Discussion: Part-I

Anti-inflammatory Effect of the Plant C. asiaticum on various in-vitro and in-vivo Models of Acute Inflammation Developed in Rats

Inflammation is a very complex and tightly regulated sequence of events acting as the primary line of protection by restricting the tissue damage or pathogen invasion to the site of tissue injury or infection (Vane and Regina, 1987). Inflammatory responses can be triggered by a variety of stimuli of both exogenous as well as endogenous origin such as microbial products, chemical agents, tissue injury, auto-immune responses, cytokines etc. The phenotype of inflammation is mainly dependent on the kind of stimulus or irritant (Gordon S, et al., 1995, Hamilton TA, et al., 1999).

Various methods and medicines are used to combat and decrease the inflammatory response generated by the body in case any infection or auto-immune dysfunction. In order to restore the normal homeostasis and recover from various inflammatory responses which occur inside the body, plants have been considered as one of the major resources provided by the nature since ages.

Before checking the anti-inflammatory property of Crinum asiaticum we assayed whether the plant extract had any kind of side effect such as ulcerogenic effect which is generally associated with the commercially available drugs like acetyl salicylic acid, indomethacine or dichlofenac prescribed in case of several inflammatory diseases. In order to check the ulcerogenic effect the rats were fasted for 24 hours and then treated with the plant extract and were compared with acetyl salicylic acid (ASA) which was used as positive control. The plant exhibited protection from DMSO (solvent) induced ulcerogenic effect in a dose related manner. At a dose of 400mg/kg b.w., plant exhibited significant decrease in ulcer formation. On the contrary, the conventional NSAIDs treatment like acetyl salicylic acid (ASA) resulted in increase in ulceration as reported earlier (Schaffer D, et al., 2006). It is already reported that since gastric ulceration or irritation is common when treated with NSAIDs as inhibition of prostaglandin synthesis can be involved in case of their mechanism of
Discussion: Part-I

action. Hence, it can be safely speculated that the preliminary mode of action of the plant extract may be mediated by inhibition of prostaglandins synthesis, which belongs to the group of prostanoid mediators of inflammation.

Next, we tried to check the anti-pyretic properties of the plant extract. One of the initial symptoms of inflammation is rise in body temperature. One of the basic property which needs to be possessed by any agent having anti-inflammatory effect should be also effective in restoring the normal body temperature. It was found that the treatment with *C. asiaticum* extract exhibited ant-pyretic effect in a dose dependent manner and its effect was comparable to the effect observed in positive control rats which were treated with a conventional NSAIDs drug i.e. paracetamol thus clearly indicating its mode of action to be through inhibition of prostaglandin synthesis, as rise in body temperature during inflammation is mainly mediated by the prostaglandin release.

Next, in order to evaluate the anti-inflammatory property of the methanolic extract of the plant *C. asiaticum* leaves we performed various *in-vivo* and *in-vitro* experiments using agents which are established mediators of inflammation like xylene, dextran, histamine etc. (ref.)

We started to evaluate the anti-inflammatory effect of the extract by assaying the effect on topical ear edema induced by xylene. It was observed that the plant extract when topically applied on the ear of the rat simultaneously along with xylene on posterior surface it exhibited a marked decrease in the development of ear edema, thereby suggesting that the plant may possess anti-inflammatory activity. This was further substantiated by studying the effect of *C. asiaticum* extract on two very well studied acute inflammation model systems such as the dextran- induced acute inflammation model and histamine- induced inflammation model.

It has been reported earlier that dextran- induced paw oedema is mainly mediated through the action of histamine, serotonin and prostaglandins (*McCarthy RE, et al., 1976*). In our study, the *C. asiaticum* extract showed a dose dependent anti-inflammatory response in dextran- induced paw edema model. The extract at a lower dose of 200mg/kg bw. reduced the hind paw edema similar to the reduction observed with acetyl salicylic acid (ASA), the positive control. Further, the effect of the extract...
at the higher dose of 400mg/kg bw. was significantly higher than the positive control. Thus, the results further corroborated the anti-inflammatory effect seen with topical inflammation model.

