Chapter 6
Discussion
6. DISCUSSION

Natural products continue to be viewed as one of the few de novo sources of drug discovery, yielding unorthodox and often unanticipated chemical structures that offer novel points of departure for molecular modifications leading to new therapies.

Plant constituents along with other natural products, are often extremely useful as "lead" compounds for the synthetic design of analogues with either improved therapeutic activity or reduced toxicity. This provides a strong justification to discover new structural leads from natural sources that can effectively interact with therapeutic targets. There is also a growing interest in plant-based drugs, reflected from the fact that the consumption of botanical products has doubled in the last 10 years in the Western Europe (Hamburger et al., 1991). On the other hand, chemical synthesis and semi synthesis yield the majority of heart drugs, central nervous system agents, antihistamines, analgesics and anti-inflammatory drugs. The vast majorities of plant-derived drugs in the US market today are either glycosides or alkaloids, which are water soluble or can easily be rendered so by salt formation (Kinghorn, 1992). It is envisioned that broader structural range of plant constituent, somewhat more difficult to synthesize within laboratory, will become future drug candidates, such as aromatic substances, terpenoids, oligopeptides and even complex carbohydrates and proteins (Tyler, 1986).

Traditional medicine has some strength that western medicine is lacking, namely the holistic view of the patient's situation. In traditional practice, the psychological, spiritual and social aspects play a major role, and this holistic treatment can to some extent make up for the often weaker aspect, the medicinal treatment, when compared to western biomedicine.

25% of today's drugs have originated from nature (Newman et al., 2003). Despite the great successes already achieved in natural products chemistry and drug development, we have barely begun to tap the potential of our molecular diversity. Only an estimated 5% to 15% of the 250,000 species of higher terrestrial plants in existence have been chemically and pharmacologically investigated in systematic fashion.

People in separate cultures and places are known to have used the same plants for similar medical problems. A larger number of these plants and their isolated
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constituents have shown beneficial therapeutic effects, including anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial and immunomodulatory effects. Several plant products are known to exhibit immense medicinal value against human diseases. As said earlier, the restorative and rejuvenating power of these herbs may be due to their action on the immune system. Oxidative damage to biological structures has also been implicated in the toxicity-induced pathophysiology of several diseases (Maxwell, 1999). It has been reported that the health promotive, disease preventive and rejuvenation approach based on using medicinal plants in ‘Ayurveda’, is due to the anti-oxidant effects of these plants (Govindrajan et al., 2005).

Inspite of the tremendous advances made in the modern sciences, there are still a large number of ailments for which suitable drugs are not found. With the potential of uncovering new compounds with idealistic pharmacological profiles (i.e., no side effects, no addictive potential), natural products still hold great promise for the future of drug discovery especially in the treatment of pain disorders and potentially drug addictions. The alternative and complementary systems of medicine have not adequately been explored for the safe and effective anti-inflammatory drugs. Plants and other natural products described in historical ethnobotanical and ethnopharmacological literature are being utilized to aid in the identification of natural products that have been historically employed in the alleviation of pain. Over the last several decades, more analgesic substances have been purified from natural products such as polysaccharides, terpenes, curcuminoids, alkaloids, etc. resulting in novel structural classes and mechanisms of actions (Darshan and Doreswamy, 2004). Many of these act by their radical scavenging effect or their immunomodulatory activity. Immunomodulators act by stimulating or suppressing both the humoral and cellular immune system thereby promoting the phagocytosis and further neutralizing the toxins whereas antioxidants protect, by preventing the harmful effects of free radical mediated chain reactions in the cell membranes and by reducing the susceptibility of the tissues to oxygen stress.

6.1 Pharmacognostical and phytochemical analysis

_Inula cappa_ (Buch.-Ham. ex D. Don) DC., (F:Compositae), a perennial shrub commonly known as ‘Chirchitta’ and ‘Poshkanmul’, is found mainly in the temperate
In the traditional system of medicine, the roots of the plant are used as an anti-inflammatory agent, anodyne, and in the treatment of gastric disorders. In the present study, the *Inula cappa* plant was authenticated by its pharmacognostical and phytochemical studies and was further evaluated for its pharmacological activity, mainly anti-inflammatory, immunomodulatory, and antioxidant activity with an aim to substantiate claims made of this plant in traditional systems of medicine.

The macroscopy and microscopy studies were carried out to authenticate the *Inula cappa* roots. Microscopic study showed the presence of cork, cortex, and stelar regions. Cork consisted of 12-15 layers of cells of which few cells were filled with brown-colored pigment followed by a uni-bi layered cork cambium. Cortex was wide, traversed with oil cells and patches of stone cells and sclerides. Phloem was wide and parenchymatous. Peripheral phloem consisted of groups of fibrous, lignified sclereids, and stone cells. Cambium was very narrow and indistinct. Xylem was wide; made up of xylem vessels, xylem fibres, and xylem parenchyma; vessels were bordered pitted and radially arranged, associated with lignified pitted xylem fibres and lignified, pitted, xylem parenchyma. The plant was identified and authenticated by Prof. Minoo Parabia, Head, Botany Department, South Gujarat University, Surat, India. The macroscopy and the microcopy study carried out in the present investigation revealed the authenticity of the *Inula cappa*.

Phytochemical evaluation is one of the tools for the quality assessment, which included preliminary phytochemical screening, chemoprofiling, and marker compound analysis using modern analytical techniques like HPTLC and HPLC, which have emerged as one of the tools for the quality assessment of the ayurvedic drugs. HPTLC can be used for the qualitative, semi-quantitative, and quantitative phytochemical analysis of herbal drugs and formulations. This includes developing TLC fingerprint profiles and estimation of chemical markers and biomarkers.

To further confirm the authentication, preliminary phytochemical analysis was carried out, which showed the presence of triterpenoids, flavonoids, and coumarins in *Inula cappa* roots. Study of the quality parameters revealed the presence of high extractive and low ash values of the herb indicating its good quality. These values are indirectly helpful to detect the quality of the herb.
TLC finger printing was developed for the methanolic extract using various solvent systems. In our studies, \(\beta\)-sitosterol, lupeol, \(\alpha\)-amyrin, chlorogenic acid and thymol isobutyrate were used as the marker compounds. Methods were developed for their qualitative studies using different spray reagents and quantitative studies using HPTLC.

