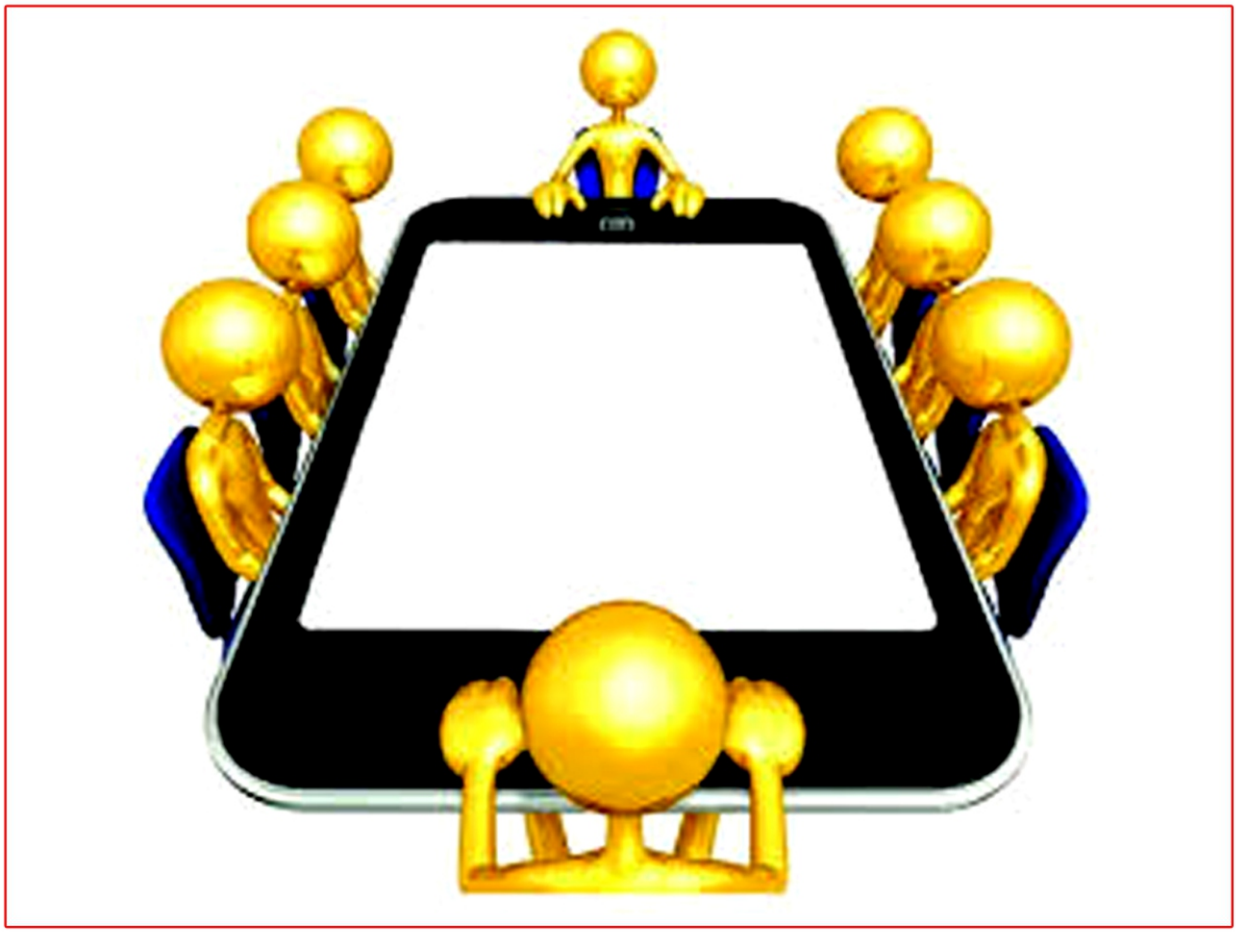
DISCUSSION



DISCUSSION:

Medicinal plants plays very fundamental role to create a large number of various organic chemicals, which are of pharmaceutical and industrial interest. A huge number of pharmaceutically essential chemicals are extracted from plants. For thousands of year's natural products have played a vital role in health care system. Throughout the evolutions, the value of natural products for medicine and health has been enormous. About 25% of drugs prescribed worldwide are basically come from plants, 121 such an active compounds are being in current use. About 252 drugs as basic and essential by the World Health Organization (WHO), 11% are exclusive of plant origin and significant number of synthetic drug obtained 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).

