International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.5, No.3, pp 1426-1433, July-Sept 2013

Anti-Inflammatory Activity Of Methanol Extract Fractions Of Abrus precatorius Leaves

Khadse C.D.[1]*, Kakde R.B.[2] , Chandewar A.V.[3]

[1]MES College of Pharmacy, Sonai Ta. Newasa, Dist. Ahmednagar (M.S.), India
[2]Department of Pharmaceutical Sciences, RTM Nagpur university, Nagpur (M.S.), India
[3]P.Wadhwani College of Pharmacy, Yavatmal, Maharashtra, India

***Corres.author: khadsecd@rediffmail.com**
Mob. No. +919921357845

Abstract: Methanol extracts fractions obtained by column chromatography of *Abrus precatorious Linn.* leaves were investigated for its anti-inflammatory activity using carragennan-induced rat paw edema model, protein denaturation and membrane stabilization method. The results showed that MeOH:water(9:1) fraction(FR-II) and MeOH:water (1:9) fraction(FR-III) at (400mg/kg) have shown significant 51.92 % and 46.34% inhibition of rat paw edema respectively at the end of 4 hr. when compared to control. Fraction FR-II and FR-III have shown significant percentage inhibition of haemolysis 67.1% and 58.3% respectively and FR-II & FR-III also have shown significant percentage inhibition of protein denaturation 57.23% and 51.92% at a concentration of 200µg/ml, which was compared with control. The anti-inflammatory action of fractions were compared with indomethacin as reference drug. The anti-inflammatory activity may be due to an inhibitory effect on mediators of inflammation.Thus the results obtained showed that methanolic extract fractions possess anti-inflammatory effect and thus this plant effectively use in the treatment of inflammatory disease conditions.
Keywords: *Abrus precatorious*, anti-inflammatory activity, indomethacin, protein Denaturation, membrane stabilization

Introduction:

Abrus precatorius Linn.(Fabaceae) is a perennial climber plant with multiple branches. Flowers are pale purple color, seeds are scarlet and black[1,2] Leaf, stem and roots of this plant traditionally used for the treatment of arthritis, swellings[3], pain, mild to moderate depression, root paste is applied on skin infection[4,5]. The major activity of the plant seed till now reported are anticancer[6,7], antioxidative antiplatelets[8], seeds have shown anti-inflammatory, antimicrobial[9], antifertility activity in male and aborfacients activity in female[10]. Abrin from the Seeds are found to have antitumor activity[11,12], Seed kernel contain flavonoids abrectorin and glycoside desmethoxycentaureidin 7-O-rutinoside[13], 8-c glucosylscutellarine 6,7- dimethyl ether, 2-O-apioside flavones C-glycoside[14], alkaloids methyl ester of N-N dimethyltryptophan metho cation and precatorine.[10] Inflammation is a normal protective response shown by living tissue against the injury caused by physical trauma, noxious chemicals or microbiological agents[15,16]. It is one of the most common reasons for which people try to find medical attention, thus, anti-inflammatory are among the most commonly prescribed medications in clinical practice. But on chronic use of more commonly anti-inflammatory drugs are showing toxic side effects like; NSAIDs induce gastric ulcerations, while acetaminophen causes liver damage[17]. Consequently, it is important to search for anti-inflammatory agent from plant source which are able to manage inflammation of various

etiologies and have less or negligible adverse side effects. Therefore in the present study efforts have taken to evaluate anti-inflammatory activity of this plant.

Material And Method:

Plant material:

The leaves of *Abrus. precatorius* Linn. were collected in the month of January 2010 at Sonai place, Dist. Ahmednagar. (M.S.), India and identified by Dr. P. G. Diwakar at Botanical Survey of India (BSI), Pune.(M.S.). A voucher specimen (CHADABRUP3) is deposited in the herbarium of BSI Pune for future reference.

Chemicals and drugs

Caragennan (Sigma Aldrich US), Eggs albumin (Himedia Lab.,Mumbai), Indomethacin (E Merk India), All the solvents used were of Analytical grade. Saline water (Claris life sci., India)

Preparation of Extracts:

Shade dried and coarsely powdered of *A. Precatorious* leaves were subjected to successive solvents extraction in soxhlet extractor used for extraction are in the order of petroleum ether, chloroform, ethanol, methanol and marc left was refluxed with distilled water, all the extracts were vacuum dried to yield PETE(0.5%),CHME(1.92%), ETHE(4.28%), MEOHE(5.24%) and AQE(4.12%) respectively.