TLC of Extract A showed 3-7 quenched bands under UV 254 nm from \(R_f\) 0.04 to 0.89, in the various solvent systems tried. TLC finger print profile of Extract A of *Inula cappa* at 366 nm showed minimum (4) bands in the solvent system III (toluene : ethyl acetate : formic acid, 7:3: 0.5, v/v/v) and maximum (10) bands in the solvent system II (Toluene: methanol, 9:1 v/v). In the solvent system I (hexane: ethyl acetate, 9: 1, v/v) Extract A showed 9 bands; whereas in the solvent system IV (chloroform : methanol : water, 7 : 3 : 0.5, v/v/v), V (toluene : ethyl formate : formic acid : water, 3: 3: 0.8: 2.0, v/v/v/v) and VI (ethyl acetate : formic acid : glacial acetic acid : water, 10: 1.1: 1.1: 2.6, v/v/v/v/v) it showed 7, 8 and 5 bands respectively under UV 366 nm. Along with TLC finger print profiles, the presence of chlorogenic acid in the sample extract was confirmed in co-chromatography (\(R_f\) 0.55) and overlay of absorption spectra (\(\lambda_{max}\) 330 nm) in the solvent system I.

TLC finger print profile of Extract A of *Inula cappa* showed minimum four bands in the solvent system II and the maximum 11 bands in the solvent system VI after derivatization with anisaldehyde in sulphuric acid reagent followed by heating at 110° C for 5 minutes. Thymol isobutyrate was resolved in the solvent system VI at the \(R_f\) of 0.51 as an orange to pink colour band. \(\beta\)-sitosterol, lupeol and \(\alpha\)-amyrin were resolved in the solvent system V (Toluene : methanol) at the \(R_f\) 0.44, 0.58 and 0.92 as blue, purple and pink color, respectively. The isolated compound, isoalantolactone was resolved in the solvent system VI at the \(R_f\) 0.32 as a blue band after derivatization with anisaldehyde-sulphuric acid reagent. These TLC finger printing developed can be used as one of the tools for the standardization of *Inula cappa*.

Volatile oil was isolated from the roots of *Inula cappa* using hydro distillation and was analyzed using GCMS. Thymol and thymol isobutyrate giving a mass peak of 150 and 220 respectively were identified as the major compounds accounting for 78.42 % of the oil. The other peaks were not identified. The volatile oil was also analyzed by TLC and showed major 2 bands with the thymol being more prominent.
with the characteristic orange color and was identified by co-chromatography using a standard compound.

Further, the TLC densitometric methods for the quantification of various marker compounds in the roots of *Inula cappa* were established using HPTLC. The methods were validated in terms of accuracy, precision and repeatability. The percentage of β-sitosterol and lupeol were found in 0.022% w/w and 0.055% w/w in the methanolic extract of the root of *Inula cappa* whereas the amount of α-amyrin and chlorogenic acid was found to be 0.053% w/w and 0.047% w/w, respectively. Thymol Isobutyrate was quantified from the roots of *Inula cappa* using TLC densitometric method. The percentage of thymol isobutyrate found in the roots of *Inula cappa* was 0.0423% w/w.

Two compounds were isolated from the roots of *Inula cappa* using column chromatography, characterized by recording UV, IR, Mass and NMR spectra and identified as Isoalantolactone and Germacrenolide, by comparing them with the reported data. They were used as standards to develop TLC densitometric methods for their quantification in roots using HPTLC. The percentage of Isoalantolactone and Germacrenolide were found to be 0.042% w/w and 0.043% w/w respectively.

6.2 Antioxidant activity

Oxidation is one of the major causes of chemical spoilage, resulting in rancidity and/or deterioration of the nutritional quality, colour, flavour, texture and safety of food (Antolovich et al., 2002). Natural antioxidants not only protect food lipids from oxidation, but may also provide health benefits associated with preventing damage due to biological degeneration (Davies, 1995). Moreover, aerobic mammals use oxygen to maintain normal physiological functions, and up to 2% of oxygen consumption may end in the form of reactive oxygen species (ROS) (Davies, 1995; Yuan and Kitts, 1996). ROS are oxygen derivatives with unpaired orbital electrons and as a result are unstable and highly reactive. ROS include hydroxyl radical, superoxide radical, peroxyl radical and singlet oxygen (Halliwell, 1995). Though some ROS are a part of normal metabolism (Davies, 1995; Halliwell, 1995), cigarette smoking and exposure to environmental oxidative stress (Halliwell and Auroma, 1997) can result in the production of exogenous sources of ROS, which may contribute to several forms of human cancer (Morse and Stoner, 1993). Exogenous
antioxidants act to supplement endogenous primary antioxidants, such as α-tocopherol, in combating against cell injury induced by oxygen radicals. In addition to ROS, reactive nitrogen species (RNS), such as nitric oxide and peroxynitrite, also have high reactivity with potentially important biological significance (Halliwell, 1995). Natural plant extracts possess the activity because of the presence of many substances, including some vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens, minerals etc. (Calucci et al., 2003). Plants, particularly those with abundant polyphenolic content, have been examined for their antioxidant potential in biological models (Cuvelier et al., 1994; Teissedre et al., 1996; Marcocci et al., 1994; Hu and Kitts, 2000; Liao and Yin, 2000).

The antioxidant and free-radical scavenging activities of *Inula cappa* have not been examined until recently. Hence it was thought of interest to screen it for its possible antioxidant activity. The antioxidant activity may result from the neutralization of free radicals initiating oxidation processes, or from the termination of radical chain reactions. For this reason, some selected methods were used to determine the antioxidant activity.

Proton-radical scavenging action is an important attribute of antioxidants. α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging assay is the most widely used method to estimate this activity. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction resulting in a color change from purple to yellow (Shon et al., 2003; Chung et al., 2002).