Zingiberaceae family consist of large number of rhizomatous medicinal and aromatic plants characterized by the existence of volatile oils and oleoresins, generally, the rhizomes and fruits are aromatic, tonic and stimulant in nature; occasionally these are nutritive. Some of the plants are used as a food due to presence of starch in large quantities while others yield an astringent and diaphoretic juice (Joyet al., 1998). Zingiberaceae family is an important natural resource that provides many useful products for food, spices, medicines, dyes, perfume and aesthetics (Jantanet al., 2003). Rhizomes of certain ginger species like *A. officinarum, A. galanga, A. calcarata, Kaempfera galangal* have high medicinal values (Indranyl et al., 2009) and the ethnomedical uses of Zingiberaceous plants of Northeast India have been extensively reviewed by (Lath et al., 2010). Many *Alpinia* species are valued for the medicinal properties and are also utilized in traditional system of medicines as a spasmolytic, anti-oxidant, anti-inflammatory, bacteriastatic, hypotensive, anti-emetic, fungistatic property in India, China and other regions (Parida et al., 2011).

Standardization is an important tool used for herbal drugs in order to found their identity, purity, safety and quality (Mukherjee, 2002). In order to standardize a drug, various macroscopic, fluorescence analysis, physicochemical analyses and phytochemical analysis were done. The quantitative estimation of some pharmacognostical parameters is useful for setting standards for crude drugs. (Ravichandra et al., 2011). Raw drugs create

a problem of identification and to establish their genuineness when they not have any external diagnostic features or any organoleptic clues, during such situations, the microscopic analyses of the specimen will suggest a helping hand to set up the identity of the phytodrugs (Mukherjee, 2002).

Macroscopic studies revealed that *A. galanga* are cylindrical rhizomes and about 2 to 8 cm in diameter. Externally reddish brown color, odor is pleasant and aromatic, taste spicy and sweet, while *A. officinarum* rhizome is a slightly curved and cylindrical rhizome, 2.8 cm in length, 6. 15 mm in diameter; externally red-brown to dark brown, odor is characteristic, taste is extremely pungent. Microscopic studies of *A. galanga* revealed an outer cortical region and an inner stelar region. Cortex consists of xylem which showed presence of parenchyma, vessels, tracheids, fibers, oleoresin cells and starch grains. Transverse section of *A. officinarum* reveals the presence of epidermal cells, cortex, endodermis; vascular bundles and fibers. Calcium oxalate and starch grains were also observed.

Powdered *A. galanga* showed the presence of epidermal cells, parenchyma cells with starch grains, vessels with scalariform thickening, parenchyma contain trachied and starch grains. Powdered *A. officinarum* showed the presence of epidermal cells, parenchymatous cells, fragments of vessels, vessels with scalariform thicknings, parenchyma cells containing starch grains.

The total ash, acid insoluble ash, water soluble ash and loss on drying of *A. galanga* was found to be 10.2%, 4.1%, 5.3%, 11% respectively, and *A. officinarum* 7.5%, 1.5%, 1.6%, 1.5 % respectively. The ether soluble extractive value, chloroform soluble extractive value, ethanol soluble extractive value, methanol soluble extractive value and water soluble extractive value of *A. galanga* extract was found to be 3.47%, 1.48%, 9.04%, 4.7% and 12.50% respectively, while for *A. officinarum* extract it was found to be 0.60%, 1.8%, 14%, 2.7%, 1.6% respectively.

Preliminary phytochemical screening was useful in prediction of nature of drugs and also useful for the recognition of different constituents present in different polarity solvent. So it could be helpful to extract out particular constituents by solvent (Harborne et al., 1998). The phytochemical study of *A. galanga* revealed the presence of alkaloids, tannins, terpenoids and phenolics, alkaloids, carbohydrates, tannins, aminoacids, and

saponins, while the phytochemical study of *A. officinarum* revealed the presence of alkaloids, tannins, coumarins, terpenoids and phenolics, carbohydrates, tannins, glycosides, amino acids, phenols, gums and saponins. All the extracts of *A. galanga* and *A. officinarum* are brown to brownish yellow in color and showed semisolid consistency while aqueous extract is brown in color and showed solid consistency.

