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IN VITRO AND *IN VIVO* ANTI INFLAMMATORY ACTIVITY OF *BUTEA MONOSPERMA* STEM BARK EXTRACT

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Abstract:

In this study the anti inflammatory activity of ethanolic extracts and it's fractions of stem bark of B*utea monosperma* were evaluated *in vitro* on key enzymes of arachidonic acid cascade involved in the mediation of inflammation. The ethanolic extract inhibited the COX-1 and COX-2 enzymes with an IC_{50} of 83µg mL^{-1} and 65µg mL^{-1} respectively and it inhibited the 5-LOX and 15-LOX enzymes with an IC_{50} of 80µg mL^{-1} and 95µg mL^{-1} respectively. Among the fractions of ethanolic extract, the acetone fraction showed more inhibition on COX-1(IC_{50} of 45µg mL^{-1}), COX-2(IC_{50} of 35µg mL^{-1}), 5-LOX (IC_{50} of 38µg mL^{-1}) and 15-LOX (IC_{50} of 48µg mL^{-1}) enzymes than the petroleum ether, benzene and chloroform fractions. Based on the *in vitro* studies data the *in vivo* anti inflammatory activity of ethanolic stem bark extract and the acetone fraction was evaluated by using acute inflammatory models like; carrageenan induced paw oedema and chronic models like; cotton-pellet induced granuloma and carrageenan induced air-pouch model in rats. The biochemical parameters like reduced glutathione (GSH), lipid peroxidation and catalase were also estimated as supportive studies. The ethanolic extract and the acetone fraction significantly reduced inflammation in the carrageenan-induced rat paw oedema, cotton-pellet induced granuloma and carrageenan induced air-pouch model in rats. Acute toxicity studies were performed initially in order to ascertain the safety of ethanolic stem bark extract and the acetone fraction. From the present study the ethanolic extract and the acetone fraction of *Butea monosperma* stem bark exhibited the anti-inflammatory effect by modulating cyclooxygenase enzymes and augmenting antioxidant defense system in the inflammation bearing rat.

Keywords: *Butea monosperma, Cyclooxygenases, Lipoxygenases,* Anti inflammatory activity; Antioxidants

INTRODUCTION

Inflammation is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation (Mitchell and Cotran, 2000). Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow (Ialenti *et al*., 1995). Several experimental models of paw oedema have been described. Carrageenan-induced paw oedema is widely used for determining the acute phase of inflammation. Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation (Di and Willoughby,1971) whereas prostaglandins are detectable

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in the late phase of inflammation (Salvemini *et al*., 1996). However, inflammation that is unchecked leads to chronic inflammatory disorders. Arachidonic Acid (AA) metabolism plays a crucial role in inflammatory process and associated diseases. Some of the anti-inflammatory drugs inhibit the lipoxygenase pathway and some inhibit cyclooxygenase pathway and these two pathways can be used for potential interventions against inflammation. Unfortunately most of the anti-inflammatory drugs, particularly steroids and cyclooxygenase inhibitors are often associated with adverse side effects including, GI irritation, ulcers, hypertension and cardiac abnormalities (William, L.S *et al*., 1989, Wolfe *et al*., 1999). 5-Lipoxygenase (5-LOX) inhibitors of herbal origin on the other hand are reported to offer significant relief and devoid of adverse effects. 5-LOX inhibitors are thus becoming first choice of treatment for chronic inflammatory disease such as arthritis (Krishanu *et al*., 2008, Oliver *et al*., 2007)

