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ANTI INFLAMMATORY ACTIVITY OF FLAVONOID FRACTION ISOLATED FROM THE STEM BARK OF *BUTEA MONOSPERMA (LAM)*: A MECHANISM BASED STUDY

***A.Muralidhar, [1]K.Sudhakar Babu, [1]T.Ravi sankar, [2]P.Reddanna, [2]G.V.Reddy, [3]J.Latha**

[*, 1]Department of Chemistry, Sri Krishnadevaraya University, Anantapur, Andhra Pradesh, India.
[2]School of Life Sciences, University of Hyderabad, Hyderabad. Andhra Pradesh, India. [3]Department of Bio-technology, Sri Krishnadevaraya University College of Engineering &Technology, Sri Krishnadevaraya University, Anantapur - 515 055, Andhra Pradesh, India.

Abstract:

In this study the anti inflammatory activity of the flavonoid fraction isolated from the stem bark of B*utea monosperma* was evaluated *in vitro* on key enzymes of arachidonic acid cascade involved in the mediation of inflammation. The flavonoid fraction inhibited the COX-2 and 5-LOX enzymes with an IC_{50} of 5µg mL^{-1} and 12µg mL^{-1} respectively. Based on the *in vitro* studies data the *in vivo* anti inflammatory activity of flavonoid fraction was evaluated by using acute inflammatory models like; carrageenan induced paw oedema and chronic model like; cotton-pellet induced granuloma. The flavonoid fraction significantly reduced the inflammation in the carrageenan-induced rat paw oedema and cotton-pellet induced granuloma in rats. The flavonoid fraction did not inhibit the gastric acid secretion suggesting that its anti ulcerogenic effect can be attributed to its action on the mucosa defense factors. The phytochemical investigations revealed that the flavonoid fraction contains two isoflavones genistein and prunetine. Hence the anti inflammatory activity of the flavonoid fraction may be due to these isoflovones.The safety and efficacy profiles indicated that the flavonoid fraction of *Butea monosperma* stem bark is a safe intervention for inflammatory disorders with gastric cytoprotective properties.

Keywords: *Butea monosperma, Flavonoid fraction, Cyclooxygenases, Lipoxygenases,* Anti inflammatory activity; Gastric acid, Antioxidants

INTRODUCTION

Natural products with high medicinal values are gaining much importance in light of serious side effects posed by the medicinal derivatives from chemical origin. Flavonoids are a group of polyphenolic compounds, which are distributed throughout the plant kingdom. To date about 3000 varieties of flavonoids are known (Kuhnau, 1976). Flavonoids exhibit several biological effects such as anti-inflammatory, antihepatotoxic and anti-ulcer actions (Bors *et al.,* 1990; Colerige Smith *et al.,* 1980). Inflammation is typically a protective mechanism that is triggered in response to noxious stimuli, trauma or infection to guard the body and to hasten-up the recovery process. 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

***Corresponding author**

A.Muralidhar

E mail id:- avulamuralidhar@rediffmail.com

adverse side effects including GI irritation, ulcers, hypertension and cardiac abnormalities (William, 1989; Wolfe, 1999). The use of herbal remedies for arthritis treatment has been gaining momentum in recent years (Chrubasik *et al.,* 2007). There has been some concern over the use of COX-2 inhibitors for therapeutic intervention, especially since some of the products based on COX-2 were either withdrawn or made to carry warning by the US FDA (Naesdal *et al.,* 2006; Salmon, 2006). 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, 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). It is also reported that the powder of the stem bark is used to apply on injury caused due to an axe, the juice of the stem is applied on goiter of human beings and the paste of the stem bark is applied in case of body swellings (Patil *et al.,* 2006). 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). Recently we have reported the *in vitro* and *in vivo* anti inflammatory activity of *Butea monosperma* stem bark extract (Muralidhar *et al.*, 2010). In continuation of the studies we have investigated the anti-inflammatory activity of flavonoid fraction isolated from *Butea monosperma* stem bark and phytochemical investigations were carried out to isolate the active principles from the *Butea monosperma* stem bark.

MATERIALS AND METHODS

Plant Material:

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.

Preparation of Plant Extracts:

The bark was dried in the shed and coarsely powdered. The powder was extracted with ethanol 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.

Preliminary Phytochemical Analysis:

In our previous studies the acetone fraction was found to be having the biological activity (Muralidhar *et al.,* 2010). Hence the bioactive acetone fraction was subjected to Phytochemical screening according to the phytochemical methods described by Harborne (1998).

Chromatographic separation and Isolation of Constituents:

The acetone fraction was chromatographed by column chromatography over silica gel and eluted with n-hexane/ethyl acetate (80:20 v/v) with increasing amounts of ethyl acetate. 10 mL of the eluates were collected and monitored with TLC and the similar fractions were combined together. Total 4 fractions were collected and the fraction 3 was identified as flavonoid fraction. The bioactive flavonoid fraction was further separated by HPLC (ODS, 250X10 mm, 5µ) using ethyl acetate/methanol/formic acid (70:30:3 v/v/v) as mobile phase to afford compounds **1** and **2**. The structure elucidation of compound **1** and **2** were done by comparing with reference data previously reported from available literature (Harborne, 1994; Agrawal, 1989; Muraleedharan, 1999; Talukdar *et al*., 2000; Alexandra *et al.,* 2004), and by co-TLC with the authentic samples.

