Discussion
DISCUSSION

Pharmacognosy of the plant

"Pharmacognosy" is the study of the structural, physical, chemical and sensory characters of the crude drugs of animal, vegetable and mineral origin. The important aspect of pharmacognosy deals with the methods of identification of crude drugs. Therefore in the present investigation, it is of interest to study the pharmacognostical features of the plant *Opcrculina turpethum*. It is well known that botanical identity of a plant is an essential prerequisite before the drug is used for chemical and pharmacological studies.

The coexisting plant white variety resemble so closely to black one (*Opcrculina turpethum*) that results in mistaken identity of genuine species. From the present investigation, it clearly reveals the characteristics of the plant *Opcrculina turpethum* from its adulterant white variety which is established by the pharmacognostical studies.
Preliminary Pytochemical screening

It is generally accepted that the active principle presented in the plant are solely responsible for the therapeutic efficacy and as potent oxidant scavengers. It is obvious that different species of plants would have different chemical constituents. However, these differences can extent to different varieties or even the same variety grown in different location or harvested at a different time. Different parts of plant such as leaves, bark, seeds, roots, flowers and pods can also have different active constituents (Kokate et al., 1997; Harbone 1973). A wide array of active constituents plays a important role in recovery from various diseases like cancer, inflammation, viral, fungal and bacterial infections (Havsteen 1983, Borrelli et al., 2002; Ramesh et al., 2003).

HPTLC Fingerprinting

Phytochemical evaluation of medicinal plant is essential to study the pharmacological activities. It can be done by qualitative chemical analysis by using techniques like TLC, HPTLC, HPLC, GC etc. HPTLC is a major advancement of TLC principle requiring shorter time and better resolution.

Therefore, in the present study Operculina turpethum stem bark extracts were evaluated for different phytochemical constituents by preliminary qualitative chemical analysis by using HPTLC technique.

The HPTLC fingerprinting obtained for the methanolic and hydro-alcoholic extracts of Operculina turpethum reveals that the mobile phase used gave well resolved peaks with good separation. Spectrum 1 show...
the maximum number of peaks when compared to spectrum 2. From the results it is inferred that the methanolic extract of *Operculina turpethum* has more active constituents when compared to hydro-alcoholic extract.

The preliminary phytochemical and HPTLC analysis of HAOP and MOP showed the presence of alkaloids, polyphenolic compounds, tannins, saponins, flavonoids, proteins, carbohydrates and triterpenoids which may contribute to the various pharmacological activities.

**Toxicological Evaluation of HAOP and MOP**

The rationale for the utilisation of medicinal plants has rested largely on long-term clinical experience with little or no scientific data on their efficacy and safety (Zhu *et al.*, 2002). In this context Sofowora (1989) is of opinion that the large scale use of herbal medicines need a thorough scientific investigation to validate their traditional uses.

Medicinal plants play a key role in the human health care because of their efficacy, safety and lesser adverse effects. When herbal drugs are used as a therapeutic substance for treating various ailments, it becomes an essential requirement to fulfill the guidelines formulated by world health organization (WHO). In this connection, WHO guidelines have given one of the important criteria to establish the safety profile of the herbal preparations (WHO report, 1991).

Hence in the present study, the plant *Operculina turpethum* stem bark extracts were studied for its acute oral toxicity and 90 days repeated oral dose toxicity.
Acute toxicity

*Opeculina turpethum* stem bark extracts (HAOP and MOP) did not produce any toxic symptoms or mortality up to a dose level of 2000 mg/kg/po in rats and hence the drugs were considered to be safe for pharmacological study.

Chronic toxicity study

Food intake, water intake and body weight

In 90 days repeated oral dose study of HAOP and MOP at the dose of 1000 mg/kg/po, did not produce any significant changes in the body weight, food and water intake. Changes in body weight have been used to access the course of the disease and the response to the therapy of drugs. (Winder *et al.*, 1969). Therefore, body weight is a good indicator of toxicity (Gerald *et al.*, 1971, Rolf et al 1980, Charles *et al.*, 1993, Karl *et al.*, 1993; Teo *et al.*, 2002).

Effects of HAOP and MOP on haematological parameters

HAOP and MOP at a dose of 1000 mg/kg did not produce any toxicological symptoms on chronic administration for 90 days. There were no significant changes observed in haematological parameters like RBC count, WBC count, ESR and hemoglobin levels. This is in accordance with the findings of Vijayalakshmi *et al.*, (2000).
Effects of HAOP and MOP on Biochemical parameters

HAOP and MOP at a dose of 1000 mg/kg did not produce any toxicological symptoms on chronic administration for 90 days. There were no significant changes observed in biochemical parameters like blood urea nitrogen, plasma uric acid, serum creatinine, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and acid phosphatase (ACP). The blood glucose level, which remained constant in all the groups of animals, shows the normoglycemic activity of the extracts.