As discussed earlier, histamine is an important regulator in a number of immune as well as inflammatory responses. Histamine, a biogenic amine, is primarily synthesized and stored in mast cells and basophils. It is one of the major inflammation mediators which are found at the site of tissue injury as soon as any inflammation occurs (Jutel M, et al., 2002). The present study further revealed that the C.asiaticum extract exhibited the anti-histaminic property in a dose dependent manner being more effective at a higher dose of 400mg/kg b.w. The results thus suggested that the methanol extract of C. asiaticum leaves exhibited its anti-histamanic effect by either supressing the production, release or action of histamine at the site of inflammation.

Upon establishing, the anti-inflammatory activity of C.asiaticum extract on various topical and acute inflammation models. Further, experiments were conducted to assess the role of C.asiaticum extract for potentiating the anti-inflammatory activity of generally used drug acetyl salicylic acid (ASA) in a combination study. To test the efficacy of extract in combination animals were given the intra-peritoneal injection of the combination of acetyl salicylic acid and C. asiaticum extract at two different doses i.e. 200mg/kg b.w and 400mg/kg b.w. The results clearly showed no synergism or potentiation in anti-inflammatory activity in case of histamine- induced paw edema thus indicating that the ASA and plant extract may be utilizing the same pathway for the generation of anti-inflammatory response.

On the other hand, in the case of dextran- induced inflammation model the combination exhibited a synergistic effect at a higher dose of 400mg/kg b.w by showing a decrease in the paw oedema which was more as compared to that produced by ASA alone or plant extract (400mg/kg b.w) alone. Thus suggesting that the plant exhibited a better anti- inflammatory property than ASA probably because it may be targeting multiple pathways involved in dextran induced inflammation other than histamine i.e. either by inhibiting the serotonin or prostaglandins mediated pathway or neutrophil accumulation at the site of inflammation (Hurley JV, et al., 1966;
Meacock and Kitchen, 1978; Parente L, et al., 1979). Thus, it may serve as a better option against inflammation.

One of the major phenomenon which is observed during any type of inflammation is cellular infiltration, a role which is basically played by leukocytes (Kuby J, 6th Edition). As a part of defense mechanism, they infiltrate at the site of tissue injury or inflammation where they release certain mediators which are rich in degrading enzymes and proteases. These mediators further lead to heightened inflammation and tissue injury thus resulting in a more debilitating condition (Chou CT, 1997). Thus, one strategy for downplaying these infiltrating leukocytes could be via stabilizing their membrane permeability so that the cytoplasmic contents are not released.

When the in-vitro examination of the membrane stabilization with plant extract was assayed using the chicken erythrocytes in heat and hypotonicity induced hemolysis it was observed that the extract exhibited membrane stabilizing effect in both the condition but it was more pronounced in the case of hypo-tonicity induced hemolysis of cell at a higher dose of 400µg/ml. The exact mechanism by which the plant exhibited the membrane stabilization effect was not clear but it can be speculated that the plant constituents may directly interact with the membrane proteins and exert its effect. As the proteins form a major component of the cell membrane and, even regulate their water contents and salt concentration by movement inside the cell through protein channel thus affecting the physical properties (Guyton CA, 10th Edition).

From all the above experiments, it was clearly indicated that the extract was safe to use as it did not show any significant side effect like development of ulcers in the stomach which is a major problem observed during the usage of any conventional NSAIDs during many inflammatory condition. Further, it was also observed that the plant was effective against the acute inflammatory conditions in the rats developed with various agents like xylene, dextran and histamine. The plant extract was also effective in stabilizing the membrane integrity which is seen as a crucial phenomenon during initiation of inflammation. Hence, from the above studies we could deduce that the extract could be used as a potent anti-inflammatory agent in atleast acute inflammatory condition.
Discussion: Part-II

Development of Arthritis Model in Rat

According to the report published by the arthritis foundation, arthritis is the second most frequently reported chronic health condition and one of the nation’s common causes of disability (Arthritis Foundation News, 2008).

