Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(7):249-255

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Development and Evaluation of Polyherbal Tablet Formulation with Potent Anti-Inflammatory and COX-2 Inhibitory Activity

Nasreen Begum[1*], Srisailam K.[1] and Uma Maheshwararao V.[2]

[1]Department of Pharmacognosy, University College of Pharmaceutical Sciences, Satavahana University, Karimnagar, Telangana, 505001, India
[2]Department of Pharmacognosy, CMR College of Pharmacy, Kandlakoya (V), Medchal Road, Ranga Reddy, Telangana, 501401, India

---

ABSTRACT

The main objective of the present study was to develop a polyherbal solid dosage formulation and to evaluate anti-inflammatory activity with specific COX-2 inhibitory activity. Based on the available traditional and scientific literature, plants that possess anti-inflammatory activity were selected. The extracts were prepared by successive soxhlation using solvents like petroleum ether, chloroform, ethyl acetate, methanol and water. In vitro evaluation of the extracts was done by HRBC membrane stabilisation method using diclofenac sodium as standard to study the anti-inflammatory activity. In vitroCOX-2 inhibitory activity of the same extracts was evaluated by enzyme immunoassay method. Herbal tablets were prepared by direct compression method by using the fractions with potential COX-2 inhibitory activity obtained by fractionation of the active extracts by column chromatography. Five tablet formulations with varying concentration of the active fractions of various herbs were prepared and standardised. All the formulations were further studied for specific COX-2 inhibitory activity among which, Polyherbal formulation IV proved to show pronounced invitro COX-2 inhibitory activity.

Keywords: Polyherbal tablet, COX-2 inhibitory activity, HRBC membrane stabilisation, enzyme immunoassay

---

INTRODUCTION

Since the evolution of mankind plants have played an important role. They have been used as important sources in treatment of various ailments. Plants serve as an important source of chemicals for development of novel drugs. Throughout the world, many traditional systems of medicines have been formed based on the medicinal properties of plants thus, providing natural remedies for treatment of various diseases. A path has been laid for novel drug discovery depending on the Plant based medicines, which are being dispensed in the form of crude drug. Plants serve as an important source in providing basic chemical moieties in development of modern drugs and till date many active compounds isolated from plants are being used in modern medicine. From vast diversity of plant kingdom many active compounds of pharmacological importance can be isolated. Many herbs possess anti-inflammatory activity. For the present study plants like, *Zingiber officinale*(rhizomes), *Curcuma longa*(rhizomes), *Rosmarinus officinalis*(leaves), *Matricaria recutita*(flowers), *Berberi saristata*(roots), *Origanum vulgare*(leaves), G*aultheria procumbens*(leaves), *Ocimum sanctum*(leaves) were selected.Ginger is widely used as a traditional Chinese medicine, with beneficial effects reported in numerous diseases including inflammation. It is been used asan effective anti-inflammatory herb for arthritis and rheumatism which acts by inhibiting COX-2 and lipoxygenase pathways [1]. Turmeric is one of the mostly used Indian traditional medicines where, the active constituent like curcumin, has found to show a potent anti-inflammatory effect by inhibiting prostaglandin synthesis [2]. Rosemary is known for its anti-inflammatory and antiseptic effect. The leaves acts as natural antioxidant and also commonly used as a spice and flavouring agent[3, 4]. Chamomile has a long history as a traditional medicine with anti-inflammatory and analgesic activity [5, 6]. Barberry has been traditionally used for chronic inflammation as well as

rheumatic complaints [7]. For thousands of years oregano has been used for it's effective culinary, cosmetic and folklore medicine. It has been reported that oregano has a property to neutralise the COX-2 enzyme which is associated with tissue inflammation [8]. Gaultheria commonly called as wintergreen contains active constituent methyl salicylate which inhibits the release of COX enzyme affecting the release of prostaglandins, PGF_{2a} and PGE_2 and thromboxanes [9]. Tulsi has a long history of medicinal value. It is a natural modulator of COX-2 enzyme. Like many modern pain killers, it may act by inhibiting COX-2 enzyme. This may be due to it's high eugenol concentration [10]. It acts by blocking both cyclooxygenase and lipooxygenase pathway or metabolism of arachidonic acid [11]. Today's modern anti-inflammatory drugs are either steroidal or non-steroidal and have been reported to show undesirable side effects ranging from gastrointestinal irritation to cardiovascular effects [12]. In view of this,plant derived compounds or other naturally derived sources are especially important to be developed into anti-inflammatory drugs. Moreover, considering the resulting side effects of COX-1 inhibitors, herbs selectively inhibiting COX-2 enzyme need to be studied. Hence, in the present study an attempt was made to formulate a polyherbal tablet with specific COX-2 inhibitory activity in order to prevent or reduce the side effects caused by modern COX-2 inhibitors.

