e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2022; 14(9); 680-689

Original Research Article

Anti-Inflammatory & Anti-Arthritic Activity of Aqueous Extract of Aloe Vera in Wistar Albino Rats

Pradhan Rashmita[1], Singh Subhasish[2], Singh Nipa[3], Upadhyay Rajlaxmi[4]

[1]Assistant Professor, Department of Pharmacology, S. C. B. Medical College, Cuttack, Odisha

[2]Assistant Professor, Department of Cardiology, M. K. C. G. Medical College, Berhampur, Odisha

[3]Associate Professor, Department of Microbiology, KIMS, BBSR

[4]Assistant Professor, Department of Pharmacology. SCB MCH, Cuttack, Odisha

Received: 20-07-2022 / Revised: 20-08-2022 / Accepted: 14-09-2022

Corresponding author: Dr Rashmita Pradhan

Conflict of interest: Nil

Abstract

Objective: Currently NSAIDs, selective COX-2 inhibitors, glucocorticoids which are used for treatment of inflammation &arthritis associated with adverse effects like peptic ulcer, renal toxicity and haematological toxicity, and amounts to be expensive when used on long term basis. Hence there is a necessity to find out the new cost effective as well as less toxic drug as an alternative. The present study was taken up to evaluate the systemic anti-inflammatory & antiarthritic activity of Aloe vera aqueous extract in wistar albino rats.

Method: The anti-inflammatory activity of Aloe vera aqueous extract in the doses of 125mg/kg, 250mg/kg was evaluated by using carrageenan induced rat paw oedema model for acute inflammation, cotton pellet granuloma for subacute inflammation and Freund"s Complete Adjuvant (CFA) induced adjuvant arthritis for chronic inflammation. The antiarthritic activity was evaluated by using biophysical parameters (arthritic score, paw volume and joint diameter) hematological parameters (ESR, RF) and histopathological study of joints (arthritic index).

Results: The Aloe vera aqueous extract in the doses 250mg/kg, showed significant anti-inflammatory activity on acute, subacute models of inflammation &CFA induced arthritis as that of Indomethacin and antiarthritic property like Dexamethasone.

Conclusion: Aloevera aqueous extract at 250mg/kg has better anti-inflammatory property along with good antiarthritic property.

Keywords: Aloevera Aqueous Extract, Anti-inflammatory, Antiarthritic, CFA induced adjuvant arthritis, Carrageenan.

Background

The current anti-inflammatory agents used in management of inflammation & arthritis are of two types i.e. steroidal & nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs cease inflammation by blocking the cyclooxygenase enzyme that produces prostaglandins which causes pain & swelling. When used for a longer period these traditional NSAIDS cause adverse effects like peptic ulcer, renal dysfunction, constipation, dizziness, headache, and thrombotic cardiovascular complications [1]. In this context indigenous drugs can be used as better

alternative having low incidence of adverse effects when used on long term basis. Aloe vera (A. vera) (Liliaceae) (Aloe barbadensis) is one of the most widely in traditional medicine for treatment of disorders such as helminthiasis, constipation, gout, dermatitis, peptic ulcer and burns [2].

Hence the present study was conducted to evaluate anti-inflammatory & antiarthitic activity of aqueous extract of Aloe vera when used systemically in Wistar albino rats.

Materials and Methods

Experimental Animals*:* For our study, healthy Wistar albino rats of either sex, weighing 100-200gm were used. The animals which were kept under standard laboratory conditions and temperature (25 °C±1 °C) and light/dark cycle (12 h light: 12 h dark cycle) received rat pellets and clean water ad libitum.

They were acclimatized for 2 weeks before the experimental study was carried out [9]. The study protocol was approved by Institutional Animal Ethical committee, M.K.C.G. Medical College, Berhampur. The rats were fasted overnight with free access to water on the day before experiment.

Plant material

Preparations of Extract; The aqueous extract of aloe vera (AVE) was procured from Indichem Pvt ltd (batch no WL-243/).

Drugs and chemicals: Carrageenan CFA, formalin, paraffin, heamtoxylin and eosin, Carrageen powder, Gum acasia, was procured from merck pvt. ltd. Indomethacin & Dexamethasone, streptomycin vial, Benzyl penicillin, ether were purchased from local pharmacy.

