Discussion
Pain and inflammation are associated with pathophysiology of various diseases like arthritis, cancer and vascular diseases. A number of natural products are used in various traditional medicinal systems to relief symptoms of pain and inflammation (Ashok Kumar et al., 2010). Alternative medicines for the treatment of rheumatoid arthritis are getting more popular. Many medicinal plants provide relief of symptoms in rheumatoid arthritis whose effects are comparable to that of available conventional medicinal agents (Verpoorte, 1999). Over the centuries number of medicinal plants has been exploited for the treatment of the disorders associated with the inflammatory conditions or for the control of inflammatory aspects of diseases. These medicinal plants owe their activities due to the phytoconstituents and may exert anti-inflammatory effect by interfering generally with the inflammatory pathways or especially with certain components of the pathway, such as release of pro-inflammatory mediators, migration of leukocytes under inflammatory stimulus with consequent release of the cytoplasmic contents at inflammatory sites (Otari et al., 2010).

Throughout the evolutions, the importance of natural products for medicine and health has been enormous. Since our earliest ancestors used certain herbs to relieve pain or wrapped leaves around wounds to improve healing, natural products have often been the sole means to treat disease and injuries. In fact, it has been during past decades that natural products taken a secondary role in drug discovery and drug development, after the advent of molecular biology and combinatorial chemistry made possible the rational design of chemical compounds to target specific molecules. The past few years, however have seen a renewed interest in the use of natural products and more importantly their role as a basis for drug development. Numerous useful drugs are developed from lead compounds discovered from medicinal plants. In addition, the elucidation of the molecular structure of many natural products allowed chemists to synthesize them, rather than isolating them from natural sources, which markedly lowered the cost of drug production. Subsequently, a large number of well known natural compounds were identified, analyzed and synthesized. The structural analysis of natural compounds and the ability to synthesize them allowed chemists to modify them in order to suppress or enhance certain characteristics such as solubility, efficiency, or stability in human body. Newman and Cragg (2008) estimated that
about 60% of the drugs that are now available such as artemisinin, camptothecin, lovastatin were either directly or indirectly derived from natural products. Moreover, natural products have also been an invaluable source of inspiration for organic chemists to synthesize novel drug candidates (Beghyn et al., 2008).

It has been predicted that arthritis especially rheumatoid arthritis would rank fourth for the leading cause of disability by 2020. Arthritis is a global problem that will increase in significance with the growing elderly population. The condition affects both sexes and all races. This disease is characterized by inflammation of one or more joints, pain, wear and tear of joint and muscle strains. The traditional therapy recommended for the treatment of arthritis includes non-steroidal anti-inflammatory drugs like diclofenac, indomethacin etc, glucocorticoid therapy, disease modifying anti rheumatic drugs like methotreaxate, cyclosporine A, stem cell therapy, anti TNF-\(\alpha\) blockers etc. but it is well known that the therapeutic managements have several side effects as a result of which the past decades or two have seen a dramatic increase and growing interest in the use of alternative treatments and herbal therapies in arthritis.

*Cyathocline purpurea* (Buch-Ham ex D. Don.) Kuntze is reported to contain chemical constituents which may exert analgesic and anti-inflammatory effect; however till now there were no investigations supporting the pharmacological properties of this plant. Therefore the present investigation was designed to evaluate the use of *Cyathocline purpurea* in pain, inflammation and arthritis. Three extracts of different polarities i.e. petroleum ether extract of *Cyathocline purpurea* (PECP), methanol extract of *Cyathocline purpurea* (MECP) and aqueous extract of *Cyathocline purpurea* (AECP) were prepared and tested for their analgesic and anti-inflammatory activities.

Acute oral toxicity study performed at the dose of 2000 mg/kg, p.o. revealed the nontoxic nature of all the three extracts PECP, MECP and AECP. There were no toxic reactions or mortality found with these extracts. Therefore the doses selected for the pharmacological studies were 100, 200 and 400 mg/kg, p.o. Phytochemical analysis of this extracts has mainly demonstrated the presence of flavonoids, steroids,
alkaloids, phenols, tannins and saponins. Steroids and alkaloids have been reported to have analgesic and anti-inflammatory activity. Flavonoids and phenolic compounds have multiple biological effects such as antioxidant activity (Zeashan et al., 2009). Flavonoids have also been reported to have anti-inflammatory effect (Ilavarasan et al., 2006). Steroids can decrease inflammation and reduce the activity of the immune system, while triterpenoids impairs histamine release from mast cells and exerts anti-inflammatory effects (Mehta et al., 2012).