Arthritis is described or characterized by persistent case of synovitis, combination of systemic inflammation and production of auto-antibodies against the synovial lining (Pearson CM, et al., 1960). Various factors have been attributed for the development of arthritis which include both environmental as well as genetic factors (Scott DL, et al., 2010) further to add the modern day life style further enhances the chances of developing one or the other form of arthritis.

Development of arthritis can also be correlated with the age as according to the reports (Carbonell J, et al., 2008) prevalence of arthritis increases with the age in the population and above the age of 65 years the estimated percentage of individuals suffering from or diagnosed for arthritis is about 50-75%. According to the survey conducted by Arthritis Foundation Awareness Society in 2001 it was found that 80% of adults were either diagnosed with arthritis or they knew someone suffering from arthritis. Further, it was also found that the prevalence of arthritis to some extent was gender biased as well as it is found to be higher among women (≈28.3%) than in men (≈ 18.2%) (Symmons D, et al., 2002).

It is estimated that by 2030, approximately 67 millions of American aged between 18 years of age or older than that would be at a risk of being diagnosed for arthritis (Arthritis Foundation News, 2007). We can even state that if the prevalence rate for arthritis remains stable as in today’s date then the number of arthritis affected individuals in the age group of 65 years or older than that would nearly double to approximately 41.1 million by the year 2030. According to the report published by DNA magazine, in India arthritis is the most commonly occurring chronic ailment and by 2013 it would take the shape of an epidemic (DNA, July 2010).
Till date various models have been developed in order to study the development and pathogenesis of arthritis (Wooley PH, 2004). Some of the most commonly used arthritis model which have been widely accepted are collagen- induced arthritis, streptococcal cell wall- induced arthritis, pristane- induced arthritis. One of the oldest and most widely used arthritis models is complete Freunds’ adjuvant induced-arthritis developed by Pearson CM, et al., 1960, which has been extensively used for studying the inflammatory processes in arthritis (Jones and Ward, 1966).

Complete Freunds’ adjuvant induced arthritis is a T-cell mediated sub-acute arthritis which is accompanied with severe weight loss (Grassi W, et al., 1998), increase in the hind paw volume/ thickness of the rat (Calvino B, et al., 1987), increase in arthritis score, fusion of major joints of the bone and presence of activated macrophages after disease induction.

In order to develop the model, above mentioned parameters were checked to validate the disease induction. It was found that immediately after CFA injection in the hind paw there was no immediate effect on the body weight of the animal. But, after 7th day a gradual decrease in the body weight was seen which indicated towards the development of disease in the CFA induced animal.

Further, development of disease was monitored through monitoring the hind paw volume, as the disease development in the animal it was accompanied with increase in the hind paw thickness (Mindrescu C, et al., 2000). The results showed that the hind paw volume starts increasing immediately after the CFA injection but the rate of increase in the paw volume was more after day 7 reaching its maximum by day 14. The results clearly indicated that the disease starts developing from day 7 onwards and develops fully around day 14, and beyond day 14 there was no significant increase in the hind paw thickness. The initial increase in the paw volume was noticed because of the immediate inflammatory response generated after CFA injection (Calvino B, et al., 1987). From day 7 onwards the increase in the paw edema can be associated with the development of arthritis as it is known that acute inflammation starts subsiding by 4-5 days (Paul WE, 5th edition) after which if inflammation persists then it is known as chronic inflammation and arthritis is one such common
inflammatory disease of joints. Hence, persistence and steep increase in the paw edema can be correlated with the disease induction and arthritis development in the rat model.