Determination of BUN and serum creatinine shows that the extracts HAOP and MOP did not produce any renal dysfunction, as increase in the above parameters reveals the impaired renal function or acute renal failure (Varley, 1964).

Transaminases (AST, ALT) and alkaline phosphatases are considered to be good indices of liver and kidney functions (Viluksela et al., 1997b). Since the extracts did not produce any alterations in these enzyme levels it could be inferred that the extracts did not induce any damage to the liver and kidney. This is further confirmed by the histological assessment of these organs.

Histopathological studies of various organs

The histopathological studies of the major vital organs like liver, kidney, heart, spleen, ovary and testis were taken from control and extracts
(HAOP and MOP) treated animals. The histopathological study showed normal architecture suggesting no detrimental changes and no morphological disturbances were caused by the daily oral administration of the extracts (HAOP and MOP) at the dose level of 1000 mg/kg, for 90 days.

From these results it is inferred that the extracts HAOP and MOP are considered to be safe as they did not cause any damage to the treated animals.

**Anti-nociceptive activity**

The anti-nociceptive activity of HAOP and MOP were evaluated using both thermal and chemical method in mice. These methods are used to detect central and peripheral analgesics. Acetic acid induced writhing test was used for detecting both central and peripheral analgesia, whereas hot plate and tail flick tests are most sensitive to centrally acting analgesics. Induction with aspirin activates the COX enzymes which further activates prostaglandin synthesis (Deraedt *et al.*, 1980).

**Effect of HAOP and MOP on acetic acid induced writhing, latency period of mice exposed to hot plate and tail flick test.**

Pain is a major symptom in RA. Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. The tissue damage that causes pain is called a noxious stimulus. The tissue damage and the changes in the chemical and physical microenvironment, within and surrounding the affected tissue stimulate small diameter nociceptive nerves (Anand *et al.*, 1987).
During inflammation of the joint the intra-articular pressure is increased due to an increased blood flow, synovial effusion and tissue oedema. The increased intra-articular pressure triggers the receptive endings of noiceptive fibers, becomes receptive to noxious stimuli.

Antinociceptive activity of opioid agonist, opioid partial agonist, on non-steroidal anti-inflammatory agents can be determined using the writhing test (Vogel and Vogel, 1997). In Acetic acid writhing both the extracts showed significant analgesic activity, when compared to control group and standard drug aspirin. Acetic acid induced writhing involves the release of arachidonic acid metabolites via cyclooxygenase pathway and prostaglandin biosynthesis (Duarte et al., 1988) and this is due to increase in peritoneal fluid levels of PGE$_2$ and PGF$_{2\alpha}$ (Deraedt et al., 1980).

In hot plate and tail flick test both the extracts showed significant analgesic activity when compared to control group. The centrally acting analgesics generally elevate the pain threshold of mice towards heat. From these results it could be concluded that the extracts exhibit anti-nociceptive activity by central as well as peripheral mechanism(s).

**Antinflammatory activity**

It is important to point out the general co-relation between anti-inflammatory and analgesic activities. This could be explained in terms of the presence of compounds with a similar mechanism for both activities. By the activation of the cyclo-oxygenase enzyme, the level of PGE2 increases
markedly, and its production provokes inflammation and pain (Dannhardt and Kiefer, 2001).

**Carageenan induced paw oedema**

Carageenan induced paw oedema in rats is one of most commonly used models of inflammation and has been accepted as a useful diagnostic tool for investigating new anti-inflammatory agents (Mascolo et al., 1987). *Operculina turpethum* extracts significantly inhibited this oedematous response over all time periods and at all doses assayed. The initial phase of carageenan paw oedema is mediated by histamine and serotonin, while later phase by prostaglandins, producing oedema after mobilization of leukocytes (Castro et al., 1968).

The time course of edema development in carageenan induced paw edema model in rats is generally represented by a biphasic curve (Wintar et al., 1962). The first phase occurs within an hour and is partly due to the trauma of injection and also to the serotonin component (Crunkhorn and Meacock, 1971).

Prostaglandins play a major role in the development of the second phase of reaction, which is measured around 3 h time (Di Rosa, 1972). The presence of PGE₂ in the inflammatory exudates from the injected foot paw can be demonstrated at 3 h (Vinegar et al., 1969). The carageenan induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors (Phadke, 1988).
Both extracts of *Operculina turpethum* effectively inhibited oedema produced by serotonin, histamine and PGE2, which suggests that the anti-inflammatory activity of *Operculina turpethum* extracts is possibly mediated by either inhibiting the synthesis, release or action of these mediators.