The peripheral analgesic effect may be mediated through inhibition of cyclooxygenase and/or lipooxygenases, while central analgesic action may be mediated through inhibition of central pain receptors (Shulan et al., 2011). Therefore peripheral (acetic acid induced writhing) and central (hot plate test) models were selected to observe the analgesic effect of PECP, MECP and AECP. Acetic acid induced writhing test is a simple, reliable and affords rapid evaluation of analgesic drugs (Ishola et al., 2011). The intraperitoneal injection of acetic acid elicited writhing (a syndrome characterized by a wave of abdominal musculature contraction followed by extension of the hind limbs). The intraperitoneal administration of agents that irritate serous membranes provokes a stereotypical behavior in mice which is characterized by abdominal contractions, movements of the body as a whole, twisting of dorsoabdominal muscles, and a reduction in motor activity and coordination (Perazzo et al., 2005). The abdominal constrictions induced in mice results from an acute inflammatory reaction with production of prostaglandins E2 and F2 in the peritoneal fluid (Ramachandran et al., 2011). MECP (400 mg/kg) significantly (p<0.001) inhibited the number of wriths with 35.29% inhibition. PECP (400 mg/kg) and MECP (200 mg/kg) also significantly (p<0.05) inhibited the number of wriths compared to vehicle control group. Acetyl salicylic acid (100 mg/kg) showed maximum activity with 64.71% inhibition. It has been reported that NSAID’s prevent prostaglandin production, thus sensitization of pain receptors by prostaglandin at the inflammatory site is inhibited (Dhara et al., 2000). The mechanism of peripheral analgesic action of MECP, likewise other NSAID’s, could probably be due to the blockade of effect or due to the release of endogenous substances that excite pain nerve endings. The hot plate model has been found to be suitable for the evaluation of centrally acting analgesics (Bhandare et al., 2010). Hence, the hot plate test was
performed to check if PECP, MECP and AECP would have any central analgesic effect. There were no significant results obtained in these test with PECP, MECP and AECP. On the other hand pentazocine (5 mg/kg, s.c.) showed a significant result by elevating the pain threshold. Hence it can be assumed that PECP, MECP and AECP had no effect on central nervous system.

The anti-inflammatory activity of PECP, MECP and AECP in this study was investigated using the carrageenan induced paw edema and cotton pellet induced granuloma models. Carrageenan is a family of linear sulphated polysaccharides extracted from the red seaweed marine alga Chondrus crispus. Lambda carrageenan is used in animal models of inflammation to test analgesics because dilute carrageenan solution (1-2%) injection causes swelling and pain (Costa et al., 2004). Inflammation induced by carrageenan is an acute and highly reproducible inflammatory model. Carrageenan has been widely used as an inflammasen capable of inducing experimental inflammation (William et al., 2010). This model has frequently been used to evaluate the anti-inflammatory agents (Panthong et al., 2007). The induction of edema by using carrageenan is believed to be biphasic in nature. The first phase involved within 1 h of carrageenan administration is associated with the release of histamine and serotonin from mast cells. The second phase starts after 1 h and is characterized by an increased release of prostaglandins (PGs) in the inflammatory area. During the second phase, the macrophages are known to release the large amounts of interleukin-1 (IL-1) which led to the increased accumulation of polymorphic nuclear cells (PMNs) to the site of inflammation. The activated PMNs then release the lysosomal enzymes and active oxygen species to destroy connective tissue and induce paw swelling (Marzouk et al., 2010). Statistical analysis revealed that MECP (200 and 400 mg/kg) significantly (p<0.001) inhibited the development of paw edema induced by carrageenan from 3 h onwards. Therefore it may be assumed that MECP is associated with inhibition of later phase. PECP (400 mg/kg) also showed a significant (p<0.001) inhibition at 5 h, but was less active than MECP. Moreover, diclofenac (10 mg/kg) exhibited an enhanced effect of inhibiting the paw edema than MECP with 53.99% inhibition at 5 h.
Sub acute inflammation or proliferative phase is measured by methods for testing granuloma formation such as cotton pellet induced granuloma (Begum et al., 2010). The cotton pellet induced granuloma model is a widely used method to evaluate the transudative and proliferative components of chronic inflammation (Gupta et al., 2005). The repair phase of inflammation starts as proliferation of fibroblasts, as well as multiplication of small blood vessels. Such proliferating cells penetrate the exudates producing a highly vascularised reddened mass known as granulation tissue (Sanmugapriya et al., 2005). The fluid absorbed by the pellet greatly influences the wet weight of the granuloma and the dry weight correlates with the amount of granulomatous tissue formed (Babu et al., 2009). MECP (200 and 400 mg/kg) were significantly (p<0.001) effective in both the models of inflammation, i.e. carrageenan induced rat paw edema as well as cotton pellet induced granuloma, therefore it can be assumed that it is effective in all phases of inflammation i.e. acute, sub acute, and proliferative phases.