Butea monosperma (Fabaceae*)* is a medicinal plant growing in Burma, India and Sri Lanka, The flowers are tonic, astringent, aprodiasic and diuretic. The decoction of the bark is traditionally used in cold, cough, fever, various forms of haemorrhages, in menstrual disorders and in the preparation of tonics and elixirs. The stem bark is reported to possess antitumour, antiulcer, antifungal and antidiarrhoeal activities (Bandara *et al*., 1989; Bandara *et al*., 1990; Gunankkunru *et al*., 2005). The roots are reported in the treatment of filariasis, night blindness, helmenthiasis, piles, ulcers, and tumors (Raj and Kurup, 1968). It is reported that the ethanolic extract of seeds of *Butea monosperma*, on oral administration showed antifertility activity in mice and in rats (Razdan, *et al*., 1969). Palsonin an active principle isolated from *Butea monosperma* seeds and its piperzaine salt exhibited good anthelmintic activity *in vitro* on Ascaris lumbricoides and *in vivo* on Taxicara canis (Raj and Kurup, 1968).The petroleum ether extract and triterpene isolated from flowers of *Butea monosperma* exhibited anti convulsant activity (Kasture *et al*., 2000; Kasture, *et al*., 2002). It has been reported that the methanolic extract of stem bark of *Butea monosperma* showed anti inflammatory and analgesic activity (Carey *et al*., 2007). As there is no reference in literature to the anti-inflammatory studies based on pathway involved in the mechanism of inflammation by the ethanolic stem bark extract and it's fractions, it was considered worthwhile to study the mechanism based anti-inflammatory activity of ethanolic stem bark extract and it's fractions of *Butea monosperma*.

MATERIALS AND METHODS

Collection of samples:

The stem bark of *Butea monosperma* was collected during July 2009 from Manipal, Udipi district, Karnataka state, India. The samples were authenticated by Dr. Gopalakrishna Bhat, Professor of Botany, Poorna Prajna College, Udipi, India. A herbarium specimen has been deposited at the college for further reference.

Extraction and fractionation:

The bark was dried in the shed and coarsely powdered. The powder was extracted with Alcohol in a soxhlet apparatus for 72h. The ethanolic extract was evaporated in vacuo giving the residue (24%). The ethanolic extract obtained was suspended in distilled water in small amounts and was extracted successively and exhaustively with petroleum ether (60-80°C), benzene, chloroform and acetone in the order of increasing polarity. The extract and fractions were concentrated in a rotary evaporator at reduced pressure.

Cyclooxygenase Assay:

Enzymatic activity of COX-1 and COX-2 were measured according to the method of Copeland *et al.*, (1994) with slight modifications using a chromogenic assay based on the oxidation of N,N,N,N,-tetra methyl-p-phenylene diamine (TMPD) during the reduction of PGG_2 to PGH_2. The assay mixture contained Tris-Hcl buffer (100mM, pH 8.0), haematin (15 μM), EDTA (3 μM enzyme (100 μg COX-1 or COX-2) and the test drugs. The mixture was preincubated at 25°C for 15 min. and then the reaction was initiated by the addition of arachidonic acid and TMPD in total volume of 1 mL. The enzyme activity was measured by estimating the initial velocity of TMPD oxidation for the first 25 sec of the reaction by following the increase in absorbance at 603 nm. A low rate of non-enzymatic oxidation observed in the absence of COX-1 and COX-2 was subtracted from the experimental value while calculating the percent inhibition.

Lipoxygenase Assay:

5-LOX and 15-LOX enzyme inhibitory activity of *Butea monosperma* extracts was measured using the method of Reddanna *et al.,* (1990) modified by Ulusu *et al.,* (2002) The assay mixture contained 80 mM linoleic acid and 10 μL of enzyme 5-LOX or 15-LOX in 50 mM phosphate buffer (pH 6.3). The reaction was initiated by the addition of enzyme buffer mix to linoleic acid and the enzyme activity was monitored as the increase in absorbance at 234 nm. The reaction was monitored for 120 sec and the inhibitory potential of the test substances was measured by incubating various concentrations of test substances for two min before addition of linoleic acid. All assays were performed in triplicate. Percentage inhibition was calculated by comparing slope of test substances with that of enzyme activity.

Experimental animals:

Adult Wistar strain rats (150 to 200 gm) were used for all the experiments in the present study. The animals were maintained under standard husbandry

conditions in the animal house of the institute (temperature 25 ± 2°C) in a natural light-dark cycle and fed with standard rodent diet and water *ad libitum.* Ethical committee clearance was obtained from IAE (Institutional Animal Ethics Committee) of CPCSEA (Ref. No./IAEC/XII/08/CLBMCP/2009-2010).