Column chromatography

On preliminary pharmacological evaluation of anti-inflammatory activity, MEOHE was found most active extract and it was then subject to column chromatography. MEOH (15g) was dissolved in small volume of methanol and applied to silica gel (60-120)column(3x60 cm) which was eluted by Ethyl acetate FR-I (2.14%), Methanol: Water (9:1) yielding FR-II (48.72%), and Methanol: water(1:9) yielding FR-III (10.84%)w/w.

Animals

Wistar albino rats of either sex weighing between 150-200g were used for Anti-inflammatory studies. Animals were grouped in clean polyacrelic cages and maintained at standard laboratory condition (temp 25±2^{o}C) and relative humidity (50±5%) with dark and light cycles (12/12 hrs). Animals were allowed to free access to standard dry pellets diets and water ad libitum for two days. The institutional animal ethics committee has approved the experimental protocols and was performed in accordance with the guidelines for the care and use of laboratory animals as adopted and promulgated by institutional animal committee. (CPCSEA, India Reg. No.1211/ac/08/CPCSEA)

Acute toxicity

Acute toxicity of various fractions of methanol extracts was carried out using acute toxic class method as described in OECD [organization of economic co-operation and development]. All the fractions were found to be safe up to dose of 2000mg/kg body weight, hence 200 and 400mg/kg moderate dose was used for evaluation.

Evaluation of In-vivo Anti-inflammatory activity

Anti-inflammatory activity[18] was studies for different fractions of methanol extract of *A. precatorius* leaves by caragennan induced rat paw edema. Extract fractions were suspended into 1% carboxymethyl cellulose in saline water and administered orally. The wistar albino rats were divided into eight groups (n=6). Group-I serve as control and orally administered vehicle only. Group II, III, IV administered orally (200mg/kg) of FR I,II &III respectively and Group V,VI,VII administered orally (400mg/kg) of FR I,II&III respectively. Group VIII administered orally with Indomethacine (10mg/kg) as reference drug. One hour after the respective treatment caragennan (0.1ml of 1% in normal saline) was injected into sub planner side of the right hind paw of rats. The paw volume was measured at 1, 2, 3 and 4 hours using plethysmometer. The anti-inflammatory effects of different fractions were calculated by using the following equation[21]

% inhibition of edema = (Vc-Vt/Vc) x100

Where Vt is paw volume in test group of rats and Vc is paw volume in control group of rats.

Statistical analysis

All the experimental data was expressed as mean± SEM, significance of difference among the various groups and control group were carried out using one way ANOVA followed by dunnett's t test using Grapat Instat software. Where $P < 0.05$ was considered as significant, while $P < 0.01$ was considered as more significant of test group compared with control group.

Evaluation of In-vitro Anti-inflammatory activity:

Inhibition of albumin denaturation[19]:

Method of Sachin S Sakat was followed with minor modifications. Test solution (1ml) containing different concentration (50-250µg/ml) of extracts or indomethacine (100µg/ml) was mixed with 1ml (1%) aqueous solution of egg albumin and incubated at 37±1°C for 20 min. Denaturation was induced by keeping the reaction mixture at 57±2°C in water bath for 20 min. after cooling the turbidity was measured spectrophotometrically at 660 nm. Percentage inhibition of denaturation was calculated from control where no drug was added. Each experiment was carried out in triplicate and average was taken. Percentage inhibition of protein denaturation was calculated as follows:

% inhibition = Ac- At/Ac*100

Where, Ac= absorption of control , At = absorption of test samples

Membrane Stabilization Test:

Preparation of Red Blood Cells suspension[20]:

Method of K. Nirmala Devi was followed with minor modification. Fresh human blood (10ml) was collected and transferred to heparized centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline. The assay mixture contains the test drug at various concentration, 5ml phosphate buffer (7.4pH),2.0 ml normal saline and 0.5 ml of RBC suspension. Indomethacine (100µg/ml) was used as reference drug. Control sample consist of 0.5ml of RBC suspension mixed with normal saline solution. All the assay mixture were incubated at 37°c for 30 min. and centrifuged. Absorbance of supernant solution was estimated using spectrophotometer at 560nm. The %inhibition of haemolysis was calculated by using following equation.