Evaluation of the ulcerogenic effect of the three extracts (PECP, MECP and AECP) on the rat stomach revealed a lesser ulceration of the gastric mucosa and absence of congestion as compared to diclofenac. Ulceration of the gastric mucosa by anti-inflammatory drugs is a common side effect which usually indicates that prostaglandin synthesis inhibition may be involved in their mechanisms of action. Inhibition of the synthesis of prostaglandin, a group of prostanoid mediators of inflammation and intact gastric mucosa is largely responsible for the anti-inflammatory and gastric ulceration effects of NSAIDs.

Thus the experimental findings in the study demonstrated the peripheral analgesic, and anti-inflammatory activity of *Cyathocline purpurea* extracts. PECP and AECP showed weak analgesic and anti-inflammatory effect when compared with MECP. MECP (200 and 400 mg/kg) was found to be highly effective. The results suggested that the mechanism of action of MECP seems to be similar to NSAID’s rather than to steroidal drugs. One of the factors responsible for anti-inflammatory activity of MECP is solubility of the active constituent in the solvent system used for preparation of methanolic extract. The absence of anti-inflammatory activity in the AECP may be due to poor solubility of the active ingredients in the water. MECP showed most
Discussion

RA is a chronic inflammatory disease affecting about 1% of the population in developed countries (Amresh et al., 2007). Limb swelling, inflammatory cell infiltrations, proliferative synovitis, erosion of the bone are clinical findings common to human arthritis and adjuvant-induced arthritis rat. Owing to this similarity in pathologic features, the adjuvant-induced arthritis rat is a widely used model of RA in evaluating the efficacy of anti-arthritic drugs (Noguchi et al., 2005). Freund’s complete adjuvant arthritis is a well established model that has been used in numerous studies for identifying the potential therapeutic targets. In experimental arthritis animal model female Wistar rats were used because animal model provides more uniform experimental data and allow for extensive testing of potential therapies. Adjuvant arthritic is very similar to human RA both in pathological and serological changes, including the involvement of inflammatory mediators in the arthritic etiology (Gao et al., 2008). The acute stage of arthritis is characterized by signs of hyperalgesia, lack of mobility and pause in body weight gain; during the acute period, hind paw and fore paw joint diameter increase. In the later stage of disease (day 12+), rats with adjuvant arthritis are often relatively immobile due to severity of paw swelling (Amresh et al., 2007).

In the present study, MECP (200 and 400 mg/kg) treatment showed anti-arthritic effect in all the inflammatory parameters. It significantly decreased the inflammation compared to the arthritic control group as observed by decreased paw volume and joint diameter. The present study revealed that paw volume and joint diameter increases with ankle stiffness in FCA challenged rats. Paw swelling is one of the major factors in assessing the degree of inflammation and curative efficacy of drugs. Intraplantar injection of inflammatory agents, such as carrageenan, lipopolysaccharide (LPS), bacterial endotoxin or FCA produce mechanical or thermal hyperalgesia associated with an upregulation of IL-1β and other inflammatory cytokines in the inflamed tissue and in the dorsal root ganglia (DRG) (Ren and Richard, 2009). The analgesic effect of MECP (200 and 400 mg/kg) in rats with adjuvant arthritis is also marked as evident by the increase in pain threshold, paw withdrawal latency and
mechanical nociceptive threshold. Von Frey filaments elucidate degree of mechanical nociceptive threshold where the mechanical nociceptive threshold is measured in response to increasing pressure stimuli applied to the plantar surface of paw by Von Frey filaments. Whereas tail flick unit throws IR beam on the inflamed paw bearing hyperalgesic response. Rats with severe arthritis in the arthritic control group demonstrated low paw withdrawal threshold. The animals treated with MECP (200 and 400 mg/kg) were able to bear significantly higher pressure whereas the animals in the arthritic control group were able to bear the minimum weight due to severe arthritic condition. The decrease in body weight during inflammation is due to reduced absorption of nutrients through the intestine (Patil and Suryavanshi, 2007). Therefore the restoration of the body weight in rats by MECP treatment may involve improvement in the absorption of the nutrients through the intestine of rats.