Acute toxicity studies:

The acute toxicity of ethanolic extract and the acetone fraction of *Butea monosperma* stem bark extract was determined as per the OECD guideline no. 423 (Acute toxic class method). It was observed that the ethanolic extract and the acetone fraction were not mortal even at 2000 mg kg^{-1} dose. Hence, $1/10^{th}$ (200mg kg^{-1}) and $1/5^{th}$ (400 mg kg^{-1}) of this dose were selected for further study (OECD, 2002).

Air pouch model of inflammation:

Adult Wistar strain rats (150 to 200 gm) were used for all the experiments in the present study. Air-pouch was produced according to the method described by Salvemini *et al*. (1996). Briefly, rats were anesthetized and air cavities were produced by subcutaneous injection of 20 mL of sterile air into the intrascapular area of the back (that is, 0 day). An additional 10 mL of air was injected into the cavity every 3rd day (3rd and 6th day) to keep the space open. On the 7th day, 2 mL of 1% solution of carrageenan dissolved in saline was injected directly into the pouch to induce an inflammatory response. The rats were orally pre-treated with either vehicle or test substances or diclofenac sodium 2 h prior to the injection of carrageenan. The second dose of treatment was repeated after 24 h of the first treatment. 48 h after carrageenan injection, the rats were anesthetized with ether and the pouch was carefully opened by a small incision. The volume of exudates was collected and measured. An aliquot of the exudate was used for quantification of leukocyte concentration using a haemocytometer and differential cell count was performed using a manual cell counter after staining with Wright's stain. The results were expressed as the total number of neutrophils and monocytes.

Carrageenan induced rat hind paw oedema:

The method of Winter.*et.al*. (1962) was used with slight modification. The apparatus used for the measurement of rat paw volume was that of Buttle.*et.al*., modified by Sharma *et.al*. The animals were divided into six groups of six animals each. One group served as a standard (Diclofenac sodium) and another group served as control (1% CMC) and rest of the groups were used for the test substances. The animals pretreated with test substances or diclofenac sodium one hour before were injected with 0.05 mL of 1% carrageenan (in 1% CMC) solution into the sub-plantar region of right hind paw. The volume of the injected paw was measured with a plethysmograph immediately. The paw volume was again measured after 3 hours. Reduction in the paw volume compared to the vehicle-treated control animals was considered as anti-inflammatory response and the percentage inhibition of oedema was calculated using the formula (1).

$$\text{Inhibition (\%)} = (1 - Vt / Vc) \times 100 \quad (1)$$

Where Vt is Mean volume of the test drug and Vc is Mean volume of the control

Biochemical estimations:

Biochemical changes in carrageenan induced paw oedema were estimated. The rats were anaesthetized under light ether anaesthesia and Liver was removed and subjected for homogenization and aliquots of the homogenate were suitably processed for the assessment of reduced glutathione (GSH), Catalase and lipidperoxidation. GSH was estimated by the method of Moran *et al*., (1979), Catalase activity was assayed according to the method of Cohen *et al.,* (1970) and lipid per oxidation by the method of Ohkawa *et al*., (1979). The % inhibition of lipid per oxidation by the test or standard drug was calculated by using following formula (2).

$$[(A-B)/B] \times 100 \quad (2)$$

Where A is Control group and B is Test or Standard group.

Cotton pellet-induced granuloma:

The test was performed on the rats using the cotton pellet induced granuloma method. The rats were anesthetized under light ether and an incision was made on the lumbar region by blunted forceps, a subcutaneous tunnel was made and a sterilized cotton pellet (100 ± 1 mg) was inserted in the groin area. All the animals received either test substances or Diclofenac sodium or vehicle (1% CMC) orally depending upon their respective grouping for seven consecutive days from the day of cotton pellet insertion (Winter *et al*., 1962). On the 8th day, animals were anesthetized again and cotton pellets were removed and dried to constant mass.