Another important and common parameter used for evaluating arthritis development in rat is arthritis score. Calculation of arthritis score is based on the blind scoring done after observing the rats for the degree of redness, edema developed and the flexibility of the joints affected in the disease induced rats (Pearson CM, et al, 1960). From the results it was seen that the arthritis score increases from day 0 onwards but there is a marked increase in the arthritis score after day 5 which reaches its peak by day 14 and continues at that level around day 21 indicating the development and persistence of the disease in the CFA induced rats. The continuous increase in the arthritis score of the disease induced animal indicates towards the persistence of edema and redness in the hind paw of the rats along with the loss of joint flexibility in the hind paw bones making the movement restricted. These symptoms reached their maximal score by day 14 and then maintained their status till day 21 of the experiment clearly pointing towards the development of arthritis.

During arthritis, it is commonly seen that in the severe conditions of disease the bones of major joints of the body start showing fused condition thus leading towards the decrease in the joint spaces (Aota S, et al, 1996; Makinen H, et al., 2007; Findlay and Haynes, 2005). Further, it is also seen that in extreme conditions the bone even starts becoming more opaque thus leading to the decrease in bone strength. Hence it is necessary to also do the radiological study of the major bones during the disease (Kitamura T, et al, 2007). During the radiological examination done in the initial days, it was observed that the rats which were given the injections of complete Freunds’ adjuvant as inducer for disease development did not exhibit any kind of fusion of the bone joints of the hind paw or tail bones. But, the radiological examination on day 14 revealed complete fusion in the ankle joint and tail bone joint, confirming the fusion of joint bones in diseased condition. Fusion in joints prevailed even on day 21 as well day 28 of the experiment confirming the loss of joint spaces. This condition completely established the development of arthritis in rats with CFA
injection and the diseased conditions were similar to the conditions observed in humans with arthritis.

As mentioned earlier, macrophages are known to be a key player in any inflammatory disease and serve as a major connecting link between the developments of chronic disease from its acute form. It has also been reported that inflammatory cells like macrophages play a major role in arthritis development and progression. A huge influx of macrophages is observed in the synovial lining as well as junctions of cartilage during the arthritic condition (Mulherin D, 1996). These cells release various cytokines (Kinne RW, et al., 2000) which further leads to bone destruction and recruitment of osteoclast precursors (Yingyu and Pope, 2005). Hence, we also checked whether there was any marked change in the number of macrophages at the site of inflammation and at various other parts of arthritic rats. In order to check, peritoneal macrophages and hind paw macrophages were prepared from both the control as well as the arthritic rats. It was observed that there was a marked increase in the number of macrophages in both the peritoneum as well as hind paw tissue in the diseased animal. Further, activity of the macrophages obtained from the CFA induced rats were checked by performing phagocytosis assay. It was found that these macrophages showed an increase in the percent phagocytosis as well as phagocytosis index clearly indicating towards the activated state of the macrophages.

All the above studies together indicated towards the development of arthritis disease in the female Wistar rats using complete Freunds’ adjuvant as an inducer. The diseased condition exhibited several similarities to the human arthritic condition like loss in body weight, increase in the arthritic score indicating towards loss of movement, fusion of joint bones thus leading to decrease in the joint spaces and presence of huge number of activated macrophages. Hence, this model could be very well utilised for checking the disease progression as well as for checking the anti-arthritic potential of various synthetic as well as natural drugs.
**Discussion: Part- III**

**Effect of the C. asiaticum Extract on Chronic Inflammation Developed in Arthritis Rat Model**

Arthritis, affecting nearly 1% of the world’s total population, is one of the most common chronic inflammatory disease ([Cardninali and Esquifino, 2003](#)). The disease is characterized by gradual loss in body weight, morning stiffness, lack of mobility, edema, nodule formation in chronic condition etc. The history of existence of arthritis is as old as the history of Egyptians. Recorded evidence suggests that the disease existed even around 1500 BC ([ Elliot G, 1999](#)). Since then various efforts have been made in order to find a cure using various traditional drugs for the disease.