**Cotton pellet granuloma**

The inflammatory granuloma is a typical feature of established chronic inflammatory reaction (Spector, 1969). The cotton pellet granuloma method has been widely employed to evaluate the transudative, exudative and proliferative components of chronic inflammation. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma and the dry weight. The dry weight of the pellets correlates well with the amount of granulomatous tissue (Swingle and Shideman, 1972). Both extracts of *Operculina turpethum* significantly inhibited oedema which suggests that the anti-inflammatory activity of *Operculina turpethum*.

The early events comprise an accumulation of fluid and proteinaceous material together with an infiltration of neutrophils. The granuloma formed by day 7 is characterised by the formation of a vascularized fibrous capsule containing fibroblasts and infiltrating mononuclear cells (Bailey *et al.*, 1981). Early events occurring in the vicinity of the cotton pellet result in the generation of substances chemotactic towards neutrophils and leucocytes. Possible products of the complement system, kinin system or the generation of products of arachidonic acid oxidation are
all possible stimulators of chemotaxis. The attraction and accumulation of cells particularly neutrophilic polymorphonuclear leucocytes (PMNL) at sites of inflammation is an essential part of the host defence mechanism. However, if the inflammatory stimulus is not removed, the continuing emigration and accumulation of PMNLs, lymphocytes and macrophages can lead to the release of mediators and enzymes which are responsible for the tissue damage seen in chronic inflammatory conditions. Freeman et al. (1982) have divided the cellular infiltration into two phases; an acute phase lasting for about 2 days in which PMNL predominate, followed by a further period of several days during which the population of mononuclear cells, mainly macrophages increases and cell proliferation commences.

**Antipyretic activity**

The antipyretic activity of the extract was expected since from the anti-inflammatory and analgesic tests, the extract had consistently shown to act peripherally on inflammatory mediators especially prostaglandins. The blockade of phase 2, which antagonize cyclo-oxygenase – an enzyme which produces prostaglandins responsible for the genesis of fever (Brune and Alpermann, 1983).

In the present investigation HAOP and MOP showed a significant reduction in pyrexia induced by TAB vaccine.

The role of PGE₂ in pyrexia is postulated to increase the set point of the hypothalamic thermostat to a higher level leading to increased heat production and decreased heat loss. Fever inducing effects of endogenous
pyrogens are mediated via increase in hypothalamic PGE$_2$ activity. Hence the antipyretic activity of HAOP and MOP in TAB vaccine induced pyrexia may be attributed to its inhibition of PGE$_2$ biosynthesis (Panthong et al., 2003).

**Antiulcer activity**

Gastric ulcers arise due to various factors (McGuigan, 1991). Even though the etiology of gastric ulcers is still debated, it is accepted that ulcers are caused due to net imbalances in mucosal offensive and defensive factors (Goel and Bhattacharya, 1991).

Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and piroxicam, remain among the most commonly used pharmacological agents (Garner, 1992). However, these classes of substances may cause gastrointestinal ulceration, due to the ability of these agents to suppress prostaglandin synthesis (Wallace, 2001). Piroxicam is a preferential COX-1 inhibitor. Cyclooxygenase was constitutively expressed in the gastrointestinal tract in large amounts and has been reported to maintain mucosal integrity through continuous generation of prostaglandins (Halter et al., 2001). The ulcerogenic action of aspirin was potentiated by acute stress. Extensive ulceration occurred in stressed rats while relatively little gastric damage occurred in non-stressed rats (Meeroff et al., 1975; Brown et al., 1978).

Gastric wall mucus, an obligatory component of which is hexosamines, is thought to play an important role as a defensive factor against gastrointestinal damage (Davenport, 1968). The determined gastric wall mucus was used as an indicator for gastric mucus secretion, while mucosal
hexosamine content was used as an indicator for gastric wall mucus synthesis (Lukie and Forstner, 1972).

Aspirin decreased the concentrations of all the individual carbohydrates and also the carbohydrate to protein ratio, however, the decrease in hexosamine concentration was not significant. A similar decrease in carbohydrate/protein ratio and of individual carbohydrates has been earlier reported in the non-dialysable and lyophilized fractions of the mucus in aspirin-treated dogs (Menguy and Masters, 1965).

The increase in protein content of the gastric juice in untreated ulcer group indicates damage to the gastric mucosa as a result of which plasma proteins leak into gastric juice (Grossman, 1978; Goel et al., 1984). Both extracts showed reduction in protein concentrations, which indicates strengthening of the gastric mucosa, thereby restricting the entrance of the plasma proteins into gastric juice.