Instruments: Oral feeding tube, syringe &needle, test tubes, plethysmograph, Hot air oven, capillary tube, glass seeker, forceps & a pair of scissors, cotton pellets, weighing machine, screw gauze.

Experimental protocol

Acute Inflammation Study

Carrageenan Induced Rat Paw Oedema [7]**:** For our study, twenty four albino rats of either sex were divided into four different groups with six animals in each. They were kept in separate cages and the cages were numbered. Basal body weight and paw volume of all the animals were measured at the start of the study.

Table 1: Grouping and treatment schedule. For Acute & Sub acute inflammation model

Group	No of animals	Drugs &doses
I(Control)	6	Gum Acacia(GA)-1ml/rat
II(standard)	6	Indomethacin(2.5 mg/kg)
III(Test drug)	6	AVE(125mg/kg)
IV(Test Drug)	6	AVE(250mg/kg)

Table 2: Grouping &treatment schedule of rats in Freund's Complete Adjuvant induced Arthritis model (Chronic inflammation model)

Group	No of rats	Drugs &Doses	Route
I	6	GA(1ml/rat) -Normal Control	Oral
II	6	GA(1ml/rat)- Disease Control	
III	6	Indomethacin(2.5mg/kg)	
IV	6	Dexamethasone(0.1mg/kg)	
V	6	AVE(125mg/kg)	
VI	6	AVE(250mg/kg)	

Freshly prepared 0.2ml of carrageenan (1%w/v) was injected subcutaneously into the sub plantar region of the right hind paw of each rat for induction of acute inflammation. The Control Group was administered only vehicle (1ml of Gum Acacia) by oral route & the standard treatment group received indomethacin 2.5 ml/kg. After 1h both doses of AVE was given by oral route (Table 1). The paw volume was measured with plethysmograph before and after drug treatment up to the 6hour., 12hour and 24hour. Results were determined as the percentage increase in paw volume due to oedema up to 24hours and compared with control group and standard treatment group [3-6]. Anti-inflammatory effect of standard drug and AVE was described as the percentage inhibition of the rat paw edema as calculated after each hour of carrageenan injection up to 24 hours by the formula described by Sudjarwo Agus.

Percentage inhibition of edema

$$= \frac{V_c - V_T}{V_C} \times 100\%$$

V_T= Mean paw edema volume in drug treated group

V_C=Mean paw edema volume in control group

Subacute Inflammation

Cotton Pellet Induced Granuloma in Rats [8,9]: 10mg of autoclaved cotton pellets were implanted subcutaneously into both sides of the axilla under ether anaesthesia. Drugs were administered orally to each rat for 7days. On eighth day cotton pellets were removed surgically along with the granulation tissue by anaesthetising the rats. Immediate wet weight and constant dry weight after drying in a hot air oven at 60 0 C for 18hr were taken. The increase in weight of the dry cotton pellet was taken as a measure of granuloma formation. Weight of the exudates =immediate wet weight of pellet- constant dry weight of pellet

Weight of granulation tissue = dry weight of pellet- constant weight of pellet

The percentage inhibition of exudates and granulation tissue formation was determined as

$$\% \text{ inhibition of exudates} = \frac{W_C - W_T}{W_C} \times 100$$

Where W_T= Weight of exudates in mg of drug treated group

W_C=Weight of exudates in mg of control group

$$\% \text{ inhibition of granuloma} = \frac{W_C - W_T}{W_C} \times 100$$

Where W_T= W_t of granuloma in mg of drug treated group

W_C=W_t of granuloma in mg of control group.

Adjuvant induced arthritis model [10]: The drugs and vehicle were given for 13days at different doses and routes of drug administration as shown in table-2. In all test group animals except normal control 0.1ml of complete Freund`s adjuvant (CFA) was injected subcutaneously into the sub-planter surface of right hind paw for induction of arthritis (Mi-Jung *et al*., 2006). Paw volume & paw thickness were measured by using mercury plethysmometer & screw gauze respectively before injection of CFA. Change in paw volume & paw thickness was measured on every alternate day up to 13 days & then on 21day. In order to induce experimental arthritis, 0.1ml of FCA was injected into sub-plantar region of right hind paw of each rat on d 1st. Dosing of all the animals groups was started from d 12th once in a day p.o., after induction of arthritis, Anti-arthritic activity of extracts were evaluated by biophysical parameters such as arthritic score, body weight, paw volume and joint diameter [7].