Some of the most commonly used modern medicines for the treatment of disease include the conventional anti-inflammatory drugs such as NSAIDs ([Vane and Regina, 1987](#)), DMARDs ([ Donahue KE, et al., 2008](#)), corticosteroids ([Kirwan JR, et al., 2007](#)), etc. Recently a new line of therapy which is being heavily exploited for the treatment of arthritis is the use of biological agents such as anti-TNF-α agonist, IL-1R agonist ([Kumar S, et al., 2001](#)) etc. These drugs, though effective in subsiding the disease symptoms do not result into the complete cure from the disease. Besides this, long term usage of the drugs leads to serious side effects such as NSAIDs leads to gastrointestinal ulcers, renal toxicity, etc., ([Schaffer D, et al., 2006; Scott PA, et al., 2007](#)) DMARDs leads to the development of lymphomas, alopecia, decrease in reproductive potential etc. ([Cutolo M, et al., 2001](#)) whereas corticosteroids dependency predisposes the patients towards development of various infections and osteoporosis in some cases ([Ravindran V, et al., 2009](#)). Biological agents on the other hand also make the patients susceptible towards the development of various melanomas ([Kaiser R, 2008](#)) Hence, people are shifting towards the use of alternative therapies involving the use of natural plant products.

The present study demonstrates the anti-arthritic potential of methanol extract of plant *Crinum asiaticum*. The extract has already been shown to possess potent anti-
inflammatory effect along with the ability to stabilize the membrane integrity in order to prevent an increase in the inflammatory conditions as shown in the previous part of the work. This part deals with the anti-arthritic potential of the plant extract which is very commonly used in the folklore medicines in the north-eastern part and northern part of India as well as in some countries like Vietnam as well.

To start with, the acute toxicity of the plant extract was checked on the animal in the dose range of 100-5000mg/kg b.w. and it was observed after 72 hours that the animal did not exhibited mortality or change in behavioural activity. This suggested that the extract did show any side-effect on the animal. In order to check for the anti-arthritic potential of the plant the adjuvant induced arthritis model in rat (Pearson CM, et al., 1960) was used and the anti-arthritic property of the plant was studied at two different doses of 200mg/kg b.w. and 400mg/kg b.w.

The rats were treated with the different doses of plant extract after being induced for the disease and the results indicated that the extract was potent in showing reversal in loss in body weight which is one of the initial trait in condition of arthritis (Calvino B, et al., 1987) thus indicating towards the anti-arthritic effect. Another very important index which is used for evaluation of arthritis in any animal model is the measurement of hind paw edema using the vernier calliper and visual inspection (Mindrescu C, et al., 2000) and the extract of *C. asiaticum* was effective in reducing the hind paw swelling in a dose dependent manner. In the next experiment effect of the extract was studied on the arthritis score of the diseased animal when they were given the treatment of plant extract. The extract effectively showed a lower arthritis score as compared to the diseased animal thus inhibiting the development of arthritis in the animals. The development of arthritis in the CFA induced rats is due to the bacterial peptide-glycans and the muramyl di-peptide (Crofford and Wilder, 1993); but the exact mechanism involved in the development of disease is not clear as the composition of bacterial adjuvant administered is complex and the immune response, which is generated with the involvement of intracellular cooperation, is also a multi-stage process and still unclear (Waiz DT, et al, 1971). Hence, one of the pathway through which plant could be showing the anti-arthritic effect can be by affecting the interaction of these bacterial adjuvant with the intracellular environment of the animal.
Further, the plant extract of *C. asiaticum* was effective in reducing the joint destruction as well as loss of joint spaces in the animals in a dose dependent manner as can be seen from the radiographs. Radiographic analysis of the diseased animal is necessary to study the status of disease and remission of disease in the presence of plant extract (Kitamura T, et al., 2007). It is seen that as the disease progresses a diffused demineralization of bones occur at the extremeties (Begum and Sadique, 1988). Reduced bone formation and increased level of resorption are the main reason for bone loss as well as joint destruction and fusion of bones in case of adjuvant induced arthritis (Aota S, et al., 1996; Makinen H, et al., 2007; Findlay and Haynes, 2005). Hence, one of the way in which plant extract could prevent the loss of joint spaces as well as fusion of bones could be by preventing the condition of de-mineralization to occur as well as through decreased level of resorption.