Out of the four extracts tested for this activity, it was observed that Extract A and A2 showed more obvious results (Table 5). However, Extract A2 exhibited more potent activity compared to the Extract A indicating that the compounds with the strongest radical scavenging activity in *Inula cappa* are of medium polarity. So far plant phenolics constitutes one of the major groups of compounds acting as primary antioxidants or free radical terminators, it was reasonable to determine their total amount in the root extract. Flavonoids, as one of the most diverse and widespread groups of natural compounds, are probably the most important natural phenolics (Agrawal, 1989). It was found that the roots of *Inula cappa* contain 2.27 % of total phenolics of which 0.31 % are flavonoids. Thus, this observed radical scavenging activity could be attributed in part to the phenolic content.
Different studies have also indicated that the antioxidant effect is related to the development of reductones (Dorman et al., 2003; Shon et al., 2003). Reductones are reported to be terminators of free radical chain reactions. Thus, the antioxidant activity of *Inula cappa* extracts, may also relate to their reductive activity. Table 8 shows the reducing power of the Extract A and A2 isolated from *Inula cappa*. Both the extracts possessed the significant ability to reduce iron III (Fe$^{3+}$) and also in a linear concentration dependent fashion, when compared with standard reducing agents like gallic acid and tannic acid. The ability of the extracts to scavenge the DPPH radical and reduce iron III (Fe$^{3+}$) indicates that the activity may be due to a common underpinning mechanism, i.e. electron/hydrogen donation. As discussed earlier, the qualitative and quantitative analysis of extracts have shown the presence of phenolics, principally chlorogenic acid and thymol, which could be responsible for the effects (Chen et al., 2004; Ruberto et al., 2002).

Superoxide, hydroxyl and peroxyl radicals represent different forms of ROS (Morse and Stoner, 1993). Superoxide radicals are generated during the normal physiological process mainly in mitochondria. Although superoxide anion is by itself a weak oxidant, it gives rise to the powerful and dangerous hydroxyl radicals as well as singlet oxygen both of which contribute to the oxidative stress (Dahl and Richardson, 1978; Meyer and Isaksen, 1995; Davies, 1995). Therefore, it is important to remove excess superoxide radical to prevent potential damage to biological molecules from toxic ROS. The suppressive effect of *Inula cappa* on the superoxide radical was confirmed in this study. Comparing results of two radical scavenging tests (DPPH and superoxide radical) good correlation between them can be observed. Quite similar results were obtained for both the extracts in terms of its activity, where Extract A2 was found to be better scavenger than Extract A, showing more than 80% inhibition at a concentration of 300 $\mu$g ml$^{-1}$, although the activity was found to be lower than scavenging activity of the standards pyrogallol and ascorbic acid in the entire concentration ranges (Table 6). The enrichment of the phenolic substances in the ethyl acetate extract can be a possible reason for its more persuasive activity. Also the capacity of both the extracts A and A2 to scavenge superoxide radical divulge that these extracts may possess superoxide dismutase like activity.

Superoxide has also been observed to directly initiate lipid peroxidation (Wickens, 2001). Lipid peroxidation has been broadly defined as the oxidative deterioration of
polyunsaturated lipids (Kappus, 1991) leading to the formation of peroxy radicals which further, via a series of reactions fragment to aldehydes such as malondialdehyde (MDA) and polymerization products (Jadhav et al., 1996). MDA and 4-hydroxy nonenal are the major break down products of lipid peroxidation. MDA is usually taken as a marker of lipid peroxidation and oxidative stress (Janero, 1990). To evaluate the effect of Extract A and A2 on inhibition of lipid peroxidation, its activity was assessed on the rat liver homogenate. The decrease in the MDA formation, shown by a decrease in the absorbance, indicates the possible role of both the extracts A and A2 in inhibiting lipid peroxidation (Table 7). It is also evident from the results that the compounds contributing towards this activity are comparatively more polar in nature, as Extract A showed a better activity compared to Extract A2. EC50 value of Extract A was found to be 21.4 μg ml−1, which was found to be fairly less than that of the standard α-tocopherol (27.35 μg ml−1) signifying a strong activity. The components of the plant contributing to this activity may be phenolics like thymol derivatives (Alam et al., 1999), chlorogenic acid (Kweon et al., 2001) and terpenoids like lupeol (Nagaraj et al., 2000), α-amyrin (Filomena et al., 2005) etc. The reducing property of these chemical constituents can decrease the formation of oxidized intermediates of lipid peroxidation processes, and hence can act as primary and secondary antioxidants (Yen and Chen, 1995).

Free radicals can be generated by metabolic pathways within body tissues; also they can be introduced by external sources, with food and drugs, can be caused by environmental pollution etc. Biological molecules such as DNA and proteins are subject to pro-oxidative stresses induced by free radicals, which can result in various diseases such as cancer, cataract (Parejo et al., 2002) and aging (Finkel and Holbrook, 2000). Therefore, antioxidants that can quench free radicals may be implicated in the prevention of these diseases. Use of natural antioxidants, as food additives for inactivating free radicals receives a lot of attention nowadays, not only for their scavenging properties, but also because they are natural, non-synthetic products, and their appreciation by consumers is very favourable.

6.3 Anti-inflammatory activity
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The traditional claims of the use of the roots of *Inula cappa* in the treatment of arthritis, the preliminary phytochemical studies and the antioxidant activity prompted us to test it for the anti-inflammatory activity.

Carrageenan-induced rat paw oedema is a suitable experimental animal model for evaluating the anti-oedematous effect of natural products. It is believed to be a phase reaction in which several mediators (histamine, 5-hydroxytryptamine, kinins, and prostaglandins) of the inflammatory process are involved (Vinegar *et al.*, 1969; Willis, 1969; Willoughby *et al.*, 1969). Di Rosa *et al.*, (1971) showed that carrageenan induced inflammation is divided into 3 steps according to the mediators released. In the initial step (first 90 min), the release of both histamine and 5-hydroxytryptamine (5-HT) occurs; the second step (from 90 to 150 min) is mediated by kinins, while in the third step (from 150 min on), prostaglandin release takes place.

Histamine and 5-HT are responsible for vasodilation and increase in vascular permeability in the initial phase of the inflammatory process. Bradykinin has been implicated in acute inflammatory processes due to its ability to induce an increase in the blood vessel permeability. Its involvement in carrageenan- induced oedema was demonstrated by Ronald and Christopher (1990) and by Damas and Remacle-Volon (1992). Histamine, 5-HT and bradykinin are able to induce nitric oxide (NO) release from vascular endothelial cells *in vitro* via a mechanism involving receptor occupation and stimulation of NO synthetase. In addition, macrophages produce NO when activated by lipopolysaccharide or cytokines. Thus Salvemini *et al.*, (1996) pointed to NO as an important mediator of carrageenan- induced oedema and suggested that constitutive NO synthetase (cNOS) acts at the initial steps, while inducible NO synthetase (iNOS) is involved in the last step of the inflammatory reaction.