% Inhibition of haemolysis = $100^* (A_1 - A_2/A_1)$

Where: A_1= absorbance of control, A_2 = absorbance of test sample.

Result:

Phytochemical analysis:

Preliminary phytochemical investigation of *A. precatorius* leaves extracts shown the presence of flavonoids, glycosides, alkaloids and carbohydrates.

In-Vivo Anti-inflammatory activity:

Caragennan induced rat paw edema Test:

All the extracts were preliminary screened for In-vivo anti-inflammatory activity and MEOHE was found to be most active among all extracts. So it was further fractionated by column chromatography. These fractions were carried out for acute toxicity assay, no death was observed during 72 h period at the dose tested also does not shown any symptoms of convulsion, diarrhea or increased dieresis, thus the moderate dose of 200 and 400mg/kg

was used in the study. In the caragennan induced rat paw edema test, methanol extract fractions FR-I, II & III were given orally at a dose of 200mg/kg & 400mg/kg. the effect of various fractions in all experimental groups were studied at 1, 2, 3 & 4 h for inhibition of rat paw edema compared with control group are shown in **Table-I.** At the end of 4 h, both FR-II &III (200 mg/kg) showed significant (P<0.01) inhibition of edema with 40.62% & 38.01% respectively. While FR-II & III (400mg/kg) showed 51.92% & 46.34% significant (P<0.01) inhibition of edema respectively, which was compared with reference drug indomethacine, that have shown 53.85% significant (P<0.01) inhibition of edema.

In-Vitro Anti-inflammatory activity:

Inhibition of albumin denaturation:

The different concentrations of test fractions as shown in **Table-II,** were studied for their inhibiting heat induced albumin denaturation. Maximum inhibition 57.23% was observed at 200µg/ml. IC_{50} value was found to be 96.04±2 and correlation coefficient value (r)0.969. Indomethacine reference drug showed maximum inhibition 59.67 % at a concentration of 100 µg/ml.

Membrane Stabilization Test:

The different concentrations of test fractions were studied for their stabilization of RBCs membrane. The test fractions (50- 200µg/ml) inhibited the heat induced haemolysis of RBCs to varying degree as shown in **Table-III.** FR-II showed the maximum inhibition 67.1% at concentration of 200µg/ml and IC_{50} was observed at 123.49 ±3.67 and correlation coefficient value (r) at 0.998.

Table I: Anti-inflammatory activity of MEOHE fractions of *A. precatorius* by carrageen induced rat paw edema

Group		Increase in rat paw volume in ml ± SEM (% inhibition)			
		1 hr	2 hr	3 hr	4 hr
Control	Dose mg/kg	0.78±0.01	0.95±0.02	1.19±0.05	1.58±0.02
FR-I	200	0.70±0.11 (10.32%)	0.79±0.15 (16.92%)	0.91±0.11 (23.59%)	1.16±0.03 (26.66%)
	400	0.63±0.03 (14.86%)	0.72±0.02 (21.73%)	0.85±0.08 (28.62%)	1.10±0.06* (30.41%)
FR-II	200	0.60±0.06 (23.21%)	0.69±0.05* (27.43%)	0.82±0.07* (31.51%)	0.94±0.06** (40.62%)
	400	0.54±0.01 (30.81%)	0.61±0.02* (35.87%)	0.68±0.02** (42.98%)	0.76±0.01** (51.92%)
FR-III	200	0.62±0.01 (20.62%)	0.73±0.02* (23.27%)	0.85±0.02* (28.68%)	0.98±0.03** (38.01%)
	400	0.57±0.03 (27.08%)	0.64±0.04* (32.71%)	0.73±0.03* (38.82%)	0.85±0.01** (46.34%)
Indomethacin	10	0.53±0.02 (32.14%)	0.60±0.01** (37.03%)	0.66±0.07** (44.67%)	0.73±0.05** (53.85%)

Values are expressed as mean± SEM (n=6) animals in each group
*P<0.05 & **P<0.01 significant compared to control group by one way ANOVA followed by Dunnett's multiple comparison test.