In Aspirin + PL groups, HAQP (100 mg/kg) and MOP (100 mg/kg) pre treatment showed a significant increase in the defensive mucin secretion quantified in terms of total carbohydrates and protein (TC:P) ratio of the gastric juice (Sanyal et al., 1983). In contrast ranitidine (50 mg/kg) pretreated group showed a considerably lower mucin secretion. Mucin is viscous glycoprotein with physiochemical properties producing relatively resistant barrier (Flemstrong and Garner, 1982). Gastric mucus (mucin) is an important protective factor for the gastric mucosa and consists of a viscous, elastic, adherent and transparent gel formed by 95% water and 5%
glycoproteins that cover the entire gastrointestinal mucosa. Moreover, mucus is capable of acting as an antioxidant, and thus can reduce mucosal damage mediated by oxygen free radicals (Repetto and Llesuy, 2002). It makes up the major part of the mucus, an important pre-epithelial factor that acts as the first line of defense against ulcerogens (Zalewsky and Moody, 1979). Increase in mucin levels is seen in gastric ulcer. The mechanism by which the extracts augment the mucus secretion is uncertain.

Rat gastric mucosal damage induced has widely been used to investigate gastroprotective effect of medicinal plants (Zhu et al., 1997). In the present study, HAOP and MOP have been shown to possess anti-ulcer activity against Aspirin + pylorus ligated ulcer model. Both extracts significantly reduced the acid secretary parameters i.e. total and free acidity as well as the gastric volume and ulcer index. Sanmugapriya et al., (2005) have suggested that acid inhibition accelerates ulcer healing. The decrease in gastric volume and simultaneous decrease in acidity may be one of the causes of ulcer healing in HAOP and MOP treated groups.

The increase in individual carbohydrate content in HAOP and MOP treated groups over that of the untreated ulcer group appears to be due to stimulation of mucus secretion. The essential criterion, which determines the status of the mucosal defense against the offensive assault of acid, is the quality and quantity of mucus secretion (Barbara et al., 1974). Increased mucus secretion by the gastric mucosal cells can prevent gastric ulceration by several mechanisms: (i) lessening of stomach wall friction during peristalsis
and gastric contraction, (ii) improving the buffering of acid, and (iii) by acting as an effective barrier to back diffusion of hydrogen ions (Goel et al., 1990).

The total carbohydrates and protein (TC: P) ratio serves as a direct index of gastric mucosal defense i.e. reflection of mucin activity. Its increase represents augmented mucosal protective activity (Sanyal et al., 1971). As the extracts appear to strengthen the mucosal barrier, which is first line of defense against endogenous and exogenous ulcerogenic agents, it can be categorized as mucoprotective agent.

Further, histopathological examination of stomach mucosa shows that pretreatment with HAOP (100 mg/kg), MOP (100 mg/kg) and Ranitidine (50 mg/kg) protects the mucosal epithelium from the damage caused by aspirin. In HAOP and MOP treated groups the mucosa was found to be almost normal with mild muscularis mucosa. Ranitidine treated section shows normal mucosa with no ulcer, edema in the submucosa.

At the same time HAOP alone (100 mg/kg) and MOP alone (100 mg/kg) treated groups did not produce any significant changes in the biochemical parameters and they preserved the normal architecture of the stomach mucosa by significantly reducing the gastric volume and ulcer index as compared to PL control animals. This further establishes the fact that the extracts thereby are ulcer protective in nature.

These results suggest that both the extracts (HAOP and MOP) possess anti-ulcer activity, whereas HAOP is more effective when compared
with MOP in aspirin + pylorus ligated rat models. The results were compared with the standard drug Rantidine, a H₂ receptor antagonist.

**Adjuvant induced arthritis (AIA)**

RA is a common chronic and systemic autoimmune disorder characterized by inflammation of the synovial joints and concomitant destruction of cartilage and bone. It involves a complicated pathogenesis, with pathological changes in multiple targets. AIA in rat models is widely used for the screening and evaluation of anti-inflammatory drugs in RA.

The pathogenesis of AIA involves formation of immune complexes resulting in slow dissemination of microbial antigen around the body and deposition at various sites initiating joint lesions (Hiu, 1977). The etiology of the disease is thought to be due to a delayed hypersensitivity reaction. At least three phases have been distinguished in the development of arthritis in rats following inoculation with an adjuvant and these are pre-arthritic, arthritic and osteogenic phases (Baumgartner et al., 1974). Van Eden et al., (1985) reported that adjuvant-induced arthritis is probably caused by antigenic cross reactivity and accordingly an autoimmune disease may be triggered by structural mimicry between antigens in the environment and self-antigens in the individual.