Another characteristic of carrageenan induced oedema is the massive infiltration of polymorphonuclear leucocytes observed in the third step (Di Rosa and Willoughby, 1971; Ialenti *et al.*, 1992; Masso *et al.*, 1993).

The present work showed that Extract A, A1, A2 and A3, administered orally at a dose of 300 mg kg⁻¹ body weight in rats, inhibited carrageenan-induced rat paw oedema (Fig. 16). Extract A showed significant anti-inflammatory activity (30-62%,
p<0.05) comparable to that of indomethacin (78.29%) against carrageenan induced acute pedal oedema.

The inhibition was significant after 3 hour, when bradykinin is being released and there occurs an accumulation of prostaglandins and infiltration of polymorphonuclear cells. At this stage, there is also production of free radicals (Boughton-Smith et al., 1993; Bourich et al., 2003), among other mediators. This indicates that the Extract A may be acting by inhibiting release of the kinins in the initial phase and also inhibiting the infiltration of the polymorphonuclear leucocytes (PMN) in the later phase. Carrageenan paw oedema is sensitive to antioxidants (Boughton-Smith et al., 1993). Thus, it may be inferred that the antioxidant activity of the Extract A helps in mediating impairment in the late phase of the anti-inflammatory response. However, it was found that the inhibitory activity on the release of chemical mediators was less compared to the Extract A2 (Table 9).

Extract A2 was found to be more potent in the initial hours but was less active in the later stages than Extract A. This indicates that the activity may be mainly due to the inhibition of the release of chemical mediators. The Extracts A1 and A3 were found to be inactive in this model of inflammation. Although, extracts A and A2 were found to be equipotent, only Extract A was tested further at lower doses, because the yield of Extract A2 was very low (2.5 %).

From the results of carrageenan induced pedal oedema in rats, it was proved that both the extracts A and A2 possess anti-inflammatory activity. It was also found that Extract A2 was more active in the initial phase than the Extract A. To evaluate the effect of extracts A and A2 on the inhibition of the release of chemical mediators, histamine induced pedal oedema model was selected. In this model, oedema was induced by subcutaneous injection of 50 μl of 1% freshly prepared solution of histamine into the hind paws of the rats after 30 min of oral administration of extract A, A1, A2 and A3.

Histamine is stored in mast cells and basophils largely complexed to mucopolysaccharide (glycosaminoglycans) such as heparin. Histamine is said to have a role in diverse functions including primary, local dilation of small vessels; widespread arteriolar dilatation; local increased vascular permeability by contracting
endothelial cells (Rowley and Benditt, 1956); the contraction of nonvascular smooth muscle; chemotaxis for eosinophils; and blocking T lymphocyte function.

Histamine and 5- hydroxytryptamine (5-HT) are responsible for vasodilation and increase in vascular permeability in the initial phase of the inflammatory process (Rowley and Benditt, 1956). Furthermore, histamine also induces nitric oxide (NO) release from vascular endothelial cells which leads to the inflammatory conditions. Therefore, the effects of extracts A and A2 on oedema induced by histamine in rats was investigated.

Both extracts A and A2 inhibited the histamine induced hind paw oedema at the dose of 300 mg kg⁻¹ per oral. However activity of the Extract A2 (53.53% inhibition, p<0.05) was stronger compared to Extract A (43% inhibition). This strong antihistaminic activity could impair the microvascular leakage induced by carrageenan thereby inhibiting the initial phase of inflammation (Kuriyama et al., 2000).

This antihistaminic activity of both the extracts could be due to the presence of terpenoids and flavonoids which are known to impair histamine release from the mast cells and exert anti-inflammatory effects (Janaki et al., 1999). Extract A1 and A3 were found to be inactive against histamine induced mice paw edema model.

Chronic inflammation may be defined as inflammation of prolonged duration (weeks to months to years), in which active inflammation, tissue injury and healing occur at the same time. Chronic inflammation is a reaction arising, when the acute response is insufficient to eliminate proinflammatory agents.

The inflammation may be due to the persistent release of chemical mediators which induce tissue destruction, persistent increase in blood flow, increased vascular permeability, recruitment of inflammatory cells, macrophages, lymphocytes, plasma cells and proliferation of parenchymal cells (epithelial) and supportive cells (fibroblasts, capillary endothelial cells) (Dunne, 1990; Arrigoni-Maratellie, 1988). These cells can either spread or remain in granuloma form.

The cotton pellet test is considered as a suitable model for studies on chronic inflammation (Dhawn and Srimal, 2000); and inflammatory granuloma is considered as a typical feature of established chronic inflammatory reaction (Spector, 1969). Herein, a small incision was made in the dorsal region, near axila and the autoclaved
cotton pellet weighing 20 mg was implanted subcutaneously and the wound was then closed by suturing. The animals were treated with extracts A and A2 (300 mg kg⁻¹ body wt. per oral) for 7 days and sacrificed on 8th day; the granulomas dissected out and dried at 80°C to constant weight. The mean weights for different groups were determined and compared to the control group. For this and further experiments, only extract A and A2 were used as the other extracts were found to be inactive.

The extracts A and A2 were effective in suppressing granuloma formation in this model by more than 50 % (p<0.01), indicating a possible activity in the chronic inflammatory conditions. This also reflects its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation (Arrigoni-Martellie, 1977; Recio et al, 1995).

Presence of chemical constituents like volatile oil, triterpenes and flavonoids in the plant may be contributing to the activity as they are reported to possess analgesic, anti-inflammatory, fever reducing and antioxidant properties (Al-Rehaily, 2001, 2002). Since cotton pellet-induced granuloma corresponds to the proliferative phase of inflammation, it is possible that, in this inflammatory model, the extracts A and A2 act by inhibiting neutrophil migration.

In the present study, macroscopic examination of the gastric mucosa did not reveal any treatment-related tissue damage, which is a collateral effect of many anti-inflammatory drugs, including aspirin and related compounds (Quellet and Percival, 1995; Terlains et al., 1995). The anti-inflammatory activity without gastric lesions led us to believe that the active principle/s of extracts A and A2 did not interfere with prostanoid production. They might also act by selective inhibition of COX-2, and this could be of therapeutic advantage, because this isoenzyme is probably involved in prostaglandin production at the site of inflammation but not at any other site such as the gastrointestinal tract or kidney.