Statistical Analysis:

For *in vitro* assays linear regression analysis was used to calculate the IC_{50} values. In case of *in vivo* studies the experimental results were expressed as mean ± SEM. Results were analyzed by the one- way ANOVA followed by Tukey-kramer post hoc multiple comparison test using Graph pad InStat version 3.00. P value of <0.05 was considered as statistically significant.

RESULTS

Cyclooxygenase Assay:

The ethanolic extract of stem bark of *Butea monosperma* inhibited the COX-1 and COX-2 enzymes with an IC_{50} of 83µg mL^{-1} and 65µg mL^{-1} respectively. The acetone fraction showed more inhibition compared to petroleum ether, benzene and chloroform fractions

with IC_{50} of 45µg mL^{-1} for COX-1 and 35µg mL^{-1} for COX-2 enzymes. Where as the standard drug Celecoxib inhibited the COX-2 enzyme with an IC_{50} of 52nM and indomethacin inhibited the COX-1 enzyme with an IC_{50} of 28nM. The results are shown in Table 1.

Lipoxygenase Assay:

The ethanolic extract of stem bark of *Butea monosperma* inhibited the 5-LOX and 15-LOX enzymes with an IC_{50} of 80µg mL^{-1} and 95µg mL^{-1} respectively. The acetone fraction showed more inhibition compared to petroleum ether, benzene and chloroform fractions with IC_{50} of 38µg mL^{-1} for 5-LOX and 48µg mL^{-1} for 15-LOX enzymes. The ethanolic extract and the acetone fraction exhibited moderate 5-LOX and 15-LOX inhibitory activity, when compared with known standard Nordihydroguaretic acid (NDGA). The results are shown in Table 2.

Air pouch model of inflammation:

The effect of ethanolic stem bark extract of *Butea monosperma* and the acetone fraction in carrageenan induced air pouch model of inflammation in rats is shown in Table 3.The ethanolic stem bark extract of *Butea monosperma* and the acetone fraction significantly reduced the carrageenan induced inflammation in the air pouch. The ethanolic extract and the acetone fraction dose-dependently elicited significant ($P < 0.05$) reduction in excudate volume and infiltration of neutrophils and monocytes into the air-pouch compared to control group. Diclofenac sodium at a dose of 100 mg kg^{-1} b.w. also showed significant ($P < 0.05$) result.

Cotton pellet-induced granuloma:

The ethanolic stem bark extract of *Butea monosperma* and the acetone fraction were screened for cotton pellet induced granuloma in rats and the results are shown in Table 4. The ethanolic extract exhibited 13.63% and 26.25% inhibition of granuloma formation at the doses 200 and 400 mg kg^{-1} b.w respectively and the acetone fraction exhibited 32.47% and 42.68% inhibition of granuloma formation at the doses 200 and 400 mg kg^{-1} b.w respectively, whereas diclofenac sodium showed 55.70% when compared to control group.

Carrageenan induced rat hind paw oedema:

The effect of ethanolic stem bark extract of *Butea monosperma* and the acetone fraction in carrageenan induced paw oedema in rats is shown in Table 5. The result obtained indicates that the ethanolic extract and the acetone fraction found to have significant ($P < 0.05$) anti-inflammatory activity in rats. The ethanolic extract at the test doses 200 and 400 mg kg^{-1} b.w. reduced the oedema induced by carrageenan by 41.60% and 59.73% respectively at 3 h and the acetone fraction reduced the oedema by 73.22% and 83.37% respectively, whereas the Diclofenac sodium at a dose 100 mg kg^{-1} b.w. showed 90.54% of inhibition as compared to the control group.

Biochemical estimations:

The results of biochemical changes in carrageenan induced rat paw oedema are shown in Table 6. Treatment with ethanolic stem bark extract of *Butea monosperma* and the acetone fraction decreased the levels of lipid peroxidation and increased the levels of GSH and catalase. The results were found to be significant ($P < 0.05$) as compared to control groups.