Arthritic score: Different set illustration criteria was monitored according to methods followed by Paval *et al* [8].

Paw volume: The volumes of left hind paw at different days by Plethysmometer.

This parameter has been carried according to methods followed by Cain *et al* [9]. Paw thickness (Joint diameter): Joint diameter was calculated by using screw gauge according to methods followed by Barbier *et al* [10]. On day 21, severity of secondary lesion was evaluated visually and graded according to the scoring system.an arthritic index was calculated using sum of scores as described.

The average in drug treated animals was compared with disease control group. For primary lesions: The percent inhibition of paw volume of the injected left paw over control was measured at day 5. For secondary lesions: The percentage inhibition of paw volume of non-injected right paw over control was measured at day 21. An Arthritic Index is calculated as the sum of the scores as indicated above for each animal. The pain threshold, degree of joint movement -Randall & Selitto *et al* [11]. with modification and grip strength -Boissier and Simon *et al* [12] (1960) with modification, 21 st day as measured by fall of time and histopathology on 22nd day.

Histopathological Assessment: After euthanasia, on day 21st, the hind paws amputated above the knee joint and were fixed in 7.4% formalin solution. The paws were then decalcified using 10% Nitric acid embedded in paraffin and sectioned in a mid-sagittal plane. The sections of articulation of the tarsal joints were stained with heamtoxylin and eosin and were examined microscopically for mononuclear infiltration, pannus formation and bone destruction (Choi *et al*., 2003; Michele *et al*., 2005). Scores are given according to infiltration of inflammatory calls (0to3),progressive loss of articular cartilage(0 to 3), cartilage & bone destruction by using following score [13-16].

0-no change
1-moderate change(pannus invasion within cartilage)
2-moderate change(pannus invasion into cartilage/subchondral bone)
3-severe change(pannus invasion into subchondral bone)

Statistical Analysis

All values were reported as Mean ± SEM. Results were analyzed using One way ANOVA, followed by Dunnet`s/Tukey`s multiple comparison t test using graph padprism-5., P value <0.001 was considered to be statistically significant.

Results

Table 3: Effect of aloe vera extract on caragennan induced paw edema at different time interval

Drugs &Doses	Paw edema volume with% inhibition						
	1hr	2hr	3hr	4hr	6hr	12hr	24hr
GA (1ml/rat)	0.58± 0.01	0.99± 0.03	1.21± 0.04	1.06± 0.03	0.9± 0.01	0.65± 0.02	0.37± 0.03
Indomethacin (2.5mg/kg)	0.3± 0.018*** 48%	0.32± 0.03*** 68%	0.37± 0.03*** 69%	0.34± 0.03*** 68%	0.32± 0.23*** 64%	0.31± 0.02*** 52	0.26± 0.03*** 30%
AVE (125mg/kg)	0.55± 0.02 5%	0.87± 0.02 12%	0.99± 0.05 18%	0.92± 0.02 13%	0.80± 0.021 11%	0.61± 0.02 6%	0.35± 0.03 5%
AVE (250mg/kg)	0.43± 0.03 26%	0.68± 0.043*** 31%	0.72± 0.032*** 40%	0.7± 0.068***	0.62± 0.04***	0.46± 0.05***	0.28± 0.03***
F	40.03	106.1	40				
Df	3,20						
P	<0.001						

Table 4: Anti-inflammatory activity of AVE on subacute inflammation by cotton pellet granuloma method in albino rats.