The prostaglandins (PGs) are a family of lipid-soluble hormone-like molecules produced by different cell types in the body. It is important to note that unlike histamine, prostaglandins do not exist free in tissues, but have to be synthesized and released in response to an appropriate stimulus. PGE² enhances vascular permeability, is pyrogenic, increases sensitivity to pain, and stimulates leukocyte cAMP, which can
have important suppressive effects on release of mediators by mast cells, lymphocytes, and phagocytes (Turini and DuBois, 2002).

Thus, PGs are also involved in both early and late phases of inflammation (Kikuchi et al., 1996) and most of the anti-inflammatory drugs act primarily by inhibiting PG synthesis. The PG synthesis involves cyclooxygenase (COX) that exists in two isoforms COX-1 and COX-2. COX-1 is thought to produce PGs, important for homeostasis and certain physiological functions and is expressed constitutively in most tissues and cells (O’Neill and Ford-Hutchinson, 1993). COX-2, that is inducible and expressed in inflammatory cells, is primarily responsible for PG production at the site of inflammation (Vane et al., 1994).

The inhibition of the prostaglandins synthesis by Extract A, as evident from the marked inhibition (47.5 %, p<0.05) of the amplitude of the uterine contraction of diestrous rats at a dose of 1 mg ml^{-1}, suggests its possible role in the COX-2 inhibitory activity. This activity is espoused by its antioxidant activity, as hydroxyl radical scavengers have been reported to suppress upregulation of COX and subsequently reduce inflammation (Feng et al., 1995; Kumagai et al., 2000). The extract may also be acting directly by inhibiting the COX enzyme because of the presence of phytochemicals like flavonoids (Carlo et al., 1999; Kim et al., 1996; You et al., 1996) tannins (Muruganadan and Raviprakash, 2001) and terpenoids (Fernandez et al., 2001, Suh et al., 1998). The ability of Extract A to obstruct the prostaglandin synthesis by restraining the expression of COX-2 enzyme also advocates for its inhibitory activity in the second phase of carrageenan induced oedema. Extract A2 was not tested for this activity as it showed a comparatively very less activity in the second phase of inflammation.

Compounds with membrane-stabilizing properties are well known for their ability to interfere with the early phase of inflammatory reactions, namely the prevention of the release of phospholipases that trigger the formation of inflammatory mediators (Aitadafoun et al., 1996).

It is well known that the vitality of cells depends on the integrity of their membranes (Ferrali et al., 1992). Exposure of red blood cells (RBC) to injurious substances such as hypotonic medium and heat results in lysis of its membrane accompanied by haemolysis and oxidation of haemoglobin (Augusto et al., 1982; Ferrali et al., 1992).
Such injury to RBC membrane will further render the cell more susceptible to secondary damage through free radical-induced lipid peroxidation (Augusto et al., 1982; Ferrali et al., 1992). This notion is consistent with the observation that the breakdown of bio-membranes leads to the formation of free radicals which in turn enhance cellular damage (Halliwell et al., 1988; Maxwell, 1995). The progression of bone destruction seen in rheumatoid patients for example, has been shown to be due to increased free radical activity (Cotran et al., 1999; Pattison et al., 2004). It is therefore expected that compounds with membrane-stabilizing properties should offer significant protection of cell membrane against injurious substances (Shinde et al., 1999; Liu et al., 1992).

Extract A of *Inula cappa* inhibited heat-induced haemolysis in a concentration dependant manner suggesting a membrane stabilizing activity (p<0.05, Table 13) when tested using the method given by Caprino (1974) in rat erythrocytes. This membrane stabilizing activity may be responsible for the inhibition of the lipid peroxidation as observed earlier. One of the possible mechanisms of the anti-inflammatory activity of the Extract A may be the inhibition of the release of phospholipases hence preventing the synthesis of prostaglandin by the degradation of arachidonic acid via cyclo-oxygenase pathway. Extract A was also shown to inhibit the COX-2 enzyme as discussed earlier.

Mediators of inflammation, such as histamine, prostaglandins and leucotrienes are released following stimulation of mast cells. This leads to a dilation of arterioles and venules to an increased vascular permeability. As a consequence, fluid and plasma proteins are extravasated and oedema is formed (Morris, 1999).

Pleurisy is a well known phenomenon of exudative inflammation in man. In experimental animals, pleurisy can be induced by several irritants, such as histamine, bradykinin, prostaglandins, mast cell degranulators, dextran, enzymes, antigens, microbes and nonspecific irritants like turpentine and carrageenan (DeBrito, 1989).

The pleurisy model has been accepted as a reliable method to study acute and subacute inflammation allowing the determination of several parameters simultaneously or successively. The activity of steroids as well as of non-steroidal drugs can be evaluated using this model (Tomlinson et al. 1994; Harada et al. 1996).
Carrageenan-induced pleurisy in rats is considered to be an excellent acute inflammatory model in which fluid extravasation, leukocyte migration and the various biochemical parameters involved in the inflammatory response can be measured easily in the exudate.

The present study was designed to evaluate the role of Extract A on the increased cell infiltration and fluid exudation involved in inflammation produced by carrageenan in rat model of pleurisy.

It was observed that Extract A not only produced significant inhibition of fluid exudation, but was also effective in reducing leukocyte infiltration. A marginal decrease in total leukocyte count (TLC, $4.5 \times 10^6$ cells ml$^{-1}$) was also obtained with Extract A at 4 hour as compared to TLC of the control group ($6.9 \times 10^6$ cells ml$^{-1}$, Table 12). The TLC of the standard dexamethasone was found to be $1.3 \times 10^6$ cells ml$^{-1}$. Injection of carrageenan in the pleural cavity induces an acute (4 hour) inflammatory reaction characterized by protein extravasation (Henriques et al., 1992). The pre-treatment with Extract A, also significantly inhibited the protein extravasations induced by carrageenan (Fig. 19).

This indicates that the Extract A reduces the capillary permeability thus leading to a reduced dilation of arterioles and venules and to a decreased vascular permeability. Furthermore, it diminishes the fluid exudation and protein extravasation. This activity is supported by its membrane stabilizing activity and also its ability to inhibit prostaglandin synthesis. Thus, it can be said that the extract A and A2 may be inhibiting the synthesis of prostaglandins by acting through the cyclo-oxygenase pathway to prevent the degradation of phospholipids to arachidonic acid. However, all the activities were less when compared to the animals pretreated with standard phenylbutazone. It is interesting to note that the Extract A showed a good anti-inflammatory activity for the acute rat paw oedema model which is strongly dependent on protein extravasation and also in cotton pellet granuloma which is dependant on the increase in vascular permeability.
6.4 Immunomodulatory activity

Immunomodulators are becoming very popular in the worldwide natural health industry as people start to realize the importance of a healthy immune system in the maintenance of health and the prevention and recovery of disease.