EXPERIMENTAL SECTION

Plants

For the present investigation, Ginger , Turmeric rhizomes, Chamomile Flowers were obtained from local market, Holy basil leaves were collected from the local surrounding areas, Rosemary leaves, Barberry Root were obtained from Yucca Enterprises, Mumbai, Oregano leaves, Gaultheria leaves, were obtained from Munnalaldawasaz, Hyderabad.

Chemical and reagents

Diclofenac sodium was obtained from Mangalam Drugs and Pharmaceuticals Ltd, Wapi, Gujarat. Starch, talc, magnesium stearate and lactose were procured from Colorcon Asia Pvt. Ltd – Mumbai, India,All the solvents were procured from E. Merck, Mumbai. The colorimetric human COX-2 inhibitor screening kit (Item No. 560131) used for *in vitro*COX-2 inhibitory activity was manufactured by Cayman Chemical, USA. The contents of the kit includes, PG screening EIA antiserum, PG screening AChE tracer, PG screening EIA standard, EIA buffer concentrate,wash buffer concentrate, polysorbate 20, mouse antirabbitIgG coated plate, 96 – well cover sheet, Ellman's reagent, reaction buffer, COX – 1 (ovine), COX – 2 (human recombinant), heme, arachidonic acid (substrate), potassium hydroxide, hydrochloric acid, stannous chloride.

Preparation of extracts

Required plant material was carefully separated and checked for foreign matter and shade dried. The drug was powdered by using a laboratory grinder and sieved after complete drying.50 g of powdered drug of each plant was subjected to successive soxhlation by using solvents, petroleum ether (60 - 80 ^{o} C), chloroform, ethyl acetate, methanol and water for 8 hours. The solvent extracts obtained were further concentrated in vacuo by using rotary vacuum evaporator and then, dried in a desiccator.

Evaluation of In Vitro Anti-inflammatory activity

In vitro anti-inflammatory activity of the extracts was performed stabilisation of human red blood cells[13].HRBCs (human red blood cells)were prepared by centrifugation of 5ml of blood from healthy donors from which supernatant was separated and the packed cells resuspended in an equal volume of isosaline and centrifuged. Centrifugation was repeated until a clear supernatant was observed and a 10% HRBC suspension was then prepared with normal saline and stored at 4 ^{0} C until use. The reaction mixture (4.5 ml) was prepared by mixing 2ml hyposaline (0.25% w/v NaCl), 1 ml of isosaline buffer solution, pH 7.4 (6.0 g TRIS, 5.8g NaCl, HCl to regulate the pH and water to make 1000 ml) and varying volumes of the extract solution in isotonic buffer (concentration, 10mg/ ml) to make the volume to 4.0 ml. Then 0.5 ml of 10% HRBC in normal saline was added. Two controls were performed. One with 1.0 ml of isosaline buffer instead of extract (control 1) and another with 1 ml of extract solution and without red blood cells (control 2). The contents were incubated at 56^{0}C for 30 min. The tubes were cooled under running water for 20 min. The mixture was centrifuged, and the absorbance of the supernatant was read at 560 nm. Diclofenac sodium was usedas standard. The percentage of membrane stabilization was determined using the formula

$$100 - \frac{\text{(Extract absorbance value – control 1 absorbance value)}}{\text{Control 2 absorbance value}} \times 100$$

The control 1 represents 100% HRBC lysis.