It has been reported that a moderate rise in the WBC count occurs in arthritic conditions due to an IL-1β mediated rise in the respective colony stimulating factors and reduction in Hb count in arthritis results from reduced erythropoietin levels, a decreased response of the bone marrow erythropoietin and premature destruction of red blood cells (Jalalpure et al., 2011). MECP and diclofenac treatments significantly decreased the WBC count and increased the Hb level. In addition to this, other characteristic haematological alterations such as the decreased RBC and increased platelet count were also significantly restored by the MECP and diclofenac.

MECP treatment significantly reduced the levels of RF and CRP dose dependently. RF could be a marker of RA, characterized by a significant increase in the incidence of distal interphalangeal arthritis (Patel et al., 2012). Also a persistent high serum level of CRP is recognized as strong indicator of RA (Pepys and Hirschfield, 2003). Challenge with FCA (0.1 ml) significantly (p<0.001) elevated the serum AST, ALT and ALP level and decreased the total protein level. Assessment of the serum levels of AST, ALT and ALP provides an excellent and simple tool to measure the anti-arthritic activity of the drug. The activities of aminotransferases and ALP increases significantly in arthritic rats, since these are good indices of liver and kidney impairment which is also considered a feature of adjuvant arthritis. Serum AST and ALT has been reported to play a vital role in the formation of biologically active
chemical mediators such as bradykinins in inflammatory process (Mythilypriya et al., 2008). The administration of MECP dose dependently decreased the level of AST, ALT and ALP and increased the level of total protein which confirms the anti-arthritic effect.

The role of oxidative stress in arthritis is not surprising since reactive oxygen species serve as mediators of tissue damage. An antioxidant can be defined as any substance that when present in low concentrations compared to that of an oxidisable substrate significantly delays or inhibits the oxidation of that substrate (Halliwell, 1991). The physiological role of antioxidant as the definition suggest is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals. It is well recognized that free radicals are critically involved in various pathological conditions like cancer, arthritis, inflammation and liver diseases (Vijayakumar et al., 2012). Lipid peroxidation is a critical mechanism of the injury that occurs during RA, which is often measured by analysis of tissue MDA. The large amount of MDA in arthritic control group is consistent with the occurrence of damage mediated by free radicals (Arulmozhi et al., 2011). Treatment with MECP (200 and 400 mg/kg) produced a significant reduction of MDA level. GSH reflect the endogenous defense against damage caused by ROS and organic peroxides as they act as an intracellular reductant in oxidation reduction processes. The decreased levels of GSH in liver of arthritic rats might be due to the excessive consumption of GSH by the system to defend oxidative damage (Hemshekhar et al., 2013). The production of oxygen free radicals that occurs with the development of arthritis leads to decreased GSH and SOD levels as a consequence of their consumption during oxidative stress and cellular lysis (Kizilntuc et al., 1998; Hassan et al., 2001) which is evident by decreased levels of GSH and SOD in arthritic control group. Oral administration of MECP to the rats significantly re-established the depleted levels of GSH and SOD, probably by competing for scavenging of free radicals.

From the histopathological studies of the ankle joint, it is evident that the inflammation of the connective tissue is controlled by treatment with MECP. Bone destruction, which is a common feature of adjuvant arthritis, was examined by radiological analysis (Patel et al., 2012). X-ray studies of the rat paws showed that
treatment with diclofenac and MECP inhibited the arthritis associated joint alterations.

Thus the study revealed that MECP (200 and 400 mg/kg) possess significant anti-arthritic activity which is mediated by its analgesic and anti-inflammatory effects on different parameters like decrease in paw volume and joint diameter; increase in pain threshold, mechanical nociceptive threshold, and paw withdrawal latency. The anti-arthritic activity of MECP was also supported by haematological, biochemical, antioxidant, radiological and histopathological parameters. All this results thus predict that MECP provide pharmacological rationale for the traditional use of the plant against inflammatory conditions like RA.