Recently, many immune targets have been identified as having potential for central control of inflammation and there is more use of the drugs that reduce inflammation by targeting the immune system itself rather than the site of inflammation. Immune system acts by both the specific and non specific defence mechanisms. Phagocytosis is considered to be the main non-specific defence mechanism. Mononuclear phagocytes or macrophages are central participants in both non-specific and specific immune responses (Sorensen et al., 1997). Phagocytes are located in most tissues and organs of the body and in addition to other activities macrophages govern immunological responses by the recognition and digestion of foreign substances (Seljelid and Eskeland, 1993). Potentiation of non-specific defence mechanisms may occur during microbial invasion, leading to more efficient clearance and destruction of pathogens or other harmful substances (Dalmo et al., 1997).

The most important facet of phagocytosis is its control of inflammation. Depending on the phagocytosed particle, phagocytosis can induce inflammation or, as is the case with apoptotic cells, induce resolution of inflammation. Phagocytosis is also involved in immune tolerance, which prevents inflammation against normal components of the body.

The phagocytic activity of the RES macrophages can be measured from the rate of blood clearance of intravenously injected colloidal carbon particles. Carbon particles (diameter about 200 Å) injected intravenously can not filter through the capillary membrane and remain in the circulation until they were phagocytized by the reticuloendothelial system macrophages in contact with the circulating blood (Blozzi et al, 1953).

The carbon clearance activity from circulating blood was rapidly enhanced by the extracts A and A2 at a dose of 300 mg kg$^{-1}$ in mice; the enhancement is apparent as can be seen from the values of phagocytic index (K) (Table 15). It was also observed that the clearance activity was comparable for both the extracts A and A2. However extracts A1 and A3 did not show any significant rise in the phagocytic activity at the
same dose levels when compared to the control group. So far a higher phagocytic index is associated with a proinflammatory condition. However there are some reports which establish the anti-inflammatory activity coupled with the PMN mediated phagocytosis (Gasser and Schifferli, 2004). From the earlier results, which establish the anti-inflammatory activity of the extracts, we can say that the increased phagocytic activity shown by the extracts A and A2 may be PMN regulated.

Reversible adherence of leukocytes to endothelium, basement membranes and other surfaces is an essential event in the establishment of inflammation. Their entry into tissues is controlled by the dynamic interaction between adhesion molecules expressed by these cells and the endothelium. White cells circulating in the blood have the tendency to adhere to the walls of blood vessels and this tendency is greatly increased in states of inflammation. Normally, when leukocytes collide with the vessel wall, the collision behaves elastically and the cells bounce off and back into the lumen. However, biochemical changes in inflamed tissues results in inelastic collisions of cells and an increase in their adhesion, thus initiating rolling of leukocytes along the endothelial surface. As adhesion further increases, rolling is slowed and may be followed by the cells coming to a complete stop and their migration out of the vessel (Morris, 1999).

The increased phagocytic activity of the root extract led us to evaluate it for neutrophil adhesion test using a simple, rapid, in vitro assay for granulocyte adherence developed by Mac Gregor et al. (1974) with some modifications. For this experiment, only Extract A was tested as the yield of Extract A2 was very less and also the activity was comparable to the Extract A in all the previous models tested. It was observed that Extract A, when administered orally at a dose of 300 mg kg⁻¹, reduced the granulocyte adherence rate when compared with the control group (Fig. 22). This decreased neutrophil adhesion may also be responsible for the anti-inflammatory activity of the Extract A (Stecher and Chinea, 1978).

But unlike the previous experiments the activity was found to show a different pattern with the maximum reduction observed at a dose level of 150 mg kg⁻¹. The reduction in adherence was not very significantly less at a dose level of 300 mg kg⁻¹ body weight as compared to the 75 mg kg⁻¹ and 150 mg kg⁻¹ body weight. A possible explanation could be the preactivation of the PMN’s (Kuijper et al., 1997) by the Extract A at higher dose as observed with the phagocytosis experiment.
Immunostimulants are substances which enhance the nonspecific defense mechanisms as well as specific immune responses, if the treatment is followed by infection or vaccination.

The concept of immunomodulation relates to non-specific activation of the function and efficiency of macrophages, granulocytes, complement, natural killer cells and lymphocytes, and also to the production of various effector molecules generated by activated cells (Para immunity).

Immunostimulatory effects of a drug or nutritional supplement are difficult to evaluate in healthy people and animals. Therefore, in the present study, myelosuppression was induced in mice by an intraperitoneal injection of cyclophosphamide (50 mg kg\(^{-1}\)) daily for three days (Davis and Kuttan, 1998; Shalit et al., 2001). Myelosuppression is a reduction in the ability of the bone marrow to produce blood cells. The bone marrow produces three main types of mature blood cells: platelets, red blood cells and white blood cells. Each of these cell types has a distinct and important function. Myelosuppression means that any or all of the three main types of blood cells which are normally produced in the bone marrow are decreased in number and/or may take a prolonged period of time to return to "normal levels" (Brunton et al., 2005).

In comparison to the cyclophosphamide-treated control group, animals receiving Extract A had an accelerated regeneration of total leukocyte count (Fig 25). The WBC count was low at the 75 and 150 mg kg\(^{-1}\) doses, on a par with the cyclophosphamide group, but at 300 mg kg\(^{-1}\) dose, the count increased significantly \((p < 0.01)\) after a treatment for 10 days. Herein, it particularly provided protection against cyclophosphamide induced suppression of humoral immunity, an important component of defense system of body. These results followed a pattern similar to those reported for Aloe vera, which contains phenolics having anti-inflammatory potential, and which also showed B-cell stimulation to a significant extent (Hartwell, 1969). From this study, it can be assumed that the Extract A may be having some immunostimulant properties. However, it cannot be ruled out that this stimulant property of the extract may be due to a longer duration of treatment.