Phytochemical analysis of the *A. galanga* and *A. officinarum* has mainly demonstrated the presence of phenylpropanoides, saponins, flavonoids, terpenoids and steroids. Steroids can decrease inflammation and reduce the action of the immune system, while triterpenoids impairs histamine release from mast cells and exerts anti-inflammatory effects (Mehta et al., 2012). Flavonoids are often used for their antioxidant effect against free radicals. There are also strong indications that they have antiviral, anti-inflammatory and anti-hypertensive properties (Ibrahim et al., 2012). We suggest that the anti-inflammatory activity of the *A. galanga* and *A. officinarum* could be due to combined effect of phenylpropanoides, flavonoids, saponins, steroids and triterpenoids, which are the major components of the extract *A. galanga* and *A. officinarum*.

A. galanga and *A. officinarum* was fractionated by chromatographic technique and it was screened by carrageenan induced paw edema in rat. Taking into consideration the result obtained in above study it was further fractionated by column chromatography and evaluated for anti-inflammatory potential at the dose of 10 mg/kg using carrageenan induced paw edema model.

The present study demonstrated that ACA had showed anti-inflammatory activity in carrageenan induced paw edema in rats. These results with isolated ACA and previous results with AEAG confirm that ACA is beneficial in the treatment of pain and inflammation.

The present study also demonstrated that galangin had showed anti-inflammatory activity in carrageenan induced paw edema in rats. These results with isolated galangin and previous results with MEAO confirm that galangin is beneficial in the treatment of pain and inflammation.

Health diseases are the major problem towards the advancement of human civilization. In order to overcome this problem, a worldwide approach has been made through scientific research against most important health disorder like cancer, AIDS,

heart disease and arthritis. WHO pointed out that musculoskeletal situation are a major burden on individuals, health systems and social care system? It has been predicted that arthritis particularly rheumatoid arthritis would rank fourth for the primary cause of disability by 2020. Arthritis is a global crisis that will increase in significance with the rising 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 suggested for the treatment of arthritis includes non-steroidal anti-inflammatory drugs like diclofenac, aceclophenac, glucocorticoid therapy and disease modifying anti rheumatic drugs like anti TNF-α blockers, methotreaxate, cyclosporine A and stem cell therapy. Therapeutic managements of arthritis is well known for its several side effects as a result of which the past decades there is dramatic increase and growing interest in the use of alternative treatments and herbal therapies in arthritis.

Animal models for the assessment of novel anti-rheumatic or anti-inflammatory drugs are widely used in pharmacological research (Greenwald and Diamond, 1988, Seed et al., 1991). Various agents when injected into a joint of rabbits or rats can cause arthritis via different pathogenetic mechanisms. FCA induced arthritis model have been extensively used to study the pathogenesis of rheumatoid arthritis for therapeutics testing (Mizushimaet al., 1972). FCA induced arthritis is an experimental model pioneered by (Pearson and Wood 1959) that shares several human clinical and pathological states of rheumatoid arthritis.

In experimental arthritis animal model wistar rats were used because animal model provides more uniform experimental data and allow for extensive testing of potential therapies. It has been observed that this animal model share features with human arthritis. It has also been found that a rat model has similar pathological features to human pathological features and has the capacity to predict the efficacy of a given therapeutic agent in humans (Hegen et al., 2008).

Complete Freund’s adjuvant induced arthritis is one of the most widely used models for chronic arthritis (Chillingworth et al., 2003). Freund's adjuvant (a mixture of heat killed *Mycobacterium tuberculosis* with liquid paraffin) produced inflamed lesions

in areas of the body remote from the injection site after a delay of 10 to 15 days (Newbould, 1963). In adjuvant-induced arthritis model rats developed a chronic swelling in multiple joints with influence of inflammatory cells, erosion of joint cartilage and bone destruction and remodeling, it is commonly used for preclinical studies of NSAIDs and anti-rheumatic drugs and this model is most suitable as like human arthritis (Sofia et al., 1973).

In arthritis different inflamantory mediators were involved which are the products of arachadonic acid metabolism, histamine, 5-HT, bradykinin, cytokines, and nitric acid. CFA produces a characteristic inflammation and associated hyperalgesia, which can be used to quantify the anti-inflammatory or anti-hyperalgesic actions of drugs (Sammons, 2000). Mediators, like bradykinin, which are released from injured tissue directly stimulate nociceptors, and stimulate tumour necrosis factor-alpha (TNF-α) release. The TNF-α in turn, stimulates the release of interleukin-1beta (IL-1β) and interleukin-6 (IL-6), promoting the initiation of cycloxygenase enzymes, which convert arachidonic acid to prostaglandins (Wim and Berg 1999). Tumour necrosis factor-alpha (TNF-α) also stimulates the release of cytokine-induced neutrophil chemoattractant (CINC-1) in rats or interleukin-8 (IL-8) in humans. Cytokines, like IL-1β, TNF-α and IL-6, plays imp role in rhumatoid arthritis (Carteron NL 2000), these cytokines plays imp role in hyperalgesia by sensitizing peripheral nociceptors, decreasing the peripheral nociceptor threshold (Loram et al, 2007). As per the results galangin and MEAO were significant to treat CFA induced arthritis.