Evaluation of in vitro COX-2 inhibitory activity

*In vitro*COX-2 inhibition was evaluated by enzyme immunoassay [14]. For this, enzyme immunoassay (EIA) kit (Catalogue No.560131, Cayman Chemical, Ann Arbor, MI, USA) was used according the Manufacturer's instructions. The ability of the test compound to inhibit COX-2 (human recombinant) was determined. The test compound was dissolved in DMSO, and the solution was made at the final concentration of 10 µM. A reaction buffer solution (960µl, 0.1M Tris-HCL, pH-8 containing 5 mM EDTA and 2 mM phenol) containing COX-2 enzyme (10 µl) in the presence of heme (10 µl) was added with 10 µl of 10 µM test drug solution. These solutions were incubated for a period of 10 min at 37^{o}C after then 10 µl of AA solution was added followed by stopping the COX reaction by addition of 50 µl of 1 M HCL. Prostaglandins are one of the active mediators of inflammation formed by biosynthesis arachidonic acid (AA) to PGH_2 catalysed by cyclooxygenase enzyme catalyses the first step in the biosynthesis of the arachidonic acid (AA). PGH_2 by reduction with stannous chloride (100 µl) forms $PGF_{2\alpha}$, which is measured by enzyme immunoassay. The amount of PG tracer is kept constant and varying amount of PGs is added to the well which already contains PG antiserum. PGs and PG-acetyl cholinesterase conjugation (PG tracer) compete for the limited amount of PG antiserum. The amount of PG tracer that is able to bind to the PG antiserum is inversely proportional to the concentration of PGs in the well.Ellman's reagent, which contains substrate to acetylcholine esterase, is added after washing the plate to remove any unbound reagents. This enzymatic reaction gives a distinct yellow colour, which is determined by spectrophotometerically (Micro titre Plate reader) at 412 nm, Absorbance is directly proportional to the amount of PG tracer bound to the well and inversely proportional to the amount of free PGs present in the well during the incubation:

Absorbance α [Bound PG tracer] α 1/PGs.

Percentage inhibition was calculated by the comparison of compound treated by control incubations.

Fractionation of active extracts

The active extracts showing good COX-2 inhibitory activity were subjected to column chromatography using silica gel glass column(60-120 mesh)[15].The active crude extracts were placed on the top of the column and various fractions of the active extracts were eluted by passing the suitable mobile phase through the packed column in different ratios. The COX-2 inhibitory activity of the various fractions isolated by column chromatography was observed by *in vitro* enzyme immunoassay and the fractions with potential COX-2 inhibitory activity were used for the preparation of herbal formulation.

Development of formulation

Five formulations namely formulation I, formulation II, formulation III, formulation IV and formulationVwith varying concentrations of the active fractions were developed. According to the formulation, required quantity of each isolated fractions with potential COX-2 inhibitory activity and other ingredients were weighed, ground separately. Then the ingredients were screened through sieve number 80. All the ingredients except talc and magnesium stearate were mixed together and milled in a mortar pestle. The milled mixture was passed through sieve number 80. Then acacia gum solution, was slowly added to the milled mixture. This powder mass was screened through sieve number 18to obtain granules. The granules were dried at 35°C in vacuum dryer. The dried granules were passed through sieve no. 18 in order to remove bigger granules and stored in desiccators [16].The formulation details are mentioned in table no 1.

Table 1: formulation details of polyherbal tablet"

Ingredients	Amount (mg) for one tablet				
	Formulation I	Formulation II	Formulation III	Formulation IV	Formulation V
ZF4	10	15	20	25	30
CF7	10	15	20	25	30
RF5	10	15	20	25	30
MF4	10	15	20	25	30
BF6	10	15	20	25	30
OF6	10	15	20	25	30
GF2	10	15	20	25	30
TF1	10	15	20	25	30
Starch	20	20	20	20	20
Talc	5	5	5	5	5
Magnesium stearate	5	5	5	5	5
Acacia gum	5	5	5	5	5
Lactose	385	345	305	265	225

Preparation of polyherbal tablets
Power blends according to each formulation, were compressed to 500 mg tablet by using hand rotating single punch tablet presses with appropriate compression pressure. The granules were mixed with talc which acts as lubricant, and magnesium stearate which acts as glidant, before punching. The die cavity was adjusted for required weight and the Preformulation studies [17]for various parameters were conducted before compression of the powder blend to tablets.