As discussed earlier, the immune response of the body is mainly composed of specific and non-specific immunity. The specific immune response includes humoral and cellular immunity. Humoral immunity is regulated by B cells. They arise from a
separate population of stem cells of the bone marrow. These B-cells then develop into short-lived plasma cells. The plasma cells produce antibodies and release them into the circulation at the lymph nodes. Some of the activated B-cells do not become plasma cells instead they turn into memory cells which continue to produce small amounts of the antibody long after the infection has been overcome. The B-cells are produced each with a different antigen binding specificity. If the B-cell comes in contact with the specific type of antigen to which it is targeted, it divides rapidly to form a clone of identical cells (Delves et al., 2006).

The stimulation of the humoral response against sheep red blood cells (SRBC's) by Extract A is evidenced by the increase in HA titre assay in mice. Extract A (75-300 mg kg\(^{-1}\), p.o.) produced a dose dependant increase in the antibody synthesis (Table 16). The maximum effect was observed at a dose of 300 mg kg\(^{-1}\). This result is also supported by the in-vitro plaque forming assay, where nearly three-fold increase in the IgM antibody plaque formation was observed in the spleen cells of Extract A (300 mg kg\(^{-1}\)) treated mice as compared to control animals (Fig. 24). The rise in the antibody production specifies that the extract might be having immunostimulant activity on the humoral immune system. This increase in the antibody production may be because of the presence of certain terpenoids in the plant (Dua et al., 1989; Puri et al., 1993).

A delayed type hypersensitivity (DTH) reaction is an expression of cell-mediated immunity and plays a role in many inflammatory disorders. Such reactions are characterized by large influxes of non-specific inflammatory cells, of which the macrophage is a major example. It is a type IV hypersensitivity reaction that develops when antigen activates sensitized T\(_{DTH}\) cells. These cells generally appear to be a Helper T cells (TH1) subpopulation although sometimes Cytotoxic T cells (TC) cells are also involved. Activation of T\(_{DTH}\) cells by antigen presented through appropriate antigen presenting cells results in the secretion of various cytokines including interferon-gamma (secreted by TH1 cells). The overall effects of these cytokine are to recruit and activate macrophages, thereby promoting increased phagocytic activity. Several lines of evidence suggest that DTH reactions are important in host defense against parasites and bacteria that can live and proliferate intracellularly (Luis et al., 2000).
A model of cell-mediated inflammation, SRBC induced DTH, was used to investigate the immunomodulatory effect of Extract A, and a significant difference in paw thickness was observed between Extract A fed mice and negative control (normal saline) mice. It was observed that the control group evidenced a normal cell-mediated immune response to the sheep erythrocytes with a mean 46% increase in paw thickness, whereas in the Extract A fed mice, mean paw thickness was 0.017 mm (6.89% increase). DTH reaction to SRBC is shown in Fig 23a, in which data is expressed in terms of the swelling of the footpad. Administration of the Extract A (75-300 mg kg\(^{-1}\)) produces a dose dependant decrease in the DTH reaction compared to control group, however, the difference between the groups receiving 75 mg kg\(^{-1}\) and 150 mg kg\(^{-1}\) of Extract A is not significant (Fig. 23b).

In immunoprophylactic assay (Table 18), when mice of treated and untreated groups were subjected to fatal dose of *E. coli* (2.5 \(\times\) 10\(^8\) cells suspended in normal saline), Extract A treated groups showed significantly less percentage of mortality (33.3%) compared to the control group (100% mortality).

Treatment with Extract A diminishes the DTH reaction, as reflected by the decreased footpad thickness compared to the control group, suggesting reduced infiltration of macrophages to the inflammatory site. The reports have also indicated suppression in the immune response due to impaired leukocyte and cytokine balance and the failure of T cell response to T-dependent antigen.

T lymphocytes play important roles in inflammatory responses especially in the initial phase of the inflammation and certain agents, such as immunosuppressive agents, nicotine or corticosteroids, exert anti-inflammatory effects via inhibition of T cells (Madretsma *et al.*, 1996).

Colorimetric 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to analyze the effect of the Extract A on the lymphocyte proliferation, using the splenic and thymic lymphocytes, since the cleavage of MTT has several desirable properties for assaying cell survival and proliferation. MTT is cleaved by all living and metabolically active cells and the amount of MTT formazion generated is directly proportional to the cell number (Mossmann, 1983).

Splenocyte proliferation is a complex event that involves interaction of Interleukin 2 (IL-1) and Interleukin 2 (IL-2) and expression of their receptors (Smith, 1988). IL-2
is required for T cell proliferation (Rothenberg et al., 1990), and the inhibition of IL-2 production might reflect a decrease in the proliferation of lymphocytes from spleen. Immunosuppressants act by decreasing the production of IL-2 and T lymphocyte proliferation and increasing the expression of IL-1, nitric oxide (NO) and interferon (IFN).

In the present study, it is observed that the Extract A inhibited the proliferation of the splenocytes under ex-vivo conditions (Table 19 and Fig. 25). It is interesting to note the pattern of action of Extract A. It enhances the proliferation at 1 µg ml⁻¹ concentration of Con A but at higher concentrations decrease the proliferation.

The action produced by Extract A ex-vivo was not associated with a parallel effect on lymphocyte proliferation under in-vitro conditions (Fig. 26). In the in vitro conditions, Extract A shows a dose dependent increase in the proliferation (17-45%) when compared to the control group. Figure 27 and 28 reveals that Extract A is not effective in stimulating the thymocytes as well, treated in a similar manner, under the ex-vivo conditions but shows a dose dependent increase under the in-vitro condition with the maximum at a dose of 80 µg ml⁻¹.

Extracts A1 and A2 both inhibit the proliferation at all the concentrations of Con A under ex-vivo and in-vitro conditions when compared to control group, though under the in-vitro conditions it enhances the proliferation at a concentration of 40 µg ml⁻¹ of the respective extracts. Extract A3 on the other hand shows an opposite effect to Extract A in both the conditions. From the proliferative indices (Table 19), it can be assumed that the components responsible for the proliferation of lymphocytes are highly polar in nature.

The inability of Extract A to enhance the Con A stimulated mitogenic response of lymphocytes obtained from spleen and thymus under the ex-vivo conditions, is likely due to the interference of the other components of the immune system and metabolic degradation of the active constituents. It is also reasonable to postulate that Extract A at increasing doses facilitate more suppressor cell clones to the extent that a great deal of suppressor cell activity is established and the immune response gets insignificant as we can see a similar response in the delayed type hypersensitivity response model.