Table 1. IC_{50} Values of ethanolic extract and different fractions of *Butea monosperma* stem bark on Cyclooxygenases *in vitro*

Drug/Extract	COX-1	COX-2
Celecoxib	-	52 nM
Indomethacin	28nM	-
Ethanolic extract	83 µg mL^{-1}	65 µg mL^{-1}
Petroleum ether fraction	65 µg mL^{-1}	60 µg mL^{-1}
Benzene fraction	85 µg mL^{-1}	90 µg mL^{-1}
Chloroform fraction	76 µg mL^{-1}	60 µg mL^{-1}
Acetone fraction	45 µg mL^{-1}	35 µg mL^{-1}

Table 2. IC_{50} Values of ethanolic extract and different fractions of *Butea monosperma* stem bark on lipoxygenases *in vitro*

Drug/Extract	5-LOX	15-LOX
NDGA	1.5µM	200nM
Ethanolic extract	80 µg mL^{-1}	95 µg mL^{-1}
Petroleum ether fraction	55 µg mL^{-1}	78 µg mL^{-1}
Benzene fraction	105 µg mL^{-1}	110 µg mL^{-1}
Chloroform fraction	70 µg mL^{-1}	80 µg mL^{-1}
Acetone fraction	38 µg mL^{-1}	48 µg mL^{-1}

Table 3. Effect of ethanolic extract and the acetone fraction of *Butea monosperma* stem bark on leucocyte infiltration and exudate volume in carrageenan induced air pouch inflammation

Groups	Dose(mg kg^{-1})	Excudate volume (mL)	Neutrophils(x 10^6 cells)	Monocytes (x 10^6 cells)
Control	1 % CMC	4.475± 0.04951	352.333±2.231	143.1666±1.195
Standard	100	1.3083 ±0.01621**	96.5±0.922**	44.5±1.335**
ETEX 200	200	4.2916±0.04167*	340.833±2.386*	136.833±0.7032*
ETEX 400	400	4.1333±0.02108**	184±1.949**	124.1666±1.138**
ACEF200	200	3.5± 0.01826**	141 ±1.862**	67±0.7746**
ACEF400	400	2.25 ±0.02236**	130.1666±1.537**	55±0.8944**

Standard: Diclofenac sodium (100mg kg^{-1} b.w.), ETEX200: Ethanolic extract at dose 200 mg kg^{-1} b.w. ETEX 400: Ethanolic extract 400 mg kg^{-1} b.w., ACEF200: Acetone fraction 200 mg kg^{-1} b.w, ACEF400: Acetone fraction 400 mg kg^{-1} b.w. Each value is the Mean ± S.E.M for 6 rats. *P < 0.01; **P < 0.001 compared with control.

Table 4. Effect of ethanolic extract and the acetone fraction of *Butea monosperma* stem bark on cotton-pellet induced granuloma in rats

Groups	Dose (mg kg^{-1})	Granuloma dry weight (mg)	%Inhibition
Control	1 % CMC	68.2466±0.1613	-
Standard	100	30.2316±0.09931**	55.70
ETEX200	200	58.945±0.1105**	13.63
ETEX400	400	50.335±0.1848**	26.25
ACEF200	200	46.0883±0.09796**	32.47
ACEF400	400	39.1133±0.1891**	42.68

Standard: Diclofenac sodium (100mg kg^{-1} b.w.), ETEX200: Ethanolic extract at dose 200 mg kg^{-1} b.w. ETEX 400: Ethanolic extract 400 mg kg^{-1} b.w., ACEF200: Acetone fraction 200 mg kg^{-1} b.w, ACEF400: Acetone fraction 400 mg kg^{-1} b.w. Each value is the Mean ± S.E.M for 6 rats. **P<0.001 compared with control

Table 5. Effect of ethanolic extract and the acetone fraction of *Butea monosperma* stem bark on carragenan induced paw oedema in rats.

Groups	Dose (mg kg^{-1})	Mean oedema Volume 0-3h	%Inhibition
Control	1 % CMC	0.945±0.0099	-
Standard	100	0.0893± 0.0011**	90.54
ETEX200	200	0.5516±0.0094**	41.60
ETEX 400	400	0.38±0.0057**	59.73
ACEF200	200	0.2533±0.01229**	73.22
ACEF400	400	0.1566±0.0092**	83.37

Standard: Diclofenac sodium (100mg kg^{-1} b.w.), ETEX200: Ethanolic extract at dose 200 mg kg^{-1} b.w. ETEX 400: Ethanolic extract 400 mg kg^{-1} b.w., ACEF200: Acetone fraction 200 mg kg^{-1} b.w, ACEF400: Acetone fraction 400 mg kg^{-1} b.w. Each value is the Mean ± S.E.M for 6 rats. **P<0.001 compared with control.