Table-II: In-vitro anti-inflammatory activity of *Abrus precatorious* leaves by Albumin Denaturation

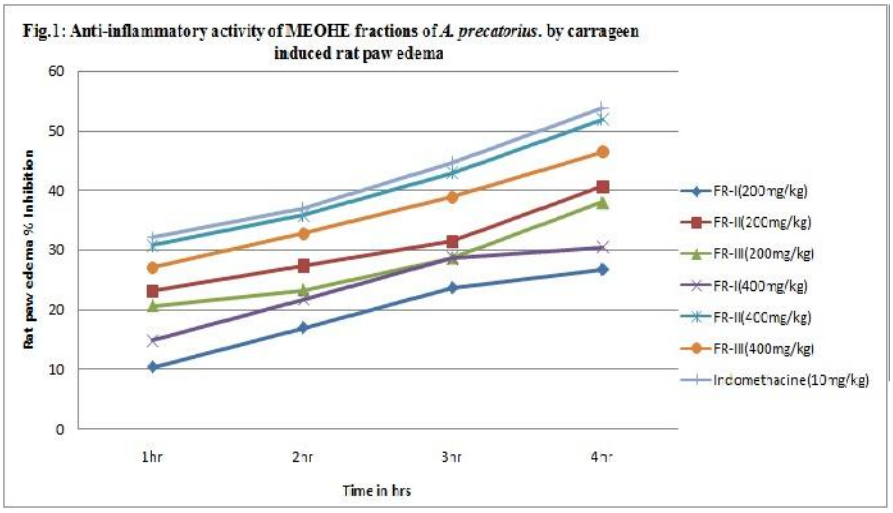
Treatment	Concentration(µg/ml)	Absorbance (nm)	%inhibition
control	-	0.49	-
Fr-I	50	0.45±0.013	08.21
	100	0.43±0.007	12.28
	150	0.39±0.026	20.42
	200	0.35±0.011	28.60
Fr-II	50	0.27±0.041	44.94
	100	0.24±0.008	51.47
	150	0.22±0.013	55.26
	200	0.21±0.002	57.23
Fr-III	50	0.29±0.004	42.35
	100	0.27±0.003	44.45
	150	0.26±0.014	46.98
	200	0.24±0.015	51.92
Indomethacine	100	0.20±0.003	59.67

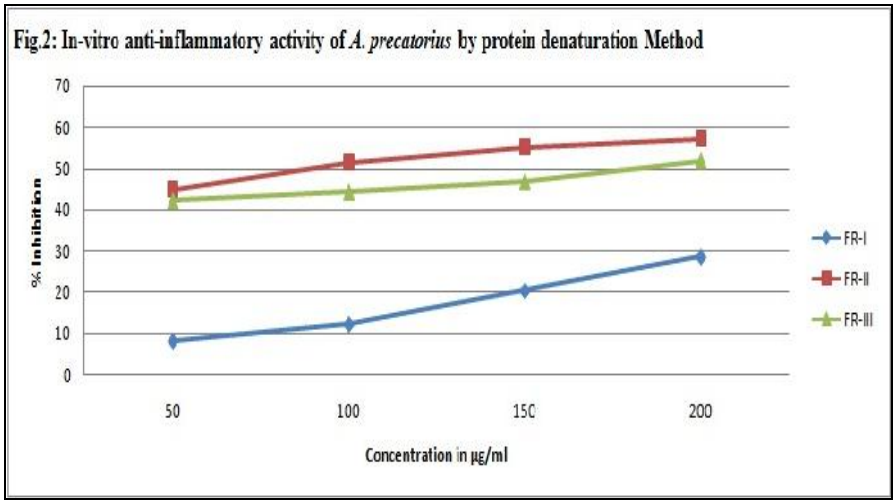
Values are expressed as SEM of three readings

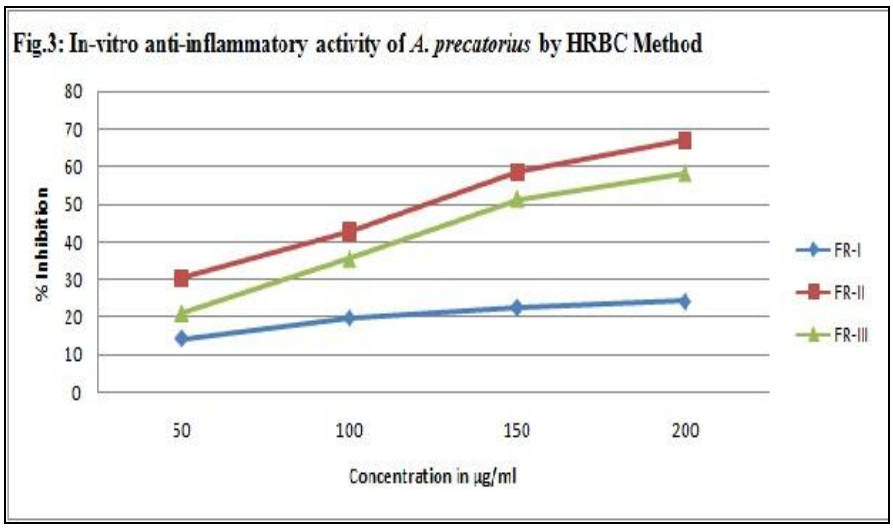
Table-III: In-vitro anti-inflammatory activity of *Abrus precatorious* leaves by HRBC Membrane