As per the results of our study ACA and AEAG exhibited a significant anti-arthritic activity by inhibition of paw volume and reduced joint diameter in arthritic treated rats. ACA and AEAG also show significant anti- hyperalgesic activity. The actual mechanism may be due to the suppression of inflammatory mediators like IL-1β, TNF-α and IL-6 released due to induction of Freund's adjuvant in arthritic rats.

Radiographic changes in RA conditions are useful diagnostic measures which indicate the severity of the disease. Soft tissue swelling is the earlier radiographic sign, whereas prominent radiographic changes like bony erosions and narrowing of joint spaces can be observed only in the developed stages (final stages) of arthritis (Harris 1991). The standard drug diclofenac prevented the bony destruction and also there was no

swelling of the joint. Treatment with AEAG (400 mg/kg) and ACA (20 mg/kg) for 28 days prevented bony destruction as there was less soft tissue swelling and narrowing of joint spaces than that observed on 14^{th} day.

Free radicals production that occurs during development of arthritis in the articular cartilage leads to decreased GSH and SOD levels, increased ROS levels in rhumatoid arthritis may result in a pro-oxidation environment, which in turn could result in increased MDA levels. As a result, lipid peroxidation may have a role in the pathogenesis of the rhumatoid arthritis (Bhowmicket al., 2008). Pathogenesis of arthritis is associated predominantly with the formation of free radicals at the site of inflammation. In rheumatic condition oxidative injury and inflammatory status was confirmed by increased levels of prostaglandins in serum and synovial fluid compared to controls. T cells isolated from the synovial fluid of patients with rheumatoid arthritis showed signs of decreased intracellular GSH level (Valko et al., 2007). In the present study, the levels of SOD and GSH were increased, while the level of MDA was reduced by AEAG, MEAO, ACA and galangin.

From the results it is clear that reduction in RBC count and hemoglobin level represents the anemic condition in arthritic rats. More significant causes are the irregular storage of iron in the reticulo endothelial system and synovial tissue and the breakdown of bone marrow to respond to anemia (Mowat, 1971). Anaemia is the most common haematological deformity seen in patients with rheumatoid arthritis (Weiss and Goodnough, 2005). Inflammation causes increase in the WBC (Castro and Gourley, 2010). The increase in both WBC and platelet counts might be due to the stimulation of immune system against the invading pathogenic microorganism (Maria et al., 1983). It is clear by the infiltration of inflammatory mononuclear cells in the joints of arthritic rats. In the present study, the level of Hb and RBC was significantly increased, while the level of WBC and platelets was significantly reduced by AEAG, MEAO, ACA and galangin.

Lysosomal enzymes play an important role in the physiology and pathology of the joint tissues in arthritis (Dingle, 1973). Measurement of their level provide an excellent tool for anti-arthritic activity of drugs, the activities of aminotransferases and ALP were significantly increased in arthritic rats, since these are excellent indices of liver impairment, which are also measured as the features of adjuvant arthritis (Vijayalakshmi

et al., 1997, Mythilypriya et al., 2008). Treatment with AEAG, MEAO, ACA and galangin significantly ($P<0.001$) decreased the levels of ALT, AST, and ALP in arthritic cases.

Presently the secondary metabolites were obtained by conventional method of cultivation, collection, extraction and isolation, which has its limitations like unforeseen environmental conditions, time consuming, desired quality, and a gap between the demand and supply. Therefore micropropagation through plant tissue culture can be an attractive alternative method. By suitable manipulation of hormones and contents of the medium, it is possible to initiate the developments of roots, shoots and complete plants from callus cultures (Evans, 2001).

On the cellular level, auxin is essential for cell growth, affecting both cell division and cellular expansion (Mcsteen and Yunde, 2008). *A. purpurata* on MS medium supplemented with 2, 4-D + kinetin (2:2) give better results for callus. Maximum numbers (9-11) of shoots were observed in medium with NAA (0.1ppm) combination with BA (3.0ppm) after incubation. Maximum roots were found in MS media containing 3 ppm of Indole acetic acid (IAA).