Evaluation of Polyherbal Tablets
The following post-compression parameters were employed for evaluation standardisation of tablets[18, 19].

General appearance: The physical appearance of the tablet involving colour, odour and texture were observed.

Percentage Weight variation: 20 tablets were randomly selected and average weight was noted. Then each tablet was weighed individually. The deviation of each tablet from the average weight was then observed and expressed as percentage deviation from the average weight.

Hardness test: In order to resist the mechanical shocks during handling processes a certain amount of strength or hardness is required for the tablet. Monsanto hardness tester was used to determine the hardness of randomly selected 20 tablets.

Percentage friability test: The percentage of weight loss of randomly selected 20 tablets was observed after tumbling them into Roche friabilator at a speed of 25 rpm for 4 minutes Disintegration test: Digital microprocessor based disintegration test apparatus (basket rack assembly, Lab India) was used to determine the disintegration of the tablets. One tablet was introduced into each tube and a disc was added. The total assembly was suspended in a 1000 ml beaker filled in with water. The volume of water was such that the wires mesh at its highest point (at least 25 mm) below the surface of the water, and at its lower point (at least 25 mm) above the bottom of the beaker. The apparatus was operated and maintained at 37±2°C. The time required for disintegration of all the tablets was noted.
Statistical analysis

Data were expressed as mean ± SEM, where, n = 3, $p \leq 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

From the investigations it has been revealed that various herbal preparations have capability of stabilizing red blood cell membrane leading to anti-inflammatory effect. Percentage of membrane stabilisation is directly correlated to anti-inflammatory response since, HRBC membrane is similar to that of lysosomal membrane[20] and thus, stabilisation of lysosomal membrane prevents the release of lysosomal enzymes responsible for inflammation. The test extracts were studied by *in vitro* method using COX catalysed prostaglandin biosynthesis assay to evaluate the COX-2 inhibitory capacity. Fromthe *in vitro* studies it is evident that methanolic extracts of *Ginger, Rosemary, Oregano, Gaultheria, Holybasil* and aqueous extracts of *Turmeric, Chamomile, Barberry* were found to have significant anti-inflammatory response as well as COX-2 inhibitory effect. The anti-inflammatory response increased with the concentration of the extracts. The significance in the percentage protection may be due to different active ingredients present in different extracts of the various herbs studied. Observing the significant COX-2 inhibition by the extracts studied it can be concluded that the mechanism involved in anti-inflammatory activity may be due to COX-2 inhibition. The percentage of membrane stabilization and COX-2 inhibition of various extracts represented in table2 and 3.Crude extracts showing promising anti-inflammatory activity and COX-2 inhibitory activity was subjected to column chromatography and various fractions were isolated which were further studied for the specific COX-2 inhibitory activity.Active extracts, mobile phase used, the number of fractions isolated for different extracts and the active fractions showing significant COX-2 inhibitory activity are mentioned in table no 4.Considering the significant results an attempt was made to prepare a polyherbal tablet with the isolated active fractions in order to potentiate the activity of herbal extracts for COX-2 inhibition. The granules prepared from the active extracts were evaluated for preformulation parameters like angle of repose, loose bulk density, tapped bulk density, loss on drying, compressibility index and Hausner ratio. The results pertaining to preformulation parameters are tabulated in table 5. The studies indicated that the granules were within the acceptable limit. All the five tablet formulations were further evaluated for their hardness, thickness, friability, weight variation, moisture content and disintegration time. Monsanto tester was used to study the hardness of formulation which was measured in kg/cm2. An appreciable limit of hardness was showed by all the formulations which facilitated the faster disintegration of the tablets prepared.