The in vivo results in DTH model also indirectly suggest that the in vitro inhibitory effect of Extract A was not due to cell cytotoxicity, but rather to an immunomodulatory effect. In addition, 300 mg kg⁻¹ per day dose of Extract A in mice
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orally is generally believed to be a non-toxic concentration, though some plant extracts have been reported to be toxic in terms of increasing the serum concentrations of glutamate–oxaloacetate transaminase (GOT) and glutamate–pyruvate transaminase (GPT) in mice at concentrations exceeding this level (Mesia et al., 2005).

Alternatively, these results could be due to either the production of cytokines with antiproliferative effect on responding T cells or the induction of biochemical mediators from macrophages that could decrease proliferation but it cannot be confirmed without a detailed study in one similar line.

Extract A of *Inula cappa* showed radical scavenging activity in DPPH model and superoxide model with EC\(_{50}\) 116.64 µg ml\(^{-1}\) and 297.28 µg ml\(^{-1}\) respectively (Table 5 and 6). Inhibition of lymphocyte proliferation by Extract A could be due to the radical scavenging activity as there are reports confirming similar action of well-known antioxidants like resveratrol, vitamin E, gallic acid and other polyphenols (Serrano et al., 1998; Pighetti et al., 1998; Gao et al., 2001).

In different experimental models, it is proposed that NO is responsible for the inhibition of DNA synthesis in several cells (Drapier and Hibbs, 1986; Orsi et al., 2000), promotion of cytostasis in tumor target cells (Kwon et al., 1991; Pervin et al., 2001) and depression of T cell proliferation. This has been shown in allogenic mixed lymphocyte response (Hoffman et al., 1990) and in fungal infections (Zhou et al., 1995; Bocca et al., 1998). Extract A treatment could pre-activate splenic macrophages *in vivo* to produce NO, which in turn could be responsible for the inhibitory effect against lymphocyte proliferation.

Extract A also inhibited the production of the proinflammatory cytokines, IL-2 in Con A-activated splenocytes. Splenocytes isolated from Extract A fed mice were less activated than those from untreated controls, and when stimulated with Con A produced lower levels of cytokines. As seen from the results, (Fig. 33) Extract A decreases IL-2 production when compared to the control group. However the decrease is not significant (12.6%). The inhibition of the production of the IL-2 in Extract A feeding mice, indicates that it might induce a regulatory immune system *in-vivo*, such as, those described for regulatory T cells (Schwartz, 2005) or directly affect
lymphocyte proliferation. Taken together it can be said that the extract has a tendency to exhibit the suppressant effects on the immune system.

As said earlier that the immunostimulatory effects of a drug are difficult to evaluate in healthy animals therefore in this study, hydrocortisone acetate (HCA) was used as an immunosuppressant to establish an animal model with decreased immune function. The results showed that HCA suppressed the immune function of mice in many respects that agree with the literature (Gillis et al, 1979), which is evident from the significant inhibition observed in the lymphocyte proliferation rate and IL-2 production (Table 23, Fig. 33) in the HCA control group.

From the results obtained in this study, it is apparent that Extract A could bring about a significant recovery of the splenocyte proliferation (51.5%) in HCA immunocompromised mice; however it was comparatively less than the normal control group. Similar results are obtained for the thymocyte assay, too. This contradicts previous assumption that Extract A possesses an immunosuppressant effect. It is also supported by the results of the cyclophosphamide induced myelosuppression, wherein Extract A exhibited a tendency to bring back the leukocyte count to the normal. Extract A was also found to be effective in the recovery of IL-2 production in immunocompromised mice although not very significant (Fig. 33).

To summarize investigations carried out on Extract A revealed that it showed a stimulatory activity under in-vitro conditions but it was found to suppress the proliferation under the ex-vivo conditions.

This stimulatory effect may be due to the presence of certain phenolic compounds such as chlorogenic acid and monoterpenoids in the extract (Chiang et al, 2003). The phenolic derivatives are concentrated in the extracts which gives a stimulatory effect. This is again clear from its concentration dependent stimulatory effect.

Besides this, Extract A did not reveal any untoward effect on behavioral response, body weight, normal reflexes and visceral appearance in the mice. It has not produced any histopathological changes in the major organ tissues and the hematological parameters are found to be normal, too.

Histological examination of liver section of Extract A fed mice showed normal hepatic lobules. There were no signs of congestion, inflammation, cellular necrosis or
cholestasis in control liver sections. Neutrophils were present in the midzonal region around the central vein. A section of the kidney showed that the cells were well arranged and uniformly stained. Spleen cells show a normal distribution of the red pulp together with white pulp. The lung tissue was also found to show normal alveoli but in one section foci was showing branchiectesis.

In the present study, we could not identify the components of Extract A those are responsible for this immunomodulatory activity. However, *Inula cappa* is known to contain ingredients like thymol, chlorogenic acid, lupeol, α-amyрин and germacrrolides. They were identified in the plant by co-chromatography using the standards. Two sesquiterpenes were also isolated from the methanolic extract using column chromatography.

In summary, the present study sets the standards for *Inula cappa* roots in terms of its microscopical studies and phytochemical analysis. Two sesquiterpenes (Isoalantolactone and germacrnomelide) were isolated and quantified in the roots. This study gives the first report on the presence of chlorogenic acid and α-amyрин in the roots of *Inula cappa*. Thymol isobutyrate was quantified using a new HPTLC method. Lupeol and β—sitosterol were also quantified in the roots for the first time. TLC fingerprinting developed can be a useful tool for the identification of the raw material. The identification and quantification of the marker compounds provide the data for the standardization for the drug. Pharmacological investigations reveal that Extract A has profound anti-inflammatory, antioxidant and immunomodulatory activity on humoral and cell mediated immune responses. Further, the anti-inflammatory activity could be due to its ability to inhibit prostaglandin synthesis, scavenge the free radicals and to some extent because of its immunomodulatory potential to inhibit the lymphocyte proliferation. Moreover, Extract A was found to be non-toxic up to a dose level of 1.5 g kg⁻¹ body weight.

In conclusion, the studies provide supportive evidence towards the traditional claims made for the roots of *Inula cappa* as a potent anti-inflammatory and immunomodulatory agent, adding value to its actual utility in the treatment of various ailments.
6.5 References


Discussion


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