Table 6: Effect of ethanolic extract and the acetone fraction of *Butea monosperma* stem bark on various biochemical changes in carrageenan induced rat paw oedema

Groups	Dose (mg kg^{-1})	GSH (ng mg^{-1} protein)	lipid peroxidation (%)	Catalase (µg mg^{-1} protein)
Control	1 % CMC	3.225±0.05123	99.333±1.08	24.5±0.4282
Standard	100	4.7083±0.08002**	64.333±1.211**	39±0.3651**
ETEX 200	200	3.5166±0.02108*	93.833±2.137	26.333±0.4216*
ETEX400	400	4.1666±0.03801**	85.333±3.386**	28.5±0.2236**
ACEF200	200	3.9166±0.04773**	76±2.191**	35.9166±0.2713**
ACEF400	400	4.55±0.06191**	69.5±1.1049**	37.666±0.1667**

Standard: Diclofenac sodium (100mg kg^{-1} b.w.), ETEX200: Ethanolic extract at dose 200 mg kg^{-1} b.w. ETEX 400: Ethanolic extract 400 mg kg^{-1} b.w., ACEF200: Acetone fraction 200 mg kg^{-1} b.w, ACEF400: Acetone fraction 400 mg kg^{-1} b.w. Each value is the Mean ± S.E.M for 6 rats. *P < 0.01; **P < 0.001 compared with control.

DISCUSSION

The results of the present investigations revealed that the ethanolic stem bark extract of *Butea monosperma* and the acetone fraction possess significant anti-inflammatory activity against acute inflammatory models like; carrageenan induced paw oedema and chronic models like; cotton-pellet induced granuloma and carrageenan induced air-pouch model in rats in a dose dependent manner. In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have some or other side effects, whereas plants still hold their own unique place, by the way of having no side effects. Therefore, a systematic approach should be made to find out the efficacy of plants against inflammation so as to exploit them as herbal anti-inflammatory agents. The enzyme, phospholipase A2, is known to be responsible for the formation of mediators of inflammation such as prostaglandins and leukotrienes which by attracting polymorphonuclear leucocytes to the site of inflammation would lead to tissue damage probably by the release of free radicals. Phospholipase A2 converts phospholipids in the cell membrane into arachidonic acid, which is highly reactive and is rapidly metabolized by cyclooxygenase (prostaglandin synthesis) to prostaglandins, which are major components that induce pain and inflammation (Higgs *et al*., 1984; Vane, 1971).

The biosynthesis of PGs is initialized by COX isoenzymes, namely, COX-1, a constitutively expressed enzyme in numerous cell types thought to provide PGs mainly for physiological functions; and COX-2, an inducible isoform in inflammatory cells, primarily producing PGs relevant for inflammation, fever, and pain (Hawkey, 1999). After conversion of arachidonic acid to PGH2 by COX enzymes, PGH2 is subsequently isomerized by three different PGE2 synthases to PGE2. PGE2 plays a major role in the pathophysiology of inflammation, pain, and pyresis, but it also regulates physiological functions in the gastrointestinal tract, the kidney, and in the immune and nervous system (Smith, 1989). The nonsteroidal anti-inflammatory drugs (NSAIDs) reduce PGE2 biosynthesis by inhibiting both COX isoenzymes, and they are potent suppressors of inflammation, fever, and pain (Funk, 2001).

Chronic use of these drugs is associated with severe side effects, mainly gastrointestinal injury and renal irritations, apparently due to suppression of COX-1-derived PGE2(Rainsford, 2007). COX-2-selective inhibitors were designed to minimize gastrointestinal complications of traditional NSAIDs, but recent clinical studies indicated small but significantly increased risks for cardiovascular events (McGettigan and Henry, 2006).