Based upon the antiarthritic activity of MECP it was further subjected to fractionation by liquid-solid separation chromatographic technique followed by column chromatography and isolation of active compound was done. MECP was fractionated by liquid-solid separation chromatographic technique and the dose for screening the active anti-inflammatory fraction was reduced to 100 mg/kg, p.o. The anti-inflammatory activity was screened by carrageenan induced paw edema model in rats. In the fraction study (F – 1 to F – 6), the paw edema of the rats increased progressively after carrageenan injection. 30% acetone in petroleum ether fraction (F – 4) was found to exert the highest anti-inflammatory activity compared to other fractions, 30.77% inhibition of inflammation at 3rd h. Ten pools (P – 1 to P – 10) were collected from fraction F – 4 through column chromatography based on TLC, which were then screened for anti-inflammatory activity by carrageenan induced paw edema model in rats and the dose was reduced to 10 mg/kg, p.o. Pool (P – 8) showed most significant anti-inflammatory activity compared to other pools and was further subjected to preparative TLC for removal of impurities. Compound was scratched from the preparative TLC and subjected for structural elucidation using IR, $^1$H-NMR, $^{13}$C-NMR, DEPT and MS. Based on the spectral data obtained and its comparison with the reported spectral values in the literature (Nagasampagi et al., 1981) the compound P – 8 was identified as Isoivangustin, a known Sesquiterpene lactone. It had a melting point of 139 – 140 °C. Docking studies were carried out to study the binding mode of the isolated compound, isoivangustin on the active site of TNF-alpha.
converting enzyme (TACE). TACE converts membrane bound pro-TNF-β to mature and soluble TNF-α. The native ligand IH6 was successfully docked into the active site of TACE. Hydroxamate group of compound IH6 forms van der Waals interaction with zinc, the co-catalytic metal ion in the active site of the enzyme (Figure 44). The compound IH6 actively takes part in forming hydrogen bond interaction with the key amino acids Gly349 and Leu348 in the enzyme protein. The phenyl ring forms an interaction with amino acid His405 by Π-Π stacking. Furthermore, the compound is surrounded with residues, such as Ala439, Leu348, Val434, Tyr436, His415, Ile438 and Pro437 in the enzyme and makes contacts through van der Waals interactions with these amino acids and the docking score of compound IH6 with TACE was -7.432. Also, the binding studies of diclofenac with TNF-β were studied and it was found that it forms the van der Waals interaction with zinc and show Π-Π stacking with amino acid HIS405. The docking score for diclofenac with TACE was -7.358 (Figure 45). Docking analysis of isoivangustin at the active site of TACE showed hydrogen binding with amino acid Gly349 and Leu348 like in native ligand IH6 which showed hydrogen bonding with same amino acids. Also, the octahydronaphthyl ring fits into hydrophobic pocket formed by amino acid His405 in the enzyme. The docking score of isoivangustin with TACE enzyme was -5.341, which shows that it has good binding interaction with active site of TACE (Figure 46).

Validation of docking procedure:
In order to validate our docking procedure, we eliminated the co-crystallized ligand IH6 from the active site, and redocked within the inhibitor binding cavity of TACE enzyme. In this study, the root mean square deviation value was below 2Å, showing that our docking method is valid for the inhibitors studied.

Therefore it was thought necessary to evaluate the antiarthritic activity of isoivangustin by FCA induced arthritis in rats and to find out its probable mechanism of action. The doses selected for the study were 2.5, 5 and 10 mg/kg, p.o. In the investigation of FCA induced RA, it was observed that swelling developed over twenty four hour period in the foot injected with FCA. Our results showed that isoivangustin, a sesquiterpene lactone isolated from methanol extract of *Cyathocline purpurea* showed anti-arthritis effect in all the inflammatory parameters. Isoivangustin (5 and 10 mg/kg) significantly inhibited development of paw volume
and joint diameter dose dependently. Analgesic effect of isoivangustin (5 and 10 mg/kg) were observed as evident by the increase in pain threshold, thermal hyperalgesia and mechanical nociceptive threshold. The loss of body weight in the arthritic control animals could be due to reduced absorption of glucose and leucine in rat intestine in arthritic condition (Babu et al., 2009). Isoivangustin (10 mg/kg) restored the body weight in FCA injected rats; therefore it may also improve the absorption process.