SN	Concentration µg/ml	% inhibition			
		FR-I	FR-II	FR-III	Indomethacine
01	Control	--	--	--	--
02	50	14.3±0.01	30.4 ±0.01	21.2 ±0.02	
03	100	19.8±0.03	42.8 ±0.02	35.6 ±0.05	68.7 ±0.01
04	150	22.6±0.08	58.6 ±0.01	51.4 ±0.02	
05	200	24.2±0.02	67.1 ±0.03	58.3 ±0.01	

Values are expressed as SEM of three readings







Discussion

In the present study *Abrus precatorius Linn*. leaves powder was successively extracted by soxhlet apparatus, vacuumed dried extracts were preliminary screened for In-vitro anti-inflammatory activity and among all the extracts methanolic extract showed significant anti-inflammatory activity in a concentration dependent manner. So it was further fractionated by using column chromatography. These fractions were carried out for acute toxicity assay, no death was observed during 72 h period at the dose tested also does not shown any symptoms of convulsion, diarrhea or increased dieresis, thus the moderate dose of 200 and 400mg/kg was used in the In-vivo anti-inflammatory study. Inflammation is a biphasic process (Vinegar et al., 1969). Early phase (1-2 h) of caragennan model is mainly mediated by histamine and serotonin in the mast cells. The later phase is mediated by prostaglandin, bradykinin, protease and lysosome[22], the products of cycloxygenase and lipoxygenase enzymes. Formations of arachidonic acid via cycloxygenase and lipoxygenase pathway represent two important classes of inflammatory mediator. The product of cycloxygenase pathway mainly prostaglandin E2 is known to cause cardinal sign of inflammation and the product of lipoxygenase pathway mainly leukotrine B4 is mediator of leukocyte activation in the inflammation[21]. From the result FR-II & III at a dose of 400mg/kg showed significant ($P<0.01$) inhibition against caragennan induced rat paw edema which is comparable with reference drug Indomethacine. FR-II was found more potent than FR-III. Reference drug is cycloxygenase inhibitor but anti-inflammatory activity against caragennan induced rat paw edema also shown by lipoxygenase inhibitor; hence inhibition of caragennan induced paw edema by crude extract may be due to inhibitory activity of lipoxygenase enzymes.

In-vitro study was carried out by inhibition of thermally induced protein denaturation and membrane stabilization method, in which FR-II have shown more percentage of inhibition of thermally induced protein

denaturation and also shown significant HRBCs membrane stabilization at a concentration of 200µg/ml, which was comparable with reference indomethacine drug. The extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lyses of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release.

From the present study, it is concluded that MEOHE fractions of *Abrus precatorius* leaves have shown significant dose dependant anti-inflammatory activity. These result support the traditional claim of this plant in the treatment of inflammation condition. Potent inhibition of caragennan induced rat paw edema, showed inhibition of prostaglandins synthesis is major mechanism by which the plant extract may showed anti-inflammatory activity. Further studies are in progress to find out isolated phytoconstituents responsible for anti-inflammatory activity.

Acknowledgement:

Authors sincerely thanks to Shri. Prashant Gadakh Patil, Chairman , Mula Education Society, Sonai, Principal, MES, college of Pharmacy, Sonai, Director, Department of Pharmaceutical sciences, JNTU, Hyderabad for their encourage and provided required facilities during the course of this study. Authors also thanks to Dr. P. G. Diwakar, Botanical Survey of India, Pune.(M.S.) for authentification of this plant.