"Table 2: Percentage of membrane stabilization of various extracts"

Extract	PEE		CE		EAE		ME		AE	
	Concentration (µg/ml)									
Herb	300	500	300	500	300	500	300	500	300	500
	Percentage inhibition of various extracts									
Ginger	36.43 ±0.02	44.38 ±0.01	52.42 ±0.05	59.23 ±0.01	62.64 ±0.02	66.43 ±0.05	71.93 ±0.03	78.64 ±0.04	49.64 ±0.02	55.29 ±0.02
Rosemary	44.31 ±0.04	47.57 ±0.01	51.26 ±0.03	53.56 ±0.02	59.14 ±0.02	62.52 ±0.02	81.56 ±0.01	84.27 ±0.02	52.75 ±0.05	56.43 ±0.03
Turmeric	49.52 ±0.03	53.46 ±0.03	56.23 ±0.05	60.40 ±0.01	61.48 ±0.03	64.69 ±0.04	66.36 ±0.04	72.62 ±0.03	80.92 ±0.03	85.66 ±0.04
Chamomile	48.51 ±0.01	53.72 ±0.03	43.34 ±0.04	51.75 ±0.02	65.24 ±0.02	69.68 ±0.03	56.67 ±0.01	61.37 ±0.03	72.25 ±0.02	82.43 ±0.01
Barberry	46.26 ±0.02	51.12 ±0.04	35.56 ±0.03	39.48 ±0.04	49.54 ±0.01	54.52 ±0.03	59.91 ±0.02	62.56 ±0.02	79.24 ±0.01	83.67 ±0.01
Oregano	20.80 ±0.01	23.90 ±0.03	30.75 ±0.04	34.95 ±0.02	45.44 ±0.02	46.05 ±0.03	79.44 ±0.01	84.34 ±0.01	49.26 ±0.02	52.43 ±0.01
Gaultheria	38.45 ±0.01	43.07 ±0.02	46.94 ±0.03	52.23 ±0.04	58.89 ±0.01	60.02 ±0.03	65.62 ±0.03	68.69 ±0.03	30.92 ±0.05	34.69 ±0.03
Holy basil	38.64 ±0.02	44.92 ±0.05	46.69 ±0.01	51.09 ±0.02	55.33 ±0.03	58.49 ±0.04	78.22 ±0.01	86.14 ±0.04	50.69 ±0.05	53.08 ±0.03
Diclofenac (Standard)					84.73±0.01 (300µg/ml)	88.45±0.01 (500µg/ml)				

PEE – petroleum ether extract. CE – chloroform extract, EAE – ethyl acetate extract, ME – methanolic extract, AE – aqueous extract.

"Table 3: Percentage COX-2 inhibition of various extracts"

Extract	PEE		CE		EAE		ME		AE	
	Concentration (µg/ml)									
Herb	300	500	300	500	300	500	300	500	300	500
	Percentage COX-2 inhibition of various extracts									
Ginger	30.12 ±0.01	36.42 ±0.02	52.43 ±0.05	59.13 ±0.01	63.14 ±0.01	69.56 ±0.04	73.62 ±0.01	79.55 ±0.02	42.31 ±0.02	51.42 ±0.02
Rosemary	40.26 ±0.02	42.9 ±0.02	55.82 ±0.02	57.32 ±0.05	51.44 ±0.04	53.53 ±0.03	68.49 ±0.01	74.59 ±0.03	56.26 ±0.02	61.33 ±0.04
Turmeric	51.12 ±0.02	56.36 ±0.03	52.16 ±0.04	59.07 ±0.02	55.26 ±0.02	66.12 ±0.03	61.67 ±0.01	67.41 ±0.02	78.26 ±0.02	85.21 ±0.03
Chamomile	35.21 ±0.01	42.9 ±0.03	45.82 ±0.04	47.32 ±0.04	38.59 ±0.05	41.43 ±0.02	35.44 ±0.05	39.68 ±0.03	60.46 ±0.01	68.62 ±0.03
Barberry	56.11 ±0.03	60.43 ±0.02	36.38 ±0.02	40.42 ±0.04	44.65 ±0.03	50.28 ±0.02	55.66 ±0.04	61.62 ±0.03	73.66 ±0.02	78.12 ±0.01
Oregano	45.62 ±0.01	49.93 ±0.02	35.68 ±0.03	39.62 ±0.04	48.65 ±0.01	54.79 ±0.02	68.86 ±0.01	74.52 ±0.03	50.36 ±0.05	52.72 ±0.05
Gaultheria	36.52 ±0.02	43.82 ±0.04	47.16 ±0.03	52.23 ±0.34	44.69 ±0.01	50.31 ±0.02	70.32 ±0.01	78.19 ±0.02	50.23 ±0.05	54.46 ±0.03
Holy basil	42.34 ±0.01	48.12 ±0.03	38.69 ±0.02	42.59 ±0.02	46.42 ±0.01	50.32 ±0.03	62.42 ±0.02	69.46 ±0.01	52.34 ±0.05	56.24 ±0.04

