Chapter-5

CONCLUSION
Conclusion

Immune system is the complex network inside our body which plays a crucial role in defending against any harmful stimuli or invading pathogens. Immune system works by utilizing two distinct units of immunity i.e. innate immunity and adaptive immunity. The innate immune response works as the immediate line of host defense whereas adaptive immune response takes several days to develop and come into action. Whenever our body encounters the attack of any invading pathogen or harmful stimuli a complex cascade of events occur inside our body which involves the role of various inflammatory cells like neutrophils, macrophages etc. and is termed as the inflammatory response and the process is termed as inflammation. Inflammation is broadly categorised into two forms depending upon the duration and severity i.e. acute inflammation and chronic inflammation. Till date various drugs have been developed for reducing the inflammatory condition generated within body like the conventional NSAIDs, etc. But, these conventional medicines come with their associated side effects hence since ages people have looked forward towards the use of natural phytochemicals for treatment. The knowledge of Ayurveda and the folklore medicines used locally have always been looked for developing new drugs for various diseases. One such commonly used plant in folklore medicine is *C. asiaticum*. The leaves of the plant is widely used in the northern and north eastern part of India as well as Vietnam for treatment of various inflammatory disorders but no significant scientific study has been conducted till date with this plant for its efficacy in an acute as well as chronic inflammatory conditions. The present study was undertaken to study the anti-inflammatory property of *C. asiaticum* in acute inflammation models as well as in arthritis, a chronic inflammation condition developed in rats.

Before studying the anti-inflammatory property of the plant, its methanolic leaf extract was evaluated for any associated typical side effects generally observed with conventional NSAIDs. In contrast to commonly used NSAIDs such as ASA, diclofenac, the *C. asiaticum* extract did not result in any ulcerogenic effect. In addition
it was found to correct the solvent (DMSO in our case)- ulcerogenic effect. Further, even upon chronic exposure for continuously ten days to *C. asiaticum* extract the animals did not show any marked toxicity or adverse effect suggesting the extract to be safe for use.

Various acute inflammation models were developed by using different chemicals known to induce inflammatory response such as xylene, dextran, as well as histamine. It was observed that the plant was effective against all the acute inflammation models tested in a dose dependent manner being more effective at a higher dose of 400mg/kg b.w. Further in a combination study with the conventional NSAID drug ASA, the *C. asiaticum* extract did not show any synergistic effect either against histamine-induced or dextran-induced inflammation. This suggested that may be both ASA and *C. asiaticum* extract were utilizing same pathways for their anti-inflammatory response. In addition, the *C. asiaticum* extract also exhibited potent anti-pyretic effect which was revealed by significant decrease in yeast-induced fever in rats. Membrane integrity, yet another parameter which gets immensely perturbed during inflammation was also found to be significantly restored with *C. asiaticum* extract in hypotonicity-induced RBC membrane permeabilization model. From all the above studies, it was very clear that the *C. asiaticum* leaf extract possessed a potent anti-inflammatory activity and definitely merits further study to evaluate its anti-inflammatory role in chronic conditions.

In order to evaluate the effect of *C. asiaticum* on chronic inflammation, an arthritis model mimicking chronic inflammatory condition was developed using complete Freunds’ adjuvant. The arthritis was developed in the Wistar rats and various parameters, commonly also found in case of humans’ arthritis were established. The following parameters were considered such as decrease in the body weight, increase in the hind paw thickness as well as an increase in the arthritis score. To further confirm radiological analysis of the bone joints of hind paw as well as tail bone were also done. The diseased animals exhibited a loss of joint spaces, change in bone density in a similar fashion as observed in humans. Since macrophages are known to play a pivotal role in any inflammation either acute or chronic, the change in macrophage activity was also considered as one of the parameters to be studied. The
very clearly evident modulations in macrophage activity in the arthritic rats further corroborated the development of arthritis CFA injected rats.

Once all suggested parameters were well established in arthritis rat, the effect of C. asiaticum extract was evaluated for its anti-arthritic potential. It was found that the rats given treatment with C. asiaticum extract before the induction of disease with CFA showed a very significant decrease in all the measured parameters of arthritis. The usual decline in body weight with arthritis was restored very nearly to the normal levels. Further, there was marked inhibition in the hind paw swelling and arthritis score of the diseased animal with the extract treatment. The radiological examination also revealed that the animals which received the treatment of plant extract showed relatively very less or no fusion of joints as well as there was less opacity both, in the hind paw bones as well as the tail bones. In addition, in the in vitro study with RAW264.7 macrophage cells it was observed that the C. asiaticum extract also possessed very potent anti-inflammatory activity towards macrophage functions. Both the phagocytic activity as well as the release of nitric oxide by the LPS-activated macrophages was very significantly down-regulated to normal levels. Thus the above set of experiments very strongly indicated that the methanolic extract of the plant C. asiaticum possessed potent anti-arthritic effect supporting its use in traditional medicine.

Phytochemical analyses of the C. asiaticum leaf extract showed the presence of number of components. In order to carry out plant extract characterization, GC-MS technique was used and the major peaks obtained were matched with NIST-15 and WILEY-08 library. Ten major peaks were selected and checked. Major components found were: butyric acid( 3, 7- dimethyl -6- octenyl ester); 4- Hepten- 3- one, 4 methyl; Palmitate (hexa decanoic acid); Phthalic acid [1,2,- Benzene dicarboxylic acid, bis (2-methylpropyl) ester ]; 1,5- anhydro-6-deoxyhexo-2,3-diulose; Tridecan-1-ol; isoamyl nitrite; methyl 12- methyl tetradecanoate; 2, 2’, 5, 5’- tetra hydro and 2-pentynon-2-enal. From the various components detected several have already been reported to possess anti-inflammatory, anti-oxidant activity as well as having effect on the macrophage function.
Taken together, our results strongly support the anti-inflammatory and anti-arthritic potential of the plant *C. asiaticum* and its use in traditional medicine. As mentioned previously, several compounds found in methanol extract of *C. asiaticum* leaves are well known for their anti-inflammatory activities. The presence of these compounds in the *C. asiaticum* leaf extract may explain the anti-arthritic properties of this plant. Our next goal is to isolate and identify the compounds responsible for the anti-arthritic potential of this plant.