Licofelone is an anti-inflammatory drug that inhibits the COX and 5-lipoxygenase pathway and is currently undergoing phase III trials for osteoarthritis (for review, see Celotti and Laufer, 2001; Kulkarni and Singh, 2007). This effect of licofelone might be attributable to the accompanied suppression of leukotrienes (Celotti and Laufer, 2001), which significantly contribute to gastric epithelial injury as well as to atherogenesis (Peters-Golden and Henderson, 2007). Suppression of LT and PG synthesis by interfering with the 5-LOX and COX pathways represent an efficient pharmacological approach for the treatment of inflammatory diseases (Funk, 2001). The ethanolic extract and the acetone fraction of stem bark of *Butea monosperma* showed inhibitory effect preferably on COX-2 and 5-LOX enzymes. Based on the results obtained the anti-inflammatory activity of the ethanolic extract and the acetone fraction is due to inhibition of prostaglandin synthesis and leukotriens by interfering with LOX and COX pathways.

It is well known that carrageenan induced paw edema is characterized by biphasic event with involvement of different inflammatory mediators. In the first phase (during the first 2 h after carrageenan injection), chemical mediators such as histamine and serotonin play role, while in second phase (3 – 4 h after carrageenan injection). Kinin and prostaglandins are involved (Hernandez *et al*., 2002). Our results revealed that administration of ethanolic extract and the acetone fraction of *Butea monosperma* stem bark inhibited the oedema starting from the first hour and during all phases of inflammation, which is probably inhibition of different aspects and chemical mediators of inflammation.

The cotton-pellet granuloma is widely used to evaluate the transudative and proliferative components of the chronic inflammation. The moist weight of the pellets correlates with transuda, the dry weight of the pellet correlates with the amount of granulumatous tissues (Castro *et al*. 1968). Chronic inflammation occurs by means of the development of proliferate cells. These cells can be either spread or in granuloma form.

Non-steroidal anti-inflammatory drugs decrease the size of granuloma which results from cellular reaction by inhibiting granulocyte infiltration, preventing generation of collagen fibers and suppressing mucopolysaccharides (Della *et al*., 1968; Alcaraz and Jimenez, 1988). The ethanolic stem bark extract and the acetone fraction of *Butea monosperma* showed significant anti-inflammatory activity in cottonpellet induced granuloma and thus found to be effective in chronic inflammatory conditions, which reflected its efficacy in

inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation.

In order to assess the efficacy of the extract against proliferative phase of inflammation, we selected carrageenan- induced air-pouch model in which tissue degradation and fibrosis occurs. During the repair process of inflammation, there is proliferation of macrophages, neutrophils, fibroblasts and multiplication of small blood vessels occurs, which are the basic sources of forming a highly vascularised reddish mass, termed granulation tissue (Bhattacharya *et al*., 1992; Swingle, 1974). Thus, in this model the extract significantly reduced infiltration of macrophages, monocytes, neutrophils and others. These results indicate that the ethanolic extract and the acetone fraction of *Butea monosperma* stem bark may alter the action of endogenous factors that are involved in the migration of these substances to the site of inflammation.

From the above studies it is quite apparent that the ethanolic extract and the acetone fraction of *Butea monosperma* stem bark possesses significant anti-inflammatory activity by modulating cyclooxygenase, lipoxygenase enzymes and augmenting antioxidant defense system in the inflammation bearing rat.

CONCLUSION

The ethanolic extract and the acetone fraction of stem bark of *Butea monosperma* showed anti-inflammatory property similar to those observed for non-steroidal anti-inflammatory drugs. It is also suggested that the mechanism of action of *Butea monosperma* stem bark is by the inhibition of eicosanoid biosynthesis and augmentation of antioxidant defense system in the inflammation. It may represent a suitable drug for the therapy of chronic inflammatory diseases with low risks of adverse effects. However, further studies are needed to isolate and characterize the anti-inflammatory chemical constituents present in the acetone fraction of the ethanolic stem bark extract of the plant.

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