**Body weight changes**

Body weight is a important indicator feeding behaviour. has been used to quantify the chronic pain state in various animal models (Colpaert
and Vanden, 1983). After inoculation the adjuvant, the body weight remains unchanged up to 1st week thereafter the body weight drastically reduced which infers sharply the pain and flaring of inflammation in arthritis. Such a decrease in body weight may be attributed to poor feeding behaviour and delayed growth (Bernard et al., 1987).

On the other hand, drug treated animals in both developing and developed arthritis show significant restoration in body weight. This infers that weight loss is normalized by HAOP and MOP and standard drug Diclofenac sodium.

**Haematological studies**

The hematological changes associated with arthritic condition are significant decrease in RBC, Haemoglobin level and significant increase in WBC, ESR levels. Upon drug treatment the hematological changes has significantly been reverted to normal.

Development of anemia in RA is related with inflammatory cytokine in joint inflammation and which interfere with normal RBC formation and destruction (Maury et al., 1988 and Voulgari, 1999). In rheumatoid arthritic patients an increased total leukocyte and platelet counts are seen (Kjeldsen-Kragh et al., 1995).

ESR is considered as one of the diagnostic criteria for RA. Elevated levels of ESR are seen with patients suffering from RA (Chuang, 1982; Healey, 1984). ESR determines the disease by its presence, progression
and response to therapy. Increased ESR indicates the presence of infectious disease, inflammatory reaction and neoplastic changes (Susanna and Booker, 1985).

**Serum acute phase proteins**

The levels of fibrinogen and ceruloplasmin were found to be significantly increased in arthritic animals. Elevated acute phase proteins fibrinogen and ceruloplasmin indicate the progression and severity of RA (Raffaele and Joost, 1996). Ceruloplasmin and fibrinogen are synthesis by hepatocytes upon interleukin release (Rokita et al., 1994; Schultz and Arnold, 1990).

Ceruloplasmin plays an important role in inhibition of lipid peroxides formation during inflammation, through oxidation of ferrous molecules required for free radical generation (Gutteridge, 1986). Fibrinogen in seen significantly increased in kidney dysfunction in RA (Dijoseph, 1993).

Treatment with HAOP and MOP significantly reduced the serum fibrinogen and ceruloplasmin levels reflecting their efficacy in controlling the progression of the disease and the results were comparable to that of the standard Diclofenac.

**Serum proteins and A/G ratio**

The effect of HAOP and MOP on serum total protein, albumin, globulin, and A/G ratio significantly elevated in arthritic rats.
A significant reduction in A/G ratio was observed in adjuvant induced arthritic rats, which might be attributed to increased permeability of vascular tissue to albumin via increasing the level of cAMP by inflammatory mediators (Geetha et al., 1998).

There is significant decrease seen in the levels of globumin and increase in the levels of albumin during acute phase of arthritis (Lowe, 1964).

**Blood glucose, urea, uric acid and Creatinine**

The levels of blood glucose, blood urea nitrogen, uric acid and creatinine were significantly altered in arthritic animals. There seems to be significant increase in urea and creatinine levels and a significant decrease in uric acid levels was observed in arthritic animals. These changes were normalized to a greater extent after treatment with HAOP and MOP in arthritic animals.

The equilibrium between entry and utilisation of glucose is altered during inflammation in joints. Ropes et al. (1960) have reported that appearance of glucose in joint fluid was delayed in arthritis and its subsequent rate of accumulation was reduced. The impaired transport of glucose into the joints might cause the increase in blood glucose level.

**Serum and tissue marker enzymes**

Aminotransferases being an important class of enzymes linking both carbohydrates and aminoacids in their metabolism. These enzymes perform a important role as marker of liver injury since liver is the major site
of metabolism (Liss et al., 1985). Administration of HAOP and MOP normalized the above changes and the result was comparable to that of the standard Diclofenac.

The marker enzymes such as Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) were elevated in serum, liver, kidney and spleen in arthritic rats.

Vijayalakshmi et al., (1998) reported elevated levels of aminotransferases in the liver and kidney of adjuvant arthritic rats. The increase in aminotransferases might be due to their release from the cells of the damaged tissues, since liver impairment is also a feature of adjuvant arthritis (Whitehouse et al., 1974). Increased ALP activity suggests that numerous alterations take place at the onset of calcification. Bourne (1972) has reported increased ALP activity in hypertrophying chondrocytes.

Glycoproteins

The levels of glycoproteins like hexose, hexosamine and sialic acid in plasma and tissues of arthritic rats were significantly increased. These conditions were reverted to normal upon treatment with HAOP and MOP and similar observation were seen in Ramprasath et al., (2006).

Considerable evidence exists that the serum glycoprotein level may serve as an indicator of certain pathological processes (Winzler, 1948). Thus, the serum glycoprotein level rises when one finds an increase in tissue
proliferation as in neoplastic disease, renal disease, infectious conditions, rheumatic diseases, cardiovascular disorders and biliary obstruction (Winzler, 1953).