Cyclooxygenase Assay:

Enzymatic activity of COX-2 was 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-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-2 was subtracted from the experimental value while calculating the percent inhibition.

Lipoxygenase Assay:

5-LOX enzyme inhibitory activity of flavonoid fraction of *Butea monosperma* stem bark 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 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 flavonoid fraction of *Butea monosperma* stem bark was determined as per the OECD guideline no. 423 (Acute toxic class method). Based on the results obtained from this study, the dose for anti inflammatory activity was fixed to be 25 mg kg^{-1} b.w. and 50 mg kg^{-1} b.w. for dose dependent study. (OECD, 2002).

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 four 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 h. 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).

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

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 peroxidation by the method of Ohkawa *et al*., (1979). The % inhibition of lipid peroxidation by the test or standard drug was calculated by using following formula (2).

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

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.

Effect of Flavonoid fraction on Gastric acid secretion:

Albino rats weighing 150-160 g were placed in individual cages with bottoms to prevent caprophagy. The animals were kept under standard conditions at 22 ± 1°C with water *ad libitum* and deprived of food for 24 h before the experiments. The technique of ligated pylorus was used (Shay *et al.,* 1945). After anesthetizing with ether an incision was made in the abdomen and the ligature was performed 0.5 to 0.7 mm below the pylorus. Care was taken not to damage the blood supply. The animals were divided in to 3 groups of 6 animals each. After closing the incisions group 1 (Control) was orally administered with 1 mL of 0.4% CMC in saline (vehicle), Group 2 and 3 were orally administered with 25 and 50 mg kg^{-1} b.w. of flavonoid fraction respectively. All animals were placed in their cages and deprived of water and food for the rest of the experiment. Four hours after the pyloric ligation, the animals were sacrificed by decapitation. A ligature was placed at the oesophago-cardiac junction and the stomach was removed. The gastric content was collected and centrifuged. Supernatant volumes were measured and the pH of the supernatants was measured using a pH meter. The acid concentration was estimated by titration to pH 7.0 with 0.1N NaOH using an auto titrator.

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 flavonoid fraction of *Butea monosperma* stem bark inhibited the COX-2 enzyme with an IC_{50} of 5µg mL^{-1}. Where as the standard drug Celecoxib inhibited the COX-2 enzyme with an IC_{50} of 52nM. The results are shown in Table 1.

Lipoxygenase Assay:

The flavonoid fraction of stem bark of *Butea monosperma* inhibited the 5-LOX enzyme with an IC_{50} of 12µg mL^{-1}. The flavonoid fraction exhibited moderate 5-LOX inhibitory activity, when compared with known standard Nordihydroguaretic acid (NDGA). The results are shown in Table 1.

Carrageenan induced rat hind paw oedema:

The effect of flavonoid fraction of stem bark of *Butea monosperma* in carrageenan induced paw oedema in rats is shown in Table 2. The result obtained indicates that the flavonoid fraction found to have significant ($P < 0.05$) anti-inflammatory activity in rats. The flavonoid fraction at the test doses 25 and 50 mg kg^{-1} b.w. reduced the oedema induced by carrageenan by 73.18% and 83.56% respectively at 3 h, whereas the diclofenac sodium at a dose 100 mg kg^{-1} b.w. showed 90.36% 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 3. Treatment with flavonoid fraction of stem bark extract of *Butea monosperma* 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.

Cotton pellet-induced granuloma:

The flavonoid fraction of stem bark of *Butea monosperma* was screened for cotton pellet induced granuloma in rats and the results are shown in Table 4. The flavonoid fraction exhibited 29.1% and 44.5% inhibition of granuloma formation at the doses 25 and 50 mg kg^{-1} b.w respectively, whereas diclofenac sodium showed 49.5% when compared to control group.

Effect of Flavonoid fraction on Gastric acid secretion:

The effect of flavonoid fraction of stem bark of *Butea monosperma* on the gastric acid secretion in the pylorus ligation method is shown in the Table 5. The results obtained showed that the flavonoid fraction did not inhibit the gastric secretion in rats. The volume of gastric content was significantly increased.

Phytochemical analysis:

The preliminary phytochemical analysis of flavonoid fraction showed the presence of flavonoids, phenolic compounds, and steroids. From the flavonoid fraction we isolated two isoflavones where the compound 1 was identified as Genistein (Figure 1: 4^r, 5, 7-trihydroxy isoflavone) and the compound 2 (Figure 2: 4^r, 5-dihydroxy, 7- methoxy isoflavone) was identified as prunetine.