Another important parameter, in order to study the anti-arthritic effect of any plant extract is to study the effect of plant extract on activated macrophages. Macrophages are known as key plays in any chronic inflammation (Allison and Davies, 1974). In condition of arthritis huge influx of macrophages is seen at the synovial joints as well as junctions of cartilage (Mulherin D, et al., 1996). These cells release various pro-inflammatory cytokines further leading to the destruction of bones as well as recruitment of osteoclast precursors at the joints (Yingyu and Pope, 2005). LPS activated murine macrophage cell line RAW 264.7 cells were used to mimic the inflammatory condition. The effect of the plant extract on the macrophage activity was checked by using two different parameters i.e. phagocytosis and NO release. The extract exhibited a huge degree of inhibition in the % phagocytosis as well as phagocytosis index thus clearly indicating towards the down regulation of macrophage activity. Further, the extract resulted into the inhibition of NO release from the LPS activated RAW 264.7 cells suggesting to play a down regulating role in the NO production from the cells after being activated by LPS. NO is a free radical generated during inflammation and has also found to play a major role in arthritic conditions in humans as well as the rat models mimicking arthritis (Ueki Y, et al,1996). High level of nitric oxide is already shown to be present in the serum as well as synovial fluid of arthritis patient (Farrell AJ, et al 1992). Hence, one of the way in
which plant could prevent the development of disease in the rats induced with CFA could be through the inhibition of NO release from macrophages which are found in the arthritic joint spaces.

Based on the above studies conducted on the CFA-induced arthritis in rat, it could be concluded that the plant extract of leaves of *C. asiaticum* was effective in preventing the development as well as progression of the disease. Further one of the common problem faced while using the conventional drugs for arthritis treatment is development of various harmful side effects. But, it was seen that the plant did not lead to the development of any significant side effects on the animals during the complete experiment thus indicating towards its safety in using for the treatment of disease.

Hence, the plant could be effectively used for the arthritis treatment.
Discussion: Part-IV

Characterization of the Methanolic Extract of Leaves of C. asiaticum using GC-MS Analysis

Due to the huge dis-satisfaction generated over the conventional synthetic drugs used for the treatment of chronic inflammatory diseases like arthritis more and more people are turning towards herbal remedies based on the natural products for the treatment. They are effective in promoting safe health and alleviating the diseases (Craig WJ, 1999). Hence, the scientific search of new drugs for the treatment of disease has shifted towards the traditional medicine and drug discovery based on ethanopharmacology (Kong D, et al., 2009). Something around 100 new drugs, for treatment of various diseases, based on natural plant products or active components obtained from them, are under the clinical development (Harvey AL, 2008). Between the year 1981 and 2002 roughly 75 % of the drugs developed for various disease were from natural products (Gupta R, et al., 2005). Hence, new medicinal plants are being exploited for the drug discovery and development on the large scale and more and more scientific research is required to analyse the medicinal property of the plants, used in traditional and folklore medicine.

Till date various species of Crinum have been checked for their biological activities and are found to be potent source for the treatment of various diseases. Once the plant is shown to possess a specific medicinal property it is further characterized to isolate the active fractions responsible for the evident effect. One of the most widely used technique for the characterization of crude plant extract is GC-MS.