Increased excretion of glycosaminoglycans and glycoproteins is likely to be due to their excessive degradation by altered levels of lysosomal enzyme activity in AIA. Elevated levels of hydroxyproline – a non-essential amino acid found to occur almost exclusively in the collagen (Aminoff, 1961) and it indicates an alteration in collagen metabolism and synthesis (Geetha et al., 1999).

Glycosaminoglycans play an important role during inflammatory condition and wound healing (Shoshan, 1974). They influence the formation of new ground substance during inflammation with an increased rate of proteoglycan synthesis and cell proliferation (Deshmukh et al., 1976).

As a result of inflammation of collagen in bone and cartilage crosslinks, mature collagen is the extracellular matrix of cartilage contains several proteoglycans such as chondroitin sulphate, heparin sulphate, keratin sulphate and dermatan sulphate (Haraoui et al., 1994).

**Lysosomal enzymes**

The activities of lysosomal enzymes namely, acid phosphatase, cathepsin-D, β-glucuronidase and N-acetyl-glucosaminidase are useful marker in arthritic condition. The lysosomal enzyme levels of plasma, liver, kidney and spleen were found to be elevated significantly in arthritic animals.
Administration of HAOP and MOP normalized the above changes and the result was comparable to that of the standard Diclofenac.

Cathepsin D is a ubiquitous endoprotease which is involved in normal protein degradation with lysosomes (Barret, 1992).

These changes are accompanied by increased activities of alkaline phosphatase, glycoproteins, glycosaminoglycans and collagenolytic enzymes. Immune complexes are endocytoosed by leucocyte into phagocytes which then unite with fragile secondary lysosomes. This series of events is followed by release of lysosomal enzymes (Latha et al., 2001). Rupture of lysosomal membrane releases an array of hydrolytic enzymes, initiating several reactions that stimulate the synthesis of inflammatory mediators such as thromboxanes, prostaglandins and leukotrienes.

Anderson (1970) has reported an increase in the lysosomal enzymes in the homogenate of adjuvant-injected paw due to the extensive infiltration of leucocytes. There is correlation between the release of lysosomal enzymes into the extracellular compartment and inflammatory processes. Release of lysosomal enzymes is crucial in the pathogenesis of tissue injury and inflammation (Weismann, 1972).

**Lipid profile**

The levels of total cholesterol, LDL cholesterol and phospholipids were significantly increased in arthritic rats with a significant reduction in triglycerides and HDL cholesterol.
RA patients with active disease exhibit an abnormal lipid profile. LDL is sensitive to lipid peroxidation due to its high content of polyunsaturated fatty acids (Esterbauer et al., 1990). The acute phase protein ceruloplasmin is reported to be involved in oxidation of LDL.

The activated macrophages and lymphocytes with adjuvant injection or their products, monokines, may be involved in abnormal lipid and protein metabolism as well as degradation of joint cartilage. Hepatic synthesis of LCAT and lipoproteins are reported to be affected by some monokines (Yamaguchi et al., 1989). Treatment with HAOP and MOP reverted the altered levels to near normal levels in arthritic rats.

**Lipid peroxidation**

The levels of LPO was significantly increased in plasma, kidney and spleen whereas significantly reduced level was observed in liver in arthritic animals.

The increase in plasma lipid peroxide may be due to the release from neutrophils and monocytes at the site of inflammation (GreenWald, 1981). Granulocytes, which accumulate in rheumatoid joints, are known to produce oxygen-derived free radicals during phagocytosis of bacteria and immune complexes (Babior, 1981). Increased lipid peroxidation in body tissues has been observed in wide range of tissue injury such as CCL₄ toxicity, atherosclerosis and in carrageenan induced inflammation (Sharma et al., 1972). Treatment with HAOP and MOP decreased the lipid peroxides in arthritic rats.
Antioxidants

In general, every part in the body are normally protected from free radical induced damage by a variety of endogenous scavenging proteins, enzymes and chemical compounds that constitute the endogenous antioxidant systems. ROS are produced in the inflamed tissue by activated phagocytes along with protoglandin a, proteases and other mediators of inflammation. In RA patients there is a drastic decrease seen in the level of Iron during the initial phase and this metal ion accumulate in synovial tissue enhances the ROS mediated damage (Halliwell and Gutteridge, 1989; 1990; Seiss, 1991).