Figure 1: The structure of Genistein

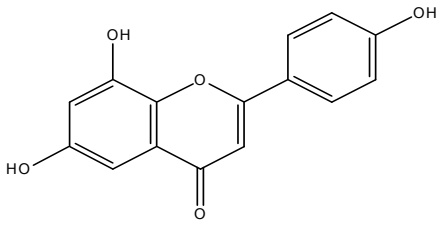

Figure 2: The structure of Prunetine

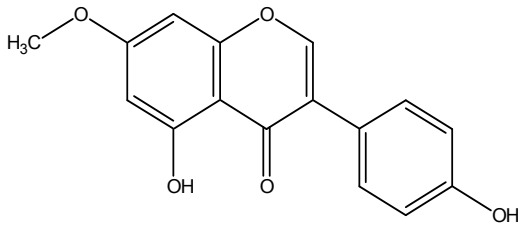

Table 1: IC_{50} Values of flavonoid fraction of *Butea monosperma* stem bark on COX-2 and 5-LOX enzymes *in vitro*

Drug/Extract	COX-2	5-LOX
Celecoxib	52 nM	-
NDGA	-	1.5µM
Flavonoid fraction	5 µg mL^{-1}	12 µg mL^{-1}

Table 2: Effect of flavonoid 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	0.962±0.0065	-
Standard	100	0.093± 0.0008**	90.36
FLAVF25	25	0.258±0.0017**	73.18
FLAVF50	50	0.158±0.0015**	83.56

Standard: Diclofenac sodium (100mg kg^{-1} b.w.), FLAVF25: Flavonoid fraction at dose 25 mg kg^{-1} b.w. FLAVF50: Flavonoid fraction at dose 50 mg kg^{-1} b.w. Each value is the Mean ± S.E.M for 6 rats. **P<0.001 compared with control

Table 3: Effect of flavonoid 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	3.57±0.0117	99.64±0.0896	25.34±0.0955
Standard	100	4.58±0.0335**	65.69±0.0939**	39.44±0.1281**
FLAVF25	25	3.93±0.0129**	75.43±0.128**	30.81±0.1047**
FLAVF50	50	4.54±0.0312**	69.28±0.0894**	37.63±0.1435**

Standard: Diclofenac sodium (100mg kg^{-1} b.w.), FLAVF25: Flavonoid fraction at dose 25 mg kg^{-1} b.w. FLAVF50: Flavonoid fraction at dose 50 mg kg^{-1} b.w. Each value is the Mean ± S.E.M for 6 rats. **P<0.001 compared with control

Table 4: Effect of flavonoid 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	70.333±0.4944	-
Standard	100	35.5±0.5627**	49.5
FLAVF25	25	49.833 ±0.4773**	29.1
FLAVF50	50	39.0 ±0.3651**	44.5

Standard: Diclofenac sodium (100mg kg^{-1} b.w.), FLAVF25: Flavonoid fraction at dose 25 mg kg^{-1} b.w. FLAVF50: Flavonoid fraction at dose 50 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 flavonoid fraction of *Butea monosperma* stem bark on the gastric acid secretion in rats

Groups	Dose (mg kg^{-1})	Volume (mL)	pH	Titrable acid conc. (µEq mL^{-1})	Total acid output (µEq mL^{-1})
Control	-	4.11±0.03	1.95±0.65	55.45±3.56	228.15±35.5
FLAVF25	25	4.62±0.06**	2.15±0.53	48.38±3.25	238.45±32.33
FLAVF50	50	5.56±0.05**	2.45±0.42	46.25±3.15	245.54±31.45

FLAVF25: Flavonoid fraction at dose 25 mg kg^{-1} b.w. FLAVF50: Flavonoid fraction at dose 50 mg kg^{-1} b.w. Each value is the Mean ± S.E.M for 6 rats. **P<0.001 compared with control

DISCUSSION

The results of the present investigations revealed that the flavonoid fraction isolated from the stem bark of *Butea monosperma* possess significant anti-inflammatory activity against acute inflammatory models like; carrageenan induced paw oedema and chronic models like; cotton-pellet induced granuloma 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 flavonoid 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 flavonoid 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 flavonoid 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 flavonoid fraction of *Butea monosperma* showed significant anti-inflammatory activity in cotton pellet 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 the pylorus ligation method it was observed that the flavonoid fraction did not inhibit gastric acid secretion at the test dose levels, so the flavonoid fraction might favours one of the defense factors of the rat gastric mucosa by increasing gastric glycoproteins. This suggests that the anti ulcerogenic effect of the flavonoid fraction against different necrotizing agents may be due to a cytoprotective activity. Histamine (H2) receptor antagonists and proton pump (H^+, K^+) ATPase inhibitors suppress gastric acid secretion and secondarily include hypergastrinemia. Sustained hypergastrinemia has atrophic effect on the fundic mucosa, resulting in enterochromaffin like ECL cell hypertrophy and hyperplasia (Hakanson *et al.,* 1992). Therefore it is of interest that the flavonoid fraction exerts an effective anti ulcerogenic action without modifying gastric acid secretion.

From the above studies it is quite apparent that the flavonoid 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 flavonoid fraction of stem bark of *Butea monosperma* showed anti-inflammatory property similar to those observed for non-steroidal anti-inflammatory drugs. The anti-inflammatory activity of flavonoid fraction is largely attributable to the presence of some bioactive compounds such as the isoflavones genistein and prunetine. Such anti-inflammatory properties may be ascribed to 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 gastric cytoprotective properties.

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