it cannot be administered for long term use (e.g. skin atrophy and hyperpigmentation of skin) (Cohen and Heidary, 2004). Therefore, it is necessary to minimize the corticosteroid dosage schedule. Owing to the low cost and safety, herbal medicines can be a newer drug therapy for treatment of AD in patients. Therefore, we have confirmed the anti-inflammatory effects of HG and combination on AD like skin lesions in Balb/c mice.

Balb/c mice are sensitive responders to OVA and are a well-established airway inflammatory disease model of human asthma (Inman et al, 1999). OVA sensitization and the subsequent OVA challenge in a murine model induces the infiltration of immune cells, including eosinophils and lymphocytes in the Bronchoalveolar lavage (BAL) fluid and mucus-secreting goblet cell hyperplasia (Camateros et al, 2007). In this model, the early response to allergen is as a result of IgE dependent type I hypersensitivity reaction driven by mast cell-derived mediators, whereas the late response develops as a result of activation of antigen-specific T cells. The secretion of pro-inflammatory cytokines by activated mast cells, T-lymphocytes and injured bronchial cells were responsible for the pathogenesis of OVA induced bronchial asthma in mice (Kumar et al, 2008). In this search, the HG and combination were investigated in the present study for their anti-asthmatic activity on OVA induced lung edema in Balb/c mice.

Complete Freund Adjuvant (CFA) induced arthritis is well recognized model and has been commonly used for evaluation of anti-inflammatory and anti-arthritis potential of various agents (Costa et al, 2004; Walz et al, 1971; Noguchi et al, 2005). It is composed of an inactivated and dried Mycobacteria tuberculosis, that contain different pathogen-associated molecular patterns including toll-like receptor 2, 4, and 9 agonists and responsible for the...
stimulation of cell mediated immunity that increases the synthesis of certain immunoglobulins (Akira et al, 2006). The CFA induced arthritis follows a biphasic response an acute local inflammatory reaction that subsides after 3-4 days and a chronic systemic reaction that shows a relapsing-remitting course after initial two weeks and can persist for several months (Neugebauer et al, 2007). Further, release of diverse inflammatory mediators such as, cytokines, lysosomal enzymes, hydrolytic enzymes, and prostaglandins (PGs) are known to participate in the pathogenesis of RA (Eric and Lawrence, 1996; Sandoval et al, 2000; Pandey et al, 2005; Naik and Wala, 2014).

**Objective**
1. To perform the preliminary pharmacological evaluation of various plant steroids
2. To perform the pharmacological evaluation of selected plant steroid in various acute and chronic inflammatory models

- **Primary pharmacological evaluation**
  - To evaluate the anti-inflammatory activity of HG and combination on Croton oil induced ear edema in mice.
  - To evaluate the anti-inflammatory activity of HG and combination on Cotton pellet induced granuloma in rats.

- **Secondary pharmacological evaluation**
  - To evaluate the anti-inflammatory activity of HG and combination on TNBS induced colitis in rats.
  - To evaluate the anti-inflammatory activity of HG and combination on DNFB induced dermatitis in mice.
  - To evaluate the anti-inflammatory activity of HG and combination on OVA induced asthma in mice.
  - To evaluate the anti-inflammatory activity of HG and combination on CFA induced arthritis in rats.

3. To perform the acute toxicity profile of Hecogenin in Swiss albino mice.
4. To perform the safety pharmacological profile of Hecogenin and combination in Swiss albino mice.