A significant increase in tissue SOD activity was observed in adjuvant-induced arthritic rats. The increased activity appears to be a reflux mechanism to guard against the extracellular oxygen derived free radicals (Marklund et al., 1987). The increased delivery of NADPH from the stimulated hexose monophosphate (HMP) shunt during inflammation may also account for the activation of SOD in arthritic rats (Roos and Weening, 1979). Ristner and Bachner (1970) have reported an increase in neutrophil SOD levels under conditions of hyperoxia. Exogenously added SOD has been shown to prevent cell and tissue injury induced by activated phagocytes (Britton et al., 1970). Tissue level of selenium correlates closely with GPx activity (Parnham et al., 1987). Selenium level was reported to be decreased in rheumatoid arthritis. (Mottonene et al., 1984). This may be the reason for decreased GPx activity. At low concentration of H$_2$O$_2$, catalase can function
as a peroxidase. The decreased activity of catalase may be due to the accumulation of $\text{H}_2\text{O}_2$ because of the increased dismutation of oxygen free radicals by SOD.

Glutathione, in reduced form (GSH) maintains cell membrane integrity against oxidative stress by acting as a substrate for the reduction of peroxides to less damaging alcohols by glutathione peroxidase (Mulherin et al., 1996). Glutathione is synthesised in the liver and is the first line of defence against lipid peroxidation (Nicotera and Orrenius, 1986). The reduced glutathione, in turn keeps up the cellular level of active forms of vitamin E (Sies and Murphy, 1991). The rate at which the level of glutathione declines in arthritis reflects its rate of utilization. The reduced level may also be due to the reduced activity of glutathione reductase. Vitamin E is a major chain breaking antioxidant. Its level was significantly decreased in adjuvant induced arthritic rats. The concentration of Vitamin E in tissues has been inversely correlated to lipid peroxidation. It prevents the cell membrane damage by donating the phenolic hydrogen atom to the peroxy radical formed, thus effecting the termination of the free radical chain reaction (Burton and Ingold, 1989).

Vitamin C was found to be significantly reduced in arthritic condition, which may be due to the decreased level of GSH and NADH- semi hydro ascorbate reductase (Bielski et al., 1975; Co Assin et al., 1991). The antioxidants were restored due to treatment with HAOP and MOP in arthritic animals.
Membrane ATPases

The activity of $\text{Na}^+$, $\text{K}^+$-ATPase, $\text{Mg}^{2+}$ ATPase and $\text{Ca}^{2+}$ ATPase in erythrocyte membrane, liver, kidney and spleen were significantly decreased in arthritic animals.

Adenosine triphosphatases are integral part of the membranes and their activity are likely to get altered upon alteration in membrane structure (Bioj et al., 1973). The $\text{Na}^+$, $\text{K}^+$-ATPase, $\text{Mg}^{2+}$ ATPase and $\text{Ca}^{2+}$ ATPase are three membrane bound enzymes that are responsible for the transport of $\text{Na}^+$, $\text{K}^+$, $\text{Ca}^{2+}$, $\text{Mg}^{2+}$ ions respectively across the cell membrane at the expense of ATP (Stehoven and Bonting, 1981). The activities of all these enzymes are found to be affected by ionic concentration (Jain and Shonet, 1981), lipid peroxidation (Ohta et al., 1989), membrane fluidity (Kimelberg, 1975) and hormones (Cohen et al., 1986).

The elevated levels of ROS in RBC, liver, kidney and spleen are responsible in decreased activity of membrane ATPases. In RA patients there is a drastic decrease seen in the level of Iron during the intial phase and this metal ion accumulate in synovial tissue enhances the ROS production (Minotti et al., 1987; Hebbel et al., 1986) and inactivates the membrane ATPases.

Membrane cholesterol inhibits the enzyme activity, which in turn reduces its availability to undergo conformational transition (Yeagle, 1989).
Increased cholesterol level in arthritic condition contributes to the reduction of Na\(^+\), K\(^+\)-ATPase activity. Inhibition of Na\(^+\), K\(^+\)-ATPase and Ca\(^{2+}\) - ATPase lead to the accumulation of intracellular Ca\(^{2+}\) to toxic levels. Increased intracellular Ca\(^{2+}\) causes cell injury and death in a variety of pathological states (Schanne et al., 1979; Farber, 1990).

Collagen

The collagen level was significantly decreased in arthritic rats. The destruction of cartilage in human rheumatoid arthritis was reported to be due to the enzymic degradation of proteoglycans by lysosomal enzymes.

Glycoproteins may play a pivotal role in maintaining the tissue integrity by stabilizing the collagen fibrils (Latha et al., 2001). Further, these activate the MMP's and results in degradation of the collagen fibers. Collagenases (MMP-1 and -13) are responsible for degradation of native collagen fibers (Goupille et al., 1998; Shingleton et al., 2000). The treatment with HAOP and MOP reversed the altered collagen levels in arthritic rats.