References:

1. Amritpal Singh Saroya, Glossary of Medicinal plants used in Ayurveda, Scientific Publication, India, 2006, 47
2. Bhattacharyajee S.K. , Handbook of medicinal plants, 2004,4th ed. pp 7
3. Nadkarni A.K., Indian plant and drugs with their medicinal properties and uses,Srishti book distributors, India, 2004, pp 2-4
4. Kirtikar K.R., Basu B.D., ,Indian medicinal plants, Vol.-I, International Book Distributors, India,1999, pp 764-768.
5. Vipin Parkash, Ashok Aggrawal, Traditional use of ethnomedical plants of lower foot hills of Himachal Pradesh-I, *Indian jou of traditional knowledge*, 2010, 9(3), 519-521.
6. Anbu J., Ravichandiran V.,M.Sumithra,B. Chowdary Sudheer, K. Swaroop Kumar. S.L.V.V.S.N., R. Kannadhasan, R. satheesh Kumar, Anticancer acticity of petroleum ether extract of Abrus precatorius on ehrlich ascitis carcinoma in mice, *International journal of Pharma and Bio sciences* , 2011, 2(3), 24-31.
7. Premalatha Balachandran Rajgopal Govindarajan, Cancer- an ayurvedic perspective, *Pharmacological Research* ,2005,51, 19-30
8. Ranju S. pal, G.Ariharasivakumar, Kundlik Girhepunje, Ashitosh Upadhyay, In-Vitro antioxidative activity of Phenolic and flavonoids compounds extracted from seeds of Abrus precatorius, *International journal of Pharmacy and pharmaceutical sci.*, 2009,1(2), 136-140
9. Jigna Parekh, Sumitra V. Chanda, , In vitro Antimicrobial Activity and Phytochemical analysis of some Indian medicinal plants, *Turk journal of Biol*2007,31, 53-58.
10. Ghosal S., Dutta S.K., Alkaloids of Abrus precatorious, *Phytochemistry* ,1971,10, 195-198.
11. Mary S Herrmann, W. David Behnke,,Physical studies on three lectins from the seeds of Abrus precatorious. *Biochemica et Biophysica Acta* ,1980,621, 43-52.
12. Tahir haji oglu Tahirov, tian- Huey Lu, A new crystal form of abrin-a from seed of Abrus precatorius., *Journal of molecular bio.* ,1994, 235, 1152-1153.
13. Bhardwaj D.K., Bisht M.S.,Mehata C.K ., Flavonoids from *Abrus precatorious*. *Phytochem.*, 1980, 19, 2040-2041
14. Kenneth R. Markham, James W. Wallace , Y. Niranjan Babu, V. Krishna Murty, M. Gopala Rao, 8-C-glucosylscutellarein 6,7- Dimethyl ether and its 2-O-Apioside from Abrus precatorious, *Phytochemistry*, 1989, 28(1), 299-301.
15. Robert A Turner, Screening methods in Pharmacology, Academic press, an Imprint of Elsevier ,2009, 152-153

16. Tripathi K.D., Essential of medical Pharmacology, Jaypee brothers medical publishers, India, 2004, 5th edn, 167-181,257-259
17. T. Sivkumar, K. Kannan, N.Kannappan, K. Kathiresan, Anti-inflamatory activity of Commiphora caudata[Wight and Arn], *Asian Journal of Chem.*, 2009, 21,(5), ,4130-4132
18. Winter CA, Risley EA,Nuss GW, "carrageenan induced oedema in hind paw of the rats as an assay for Anti-inflamatory drugs" Proc of society of Experimental Biology Medicine,1962,111,544-547
19. Sakat Sachin S., Juvekar Archana R., Gambhire Manoj N., Invitro antioxidant and Anti-inflammatory activity of methanol extract of Oxalis corniculata Linn., *International Journal of Pharmacy and Pharmaceutical Research*, 2010, (1), 146-155.
20. K. Nirmala Devi, K. Periyanayagam, Invitro anti-inflammatory activity of Plectanthus amboinicus (Lour) spring by HRBC membrane stabilization.*International journal of Pharmaceutical studies and research*,2010,1, 26-29.
21. Sharma U.S., Sharma U.K., Sutar N, Singh A, Shukla D.K., Anti-inflamatory activity of Cordia dichotoma forst f. seeds extracts, *International Journal of Pharmaceutical Analysis*, 2010,(1), 01-04
22. Achinto Saha, Muniruddin AhmedThe analgesic and anti-inflammatory activities of the extract of Albiza lebbeck in animal model, *Pakistan Journal of Pharmaceutical Sciences*, 2009,22(1), 74-77.

*****