Group (drugs & doses)	WT of exudate(mg)	WT of granuloma (mg)	% inhibition of Exudates	% inhibition of granuloma
GA(1ml)	77.6±2.96	21±0.83		
Indomethacin(2.5mg/kg)	44.5±1.41***	11.3±0.43***	43%	46%
AVE(125mg/kg)	71±1.46	17.9±0.77	9%	15%
AVE(250mg/kg	50±1.40**	13.1±0.87***	36%	37%
F	66.18	29.4		
P value	<0.001	<0.001		

Data expressed in mean± SEM, n=6, df=3,20 ***P<0.001(Vs Control group)

Table 5: Anti-Inflammatory Activity of Drugs on CFA Induced Paw Edema Volume at Different Time Intervals

Drugs & doses	Hind paw volume (ml) with % inhibition in different days				
	1st	3rd	5th	7th	21st
Gum Acacia(1ml)	1.40±0.06	1.73±0.06	2.0±0.07	2.01±0.1	1.69±0.06
Indomethacin (2.5mg/kg)	0.9±0.03*** 40%	0.91±0.05*** 48%	0.88±0.09*** 58%	0.83±0.08*** 63	0.60±1*** 64
Dexamethasone (0.01mg/kg)	0.87±0.06*** 38%	0.89±0.07*** 49%	0.85±0.07*** 58%	0.80±0.08*** 62%	.59±0.03*** 62
AVE125mg/kg	1.20±0.06 15%	1.50±0.05 14%	1.70±0.05 15%	1.77±0.21 16	1.51±0.05 11
AVE 250mg/kg	1.13±0.05**20%	1.25±0.0** 28%	1.26±0.05* 37%	1.23±0.06** 42%	1.16±0.03*** 32%
F	13.25	14.65	8.97	8.36	15.71
Df	4.25				
p	<0.001	<0.001	<0.001		

Table 6: Effect of Drugs on Paw Thickness at Different Days in CFA Induced Arthitis Model

Drugs & Doses	Paw thickness in (mm) % inhibition in different days				
	1st DAY	3rdDAY	5thDAY	7th day	21st DAY
Gum Acacia(1ml/rat)	9.69±51	12.46±39	15.49±43	16.86±53	17.18±75
Indomethacin (2.5mg/kg)	7.02±27*** 28	8.52±0.29*** 32	9.92±3*** 36	10.58±3*** 37	11.72±0.44*** 32
Dexamethasone (0.1mg/kg)	6.67±25*** 31	7.79±46*** 37%	9.14±41*** 41	9.71±46*** 42	9.87±43*** 43
AVE125mg/kg	8.38±24 13	11.04±38 11	13.71±36 12	15.00±37 11	15.4±44 11
AVE 250mg/kg	7.93±26*** 18	9.32±32*** 25	11.21±57*** 28	12.01±67*** 29	12.65±56*** 26
F	13.83	26.56	31.62	30.70	27.74
Df	4,25				
p	<0.001				

Data expressed in MEAN± SEM n=6, ***p<0.001Vs control group.

With CFA injection paw thickness was maximum on 9th day (18.14±56mm). Treatment with dexamethasone &indomethacin paw thickness was significantly reduced in diseased control group from day 1 to day 21. Aqueous extract of aloe vera at 125mg/kg did not show any significant reduction in paw thickness whereas 250mg/kg dose reduced paw thickness significantly from day 1 to21st day with maximum reduction in 7th day (29%).

Table 7: Effect of Drugs on Pain Threshold in CFA Induced Adjuvant Arthitis

Drugs & Doses	Paw Withdrawal Time(Sec)				
	mean	SEM	F	df	P
Normal Control	10.48	0.45	67.67	5.30	
Disease Control	3.27b	0.33			<0.01
Indomethacin	7.28***	0.47			<0.001
Dexamethasone	86.96**	0.66			
AVE (125mg/kg)	3.72a	0.32			
AVE (250mg/kg)	4.91*a	0.36			

b-p<0.01 Disease Control Vs Normal control; *p<0.05,**p<0.001,***p<0.001 test drug Vs disease control; Ap>0.05 AVE vs Reference standard drug

Table 8: Effect of drugs on grip strength in CFA induced adjuvant arthritis

Drugs &Doses	Fall Off Time(Sec)				
	MEAN	SEM	F	df	P
Normal Control	86.8	3	43.8	5.30	
Disease Control	20.0a	3.4			<0.01
Indomethacin	74.3**	1.6			<0.001
Dexamethasone	67.5***	0.8			
AVE(125mg/kg)	24.7b	2.8			
AVE(250mg/kg)	56.8***b	2.7			

ap<0.001 Disease Control Vs Normal Control ***p<0.001 test Vs Disease Control; bp>0.05 AVE vs Reference standard