In vitro antioxidant activity

DPPH scavenging

HAOP and MOP showed promising free radical scavenging activities of DPPH in a concentration dependant manner. The extracts HAOP and MOP showed significant scavenging activity when compared with reagent blank. Ascorbic acid is used as reference standard.
DPPH assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH'). This assay is considered as a valid and easy to evaluate the scavenging activity of antioxidants, since the radical compound is stable and does not have to be generated as in other radical scavenging assays. In methodological point of view the DPPH' method is recommended as easy and accurate with regard to measuring the antioxidant activity of fruit and vegetable juices or extracts. The results are highly reproducible and comparable to other free radical scavenging methods such as ABTS (Gil et al., 2000).

DPPH shows a strong absorption band at 517 nm in visible spectrum (deep violet colour). As the electron became paired of in the presence of free radical scavenging, the absorption vanishes and the resulting discoloration stoichiometrically coincides with respect to the number of electrons taken up. Thus by depleting the DPPH it proves that the extract might be a potent free radical inhibitor.

**Nitric oxide scavenging**

HAOP and MOP showed significant free radical scavenging action against nitric oxide (NO) induced free radicals. Ascorbic acid was used as reference standard. Sodium nitroprusside serves as a chief source of free radicals. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthylethylene diamine is used as the marker for NO scavenging activity (Mukherjee, 1989).
The chromophore formation was not complete in the presence of HAOP and MOP which scavenges the NO thus formed from the sodium nitroprusside and hence the absorbance decreased as the concentration of the extracts (HAOP and MOP) increased in a dose dependent manner.

The role of free radicals in inflammation especially that of nitric oxide is well documented (Halliwell and Guttridge, 1985). While the nitric oxide is an important second messenger in a number of physiological pathways. Recent studies suggest that in the presence of oxidative stress, nitric oxide can be converted into reactive nitrogen species that contribute to cellular injury and death (Broderick et al., 2005). Overproduction of nitric oxide is known to be an important mediator of inflammatory state (Brown et al., 2001).

**DNA mediated sugar damage**

Oxidative DNA damage is involved in various degenerative diseases like Parkinson’s disease, Hodgkin’ disease, Alzheimer’s disease etc (Jenner, 1991). The dose dependent decrease in degradation of DNA by plant extract implies that the extracts have compounds which may combat free radical mediated degradation of deoxyribose sugar moiety of DNA. The presence of various phytochemicals contribute to the antioxidant potential of the plant
Total antioxidant activity (FTC and TBA method)

FTC method was used to determine the amount of peroxide at the initial stage of lipid peroxidation. During the linoleic acid oxidation, peroxides formed and these compounds oxidize Fe$^{2+}$ to Fe$^{3+}$. The Fe$^{3+}$ ions form complex with SCN$^{-}$, which has a maximum absorbance at 500 nm. In this method, the concentration of peroxide decreases as the antioxidant activity increases. Both HAOP and MOP exhibited effective antioxidant activities at a concentration of 4 mg compared to standards vitamin E and C. Lower the absorbance values exhibited higher the antioxidant activities of the samples.

The absorbance values from TBA method showed total peroxide values produced by the oxidation of linoleic acid. The higher the absorbance values, the lower the level of antioxidant activity. The control had the higher absorbance value on day 5. The absorbance of the extracts HAOP and MOP were showed low and they were significant when compared with control. Based on the present result all the extracts (HAOP and MOP) and standard vitamin C have the highest antioxidant activity.

During the oxidation process, peroxide is gradually decomposed to lower molecular compounds that are measured by TBA method. TBA measures the amount of peroxide at the secondary stage of linoleic acid peroxidation. The extracts HAOP and MOP showed the least increase in absorbance values from day 1 to day 2, but the levels increased significantly on day 3, reached maximum levels on day-6, and finally dropped on day 7.
This reduction is due to the accumulation of malonaldehyde compounds from linoleic acid oxidation, which is not stable. Further oxidation causes malonaldehyde to be converted to secondary products such as alcohols and acids that cannot be detected. The antioxidant activity of the extracts HAOP and MOP was found to be highest in the present study. The high antioxidant activity of the extracts is due to the presence of flavonoids and phenolic compounds.

Antioxidants are known to decrease oxidative stress by scavenging free radicals and protect biological macromolecules from their toxic effect. Because oxidative stress is generally perceived as one of the major causes for the accumulation of mutations in the genome. Antioxidants are believed to provide protection against various diseases (Ames, 1983).

From the results it is clear that the both extracts of *Operculina turpethum* exhibited antioxidant, anti-inflammatory, anti-ulcer and anti-arthritisic activities and this may be attributed to the presence of phytochemicals such as lupeol and betulin (Mukherjee *et al.*, 1997).