Arthritic control rats showed a significant increase in WBC and platelet count. Treatment with isoivangustin tends to normalize the WBC and platelet count dose dependently. It has been reported that moderate rise in WBC count occurs in arthritic conditions due to an IL-1β mediated rise in the respective colony stimulating factors. The result of haematological parameters reveals that isoivangustin increased the Hb level and RBC count dose dependently, supporting the anti-arthritic activity of isoivangustin. It has been reported that reduction in the Hb level during arthritis results from reduced erythropoietin levels, a decreased response of the bone marrow erythropoietin and premature destruction of RBC (Jalalpure et al., 2011). ESR is influenced by an increase in the plasma concentration of acute-phase reactant proteins in response to inflammation (Talwar et al., 2011). Isoivangustin treatment restored the ESR count by decreasing its level dose dependently.

Significant decrease in levels of RF and CRP by treatment with isoivangustin indicates the anti-arthritic potential. The highest levels of RF are usually found in RA, also CRP is a marker for inflammation and its level rises dramatically during inflammation (Mehta et al., 2012). The animals on exposure to FCA (or mycobacteria) in the early phases induces the release of cytokines such as TNF-α, IL-1-β, IL-6, IFN-γ and several chemokines (Billiau and Matthys, 2001). It is also well known that leucocytes produce pro-inflammatory cytokines such as TNF-α and IL-1β which play important role in RA (Fan et al., 2005). TNF-α and IL-1 β originate from the activated macrophages, and TNF- α is also produced by antigen-primed helper T cells. These cytokines have been documented as critically important in RA in rats as well as in human. They contribute too many features of arthritic inflammation, including synovial tissue inflammation, synovial proliferation and cartilage and bone destruction.
damage (Zhang et al., 2009). The key mediators of RA are TNF-α, IL-1 and IL-6 that drive inflammation in RA (Eric and Lawrence, 1996). Isoivangustín significantly decreased the elevated levels of the pro-inflammatory cytokines TNF-α and IL-1β in dose dependent manners that were induced by FCA. Isoivangustín was less effective in reducing the elevated levels of IL-6. Therefore the mechanism of isoivangustín for the anti-arthritic activity may be via modulation of cytokines TNF-α and IL-1β which have been associated with the pathogenesis of RA.

Assessment of the serum levels of AST, ALT and ALP provides an excellent and simple tool to measure the anti-arthritic activity. The activities of aminotransferases and ALP increases significantly in arthritic rats, since these are good indices of liver and kidney impairment which is also considered a feature of adjuvant arthritis (Mythilypriya et al., 2008). Serum AST and ALT has been reported to play a vital role in the formation of biologically active chemical mediators such as bradykinins in inflammatory process (Mali et al., 2011). The treatment with isoivangustín significantly decreased the serum levels of AST, ALT and ALP and increased the level of total protein which confirms its anti-arthritic activity.

There are many studies which have reported the important participation of reactive oxygen species in RA pathophysiology. In these studies, free radicals have been reported to increase in joint cavity first, and then start its effects on the vessel wall with a consequent origination of edema (Tastekin et al., 2007). Lipid peroxidation is a critical mechanism of the injury that occurs during RA, which is often measured by analysis of tissue MDA. The large amount of MDA in arthritic control group is consistent with the occurrence of damage mediated by free radicals. The production of oxygen free radicals that occurs with the development of arthritis in the articular cartilage leads to decreased GSH and SOD levels as a consequence of their consumption during oxidative stress and cellular lysis, which is evident by decreased levels of SOD and GSH in arthritic control group (Arulmozhi et al., 2011). Treatment with isoivangustín significantly decreased the MDA level and increased the depleted levels of GSH and SOD, probably by competing for scavenging of free radicals.
Bone destruction is a common feature of RA which is examined by radiological analysis. Radiographic observation of the rat paw showed that treatment with isoivangustin and diclofenac inhibited arthritis associated joint alterations. From the histopathological studies of ankle joints of arthritic rats, there was destruction of joint, due to the continued migration of lymphocytes, monocytes, into the synovium and joint fluid, connective tissue proliferation and necrosis, all of which produce inflammatory cytokines. Treatment with isoivangustin and diclofenac inhibited this leukocyte migration, connective tissue proliferation and necrosis in arthritis which may have beneficial effects for joint preservation.

The study thus revealed that isoivangustin possess anti-arthritic activity which is mediated by its analgesic and anti-inflammatory effects on various parameters evaluated. The anti-arthritic activity is also supported by its effects on haematological, biochemical, anti-oxidant, radiological and histopathological parameters. The mechanism for its anti-arthritic effect is via suppression of cytokines TNF-α and IL-1β.