“Table 4: column chromatography studies”

Plant	Extract used for column chromatography	Mobile phase	No of fractions isolated	Name of the fractions	Active fraction
Zingiberofficinale	Methanolic	Pet. ether: CHCl3 in the ratio of 9:1, 7:4, 5:5	6	ZF1,ZF2,ZF3,ZF4,ZF5,ZF6	ZF4
Curcuma longa	Aqueous	$CHCl_3$:CH_3OH in the ratio of 9:1, 7:4, 5:5	8	CF1,CF2,CF3,CF4,CF5,CF6,CF7,CF8	CF7
Rosmarinusofficinalis	Methanolic	Pet. ether: CHCl3 in the ratio of 9:1, 7:4, 5:5	7	RF1,RF2,RF3,RF4,RF5,RF6,RF7	RF5
Matricariarecutita	Aqueous	$CHCl_3$:CH_3OH in the ratio of 9:1, 7:4, 5:5	7	MF1,MF2,MF3,MF4,MF5,MF6,MF7	MF4
Berberisaristata	Aqueous	$CHCl_3$:CH_3OH in the ratio of 9:1, 7:4, 5:5	8	BF1,BF2,BF3,BF4,BF5,BF6,BF7,BF8	BF6
Origanumvulgare	Methanolic	Pet. ether: CHCl3 in the ratio of 9:1, 7:4, 5:5	7	OF1,OF2,OF3,OF4,OF5,OF6,OF7	OF6
Gaultheria Procumbens	Methanolic	Pet. ether: CHCl3 in the ratio of 9:1, 7:4, 5:5	5	GF1,GF2,GF3,GF4,GF5	GF2
Ocimumsanctum	Methanolic	Pet. ether: CHCl3 in the ratio of 9:1, 7:4, 5:5	4	TF1,TF2,TF3,TF4	TF1

"Table 5: Pre-formulation studies of powder blend"

Parameters	Powder blend of				
	Formulation I	Formulation II	Formulation III	Formulation IV	Formulation V
Angle of repose	28.2±1.32°	28.1±1.51°	26.3±1.15°	30.2±1.41°	28.3±1.31°
Loose bulk density (g/cm3)	0.334±0.016	0.325±0.015	0.319±0.025	0.354±0.023	0.342±0.019
Tapped bulk density (g/cm3)	0.556±0.005	0.522±0.016	0.532±0.008	0.552±0.017	0.561±0.015
Hausner ratio	1.39±0.018	1.34±0.016	1.44±0.024	1.46±0.015	1.38±0.039
Compressibility index (%)	25.23±1.32	23.72±1.28	29.52±1.18	31.31±1.36	32.24±1.15
Loss on drying (%)	0.95±0.009	0.98±0.011	0.97±0.019	0.96±0.009	0.97±0.006

"Table 6: Evaluation of formulated herbal tablets"

Parameters	Formulation I	Formulation II	Formulation III	Formulation IV	Formulation V
Colour	Brownish Green	Brownish Green	Brownish Green	Brownish Green	Brownish Green
Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Texture	Smooth	Smooth	Smooth	Smooth	Smooth
%WeightVariation(±5%)	2.11±0.017	1.26±0.012	2.12±0.008	1.07±0.003	1.61±0.006
Hardness(Kg/cm2)	7.5±0.25	6.7±0.36	6.5±0.31	7.3±0.52	7.1±0.42
% Friability(NMT 1%)	0.57±0.012	0.68±0.024	0.65±0.0032	0.54±0.0016	0.44±0.015
Disintegration (minutes)	14±1.12	13±1.24	11±1.76	11±1.03	12±1.12

"Table7: Percentage COX-2 inhibition of various formulations"