Table 9: Effect of drugs on degree of joint movement in CFA induced arthitis model

Drugs &doses	Median score	Kw	P
Disease Control	0.25	17.5	<0.001
Indomethacin	1*		
Dexamethasone	1*		
AVE(125mg/kg)	0.25		
AVE(250mg/kg)	0.5		

*P<0.05, nsp>0.05 Drug treated group Vs Diseased control (Kruskal-Wallis followed by Dunn"s test)

Table 10: Effect of drugs on ESR in CFA induced arthitis model

Drugs &doses	ESR (Mm/Hr)				
	Mean	Se	F	Df	P
Normal Control	8.933	04153	42.27	5,30	<0.001
Disease Control	17.82a	0.9329			
Indomethacin	10.72***	0.3434			
Dexamethasone	10.63***	0.1820			
AVE(125mg/Kg)	16.65@	0.8346			
AVE(250mg/Kg)	10.98**@	0.1167			

a-p<0.001 Normal control Vs Disease control; ***p<0.001 Test group Vs Disease control group; @p>0.05 test drug Vs standard drug

Table 11: Effect of drugs on rheumatoid factor (RF) values in CFA induced arthitis model

Drugs &Doses	RF Values(IU/ml)				
	MEAN	SE	F	df	P
Normal control	14.68	0.705	176.1	5,30	<0.001
Disease control	46.68a	1.316			
Indomethacin	37.05***	1.130			
Dexamethasone	37.43***	0.6360			
AVE(125mg/kg)	44.03	0.5270			
AVE(250mg/kg)	40.03	0.4869			

a, p<0.001 Disease control Vs Normal control; ***p<0.001Drug treated group Vs Diseased control

Table 12: Effect of drugs on arthitic indices in CFA induced arthitis model

Drugs &Doses	MEDIAN SCORE	KW	P value
Disease Control	5.5	23.44	0.0001
Dexamethasone	2*		
Indomethacin	2		
AVE-125mg/Kg	2		
AVE-250mg/Kg	3		

*p<0.05, **p<0.01 Drug treated group Vs Disease control (Kruskal Wallis followed by Dunn"s test.)

Discussion

For testing the acute anti-inflammatory property of a new drug, carrageenan induced rat paw edema is one of the most common method used. Here the ability of the compound to reduce local in rat paw oedema by injection of an irritant agent (winter *et al* 1962) determines its anti-inflammatory property. Carragenan induced paw edema is mostly biphasic, the early phase (1-2h) is mediated by histamine &serotonin (Vinegar *et al*,1969) [17] and the late phase is mediated by bradykinin, leukotrienes, polymorphonuclear cells & PGs produced by tissue macrophages. (Brito &Antonio,1998: Gupta *et al*,2006) [18]. In this study the results revealed that aqueous extract of Aloe vera at a dose of 250mg/kg significantly inhibited the carrageenan induced paw oedema starting from 2nd hour over a period of 24hr which was comparable with the standard drug indomethacin. Similar results were obtained by Davis *et al*1989, & Vzquez [19] *et al* 1996. Also Kshirsagar *et al*. showed that Aloe emodin [AE] and carboxylic acid derivative of Aloe emodin [AEC] exhibited significant anti-inflammatory and antiarthritic activity [20] AE and AEC showed dose-dependent inhibition of second phase of Carrageenan induced rat paw oedema due to inhibition of Prostaglandins release [21].

Also it was observed by Egesie UG *et al* [22] that aqueous extract of the Aloe showed significant reduction in paw edema which supports its anti-inflammatory property. Also in formalin induced edema, AVE significantly reduced paw edema at the beginning of 3 hours when compared to the control group [23]. Inhibition of inflammation was observed also in the formaldehyde induced rat paw oedema and in the histamine-induced oedema further supporting the presence of anti-inflammatory substances in the plant. Bhattacharjee *et al* [23] conducted experiment on the test drug Aloe vera on Carrageenan induced paw oedema and found significant reduction in paw swelling. They recorded an increased level

of inflammatory responses at the beginning of anti-inflammatory test [0.5 hour interval] in the high dose group.