As seen from the previous chapters, the methanol extract of the leaves of plant C. asiaticum (var. sinicum) possessed potent anti-inflammatory as well as anti-arthritic effect. Hence, the plant extract was characterized using the GC-MS technique. The characterization was done using the ARS facility provided by Jawaharlal Nehru University, Delhi, India.
Out of the 24 peaks that were picked up during the GC-MS run top 10 peaks were used to check the major components which are present in the extracts using the Wiley 08 and NIST 05 library. The ten major compounds detected had majority of ester compounds.

When these compounds were searched for their respective biological activities it was found that some of the compounds were known to possess anti-inflammatory effects like 3,7 dimethyl-6 octenyl ester also known as Citronyll butyrate (Assisi RF, 2008; Zhang WH, et al., 2011), 4-Hepten-3-one, 4 methyl referred as Hazelnut ketone (Gallo and Sarachine, 2009), Hexadecanoic acid commonly known as palmitate (Bu T, et al., 2010), Methyl 12- methyl tetradecanoate (Chung IM, et al., 2012). Some of the compounds which were identified in the extract are reported to possess potent anti-oxidant and chemo preventive activities such as Citronyll butyrate (Assisi RF, 2008; Zhang WH, et al., 2011), Hexadecanoic acid commonly known as palmitate (Okoh SO, et al., 2011), 1, 5-anhydro-6-deoxyhexo-2, 3 diulose, Isoamyl nitrite (Bauer JA, et al., 1997). Methyl 12-methyl tetradecanoate has been known to possess immunotoxicity (Chung IM, et al., 2012). Since, the extract contained a rich proportion of components which have already been shown to possess anti-inflammatory effect, these compounds may either individually or in a combinatorial manner could be responsible for imparting the anti-inflammatory as well as anti-arthritic property to the methanol extract of the leaves of *C. asiaticum*.

Some of the compounds are also reported to possess the anti-microbial activity like Hexadecanoic acid (palmitate) (Pavithra PS, et al., 2009), 1,2 Benzene dicarboxylic acid, bis (2-methylpropyl) ester popularly known as Phalic acid (Naarala and Korpi, 2009), Tridecan-1-ol (Kubo I, et al., 1993; Togashi N, et al., 2007), 2, 2', 5, 5'tetra hydro-2, 2'-bifuran commonly known as Bifuran and falling in the family of furans (Logoglu E, et al., 2010).

Some of the compounds have even been shown to exhibit various other properties as well such as 3,7 dimethyl-6 octenyl ester (Citronyll butyrate) is proved to suppress mineralized nodule formation (Morozumi A, 2011), 4-Hepten-3-one, 4 methyl (Hazelnut ketone) is known to enhance arterial action potential and can be used in
cardiovascular diseases (Ozdemir S, et al., 2003). Hexadecanoic acid commonly referred as palmitate prevents macrophage invasion (Widmer KW, et al., 2012), hence can regulate the accumulation of macrophages in the synovial joints of the arthritis patients; whereas 1,2 Benzene dicarboxylic acid, bis (2-methylpropyl) ester (Pthalic acid) is known to induce apoptosis in macrophages (Naarala and Korpi, 2009) and since macrophages in the arthritic joints are known to evade apoptosis hence this component can be responsible for inducing cell death in macrophages and cure the diseased condition. 2-pentynnon-2-enal and 1, 2 Benzene dicarboxylic acid, bis (2-methylpropyl) ester (Pthalic acid) have also been shown to have effect on the male androgens (Saillenfait AM, et al., 2008; Hannas BR, et al., 2011).

Hence, from the above discussion it can be seen that the methanol extract of plant leaves of C. asiaticum is rich in compounds also been shown to possess anti-inflammatory as well as anti-oxidant activity. From this it can be concluded that these components individually or in a synergistic manner could be responsible for the anti-inflammatory as well as anti-arthritic activity of the plant extract.