Tablet formulation	% COX-2 inhibition
Formulation I	62.49±0.02
Formulation II	70.24±0.01
Formulation III	74.54±0.01
Formulation IV	86.21±0.03
Formulation V	82.62±0.02

The percentage friability studies indicated that the tablets are mechanically stable. The acceptable range of weight variation is ±5%and all the tablet formulations passed the weight variation test. An ideal tablet should disintegrate within 15min. All the tablet formulation disintegrated within 13 minutes. The post formulation results mentioned in table no 6.In view of the positive standardisation results of the herbal tablets all the five formulations were assessed for *in vitro* COX-2 anti-inflammatory activity among which, formulation IV showed significant COX-2 inhibition in comparative to other formulations (table 7). This showed the possible anti-inflammatory mechanism of the formulation is by COX-2 inhibition.

CONCLUSION

From the pharmaceutical and pharmacological evaluation of this study it can be envisaged thatplants form a better source for anti-inflammatory as well as COX-2 inhibitory drugs from which more potent drugs with lesser side effects can be prepared by isolating active constituents from the crude active fractions which holds a great promise in competing with the modern COX-2 inhibitors.

Acknowledgements
The first author is thankful to Department of Science and Technology (DST), Government of India, New Delhi for sanctioning INSPIRE fellowship.

REFERENCES

[1] KC Srivastava; T Mustafa,*Medical Hypotheses*, **1989**, 29, 25-28.
[2] MT Huang;T Lysz;T Ferrara;AH Conney. In: *Cancer Chemoprevention*, L Wattenberg;M Lipkin;CW Boone;Kelloff (eds).CRC press, **1992**;375-391.
[3] JR Chi Pault;GR Mizuno;JM Hawkins; WO Lundberg, *Food Res.*, **1952,** 17, 46-55.
[4] R Inatani*etal*,*AgriBiol Chem*.,**1983**, 47, 521-528.
[5] DL Mckay; JB Blumberg,*Phytotheraphy Research*, **2006**, 20 (7), 519-530.
[6] JK Srivastava; S Gupta. Health promotion benefits of chamomile in the elderly population. In: R Watson Ronald, editor. Complementary and Alternative Therapies in the Aging Population, Elsevier Inc, Academic Press, **2009**.
[7] N Ivanovska; S Philipov,*Int J Immunopharmacol.*, **1996**, 18, 553-561.
[8] V Lagouri; B Dimitrios, *International Journal of Food Science and Nutrition*, **1996**, 47, 493- 497.
[9] B Zhang;XL He;Y Ding;GH Du,*Eur J Pharmacol.*, January **2006**, 530 (1-2), 166-171.

[10] P Prakash;N Gupta,*Indian Journal of Physiology and Pharmacology*, April **2005**, 49 (2), 125-131.
[11] S Singh;DK Majumdar;HM Rehan, *J of Ethnopharmacol.*, Oct **1996**, 54 (1), 19-26.
[12] PN Praveen rao; EK Edward,*J Pharm Pharmaceut Sci*., **2008**,11(2), 81S-110S.
[13] E Mongeli;C Desmarchelier;J Coussio;G Ciccia,*Journal of Ethnopharmacology*,**1997**,56, 117-121.
[14] P Pradelles;J Grassi; JA Maclouf,*Annal. Chem.*, **1985**, 57, 1170-1173.
[15] S.Revathy; S. Elumalai;Merina Benny; Benny Antony,*Journal of Experimetal Sciences*,**2011**, 2, 21-25.
[16] NB Ghiware;SG Gattani;SS Chalikwar,*Int J Pharm Tech Res*., **2010**, 2(1),171-176.
[17] ME Aulton. Pharmaceutics: The science of Dosageform. Churchill Livingstone, **1996**; 304.
[18] L Lachman;HA Lieberman;JL Kanig.The theoryand practice of industrial pharmacy, 3rd edition.Varghese Publishing House, New Delhi, **1987**;293-639.
[19] Anonymous.Indian Pharmacopoeia.Governmentof India, Ministry of Health and Family Welfare Controller of Publication, New Delhi, **2007**.
[20] R Gandhisan;AThamaraichelvan;C Baburaj,*Fitoterapia*,**1991**,62(1), 81-83.