There was a sharp decline of paw circumference during 0.5 to 3.5 h supported the hypothesis. Devaraj A *et al* [24] demonstrated AVE produced dose-dependent and significant inhibition of carrageenan-induced paw oedema which was comparable to the standard drug Indomethacin [24] Cotton pellet induced granuloma is the model for testing subacute inflammation where the compound is tested for both transudative & proliferative components the wet weight of pellets relates to transudates & dry weight correlates to granulomatous tissue. (Lowry *et al*, 1958, Castro *et al*, 1968) [25,26] in our study AVE 250mg/kg showed significant anti-inflammatory activity in cotton pellet granulomas measured in terms of reduction in weight of exudates to 36% inhibition as well as weight of granuloma to 37% inhibition when compared with GA.

The standard drug, indomethacin significantly reduced weight of exudates by 43% &weight of granuloma by 46% as depicted in table-3. this corroborates with Hart *et al*,1990 [27]. Freund's adjuvant induced arthritis model is one of the most commonly used animal model for testing antiarthritic activity of a drug with features resemble to RA. In this model Aloe vera 250mg/kg showed reduction in paw oedemas compared to disease control animals. Aloe vera 250mg/kg caused reduction in paw volume & paw thickness from 1st day onwards like that of Dexamethasone & indomethacin. This indicates AVE showed significant reduction in chronic inflammation & effective against inflammatory component of arthritis.

Measurement of pain in arthritis as indicated by grip strength ,fall off time (degree of joint movement).in our study dexamethasone ,AVE 250mg/kg increased fall off time suggesting improved grip strength as compared to disease control group. Inflammation and /or nodules observed in ears, nose, tail, forepaw & hind paw are indicative of delayed response are mainly immunologically mediated, Piper *et al* 1971. Arthitic index is calculated as sum of of scores given to severity of these lesions [28]. Dexamethaxsone and indomethacin treated animals showed significant lesser arthritic index as compared with disease control animals whereas aloe vera at both doses has no significant effects. Histopathologically AVE 250mg/kg showed significant reduction in vascularity, lymphocytic infiltration with minimum inflammation angiogenesis, no thickening of synovial membrane and absence of lymphoid follicles. Our finding corroborates with Davis *et al* 1989 who evaluated anti arthitic activity of aloe vera in animal models. D Sarkar *et al*, 2005 also evaluated anti-inflammatory effect of aloe vera leaf extract in chronic model of inflammation [29].

Conclusion

Aloe vera at a dose of 250mg/kg revealed significant inhibition of carrageenan induced paw oedema in rats as well as it significantly reduced the formation of exudates and granuloma in cotton pellet granuloma models of inflammation. But this anti-inflammatory activity exhibited by aloe vera was less than that of standard NSAID, indomethacin. Again, aloe vera at dose 250mg/kg reduced inflammation and swelling without protective effect on secondary lesion and other immunological parameters (RF) in CFA induced adjuvant arthritis in rats.

References

1. Wolfe MM, and R, L. David: Gstrointestinal toxicity of Non-steroidal anti-inflammatory drugs,N Eng J, Med 1999:340:1888-1899.
2. Bensky D Gamble A, Kaptchuk TJ, Chinese herbal medicine: materia medica, seatle Wash: East land press,1993: xxxv:556.

3. Harries J, Spensen PS: A modified plethysmographic apparatus for recording volume changes in rat paw: J Pharmacy and Pharmacology, 1962; 14: BJMC, 464-466.
4. Bhatt KR, Mehta R, Shrivastava PN: A simple method for recording anti-inflammatory effect on rat paw edema: Ind. J. Phy. And Pharmac, 1997; 399-400.
5. Winter CA, Risley EA, Nues GW: Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drugs: Proc. Soc. Exp. Biol. Med. III 1982; 544-547.
6. Wang JP and Tang CM: Roles of PMN leucocyte, platelets and some mediators in rat hind paw edema: J. Pharm. and Pharmacol, 1992; 44: 300-305
7. Agus S. The Potency of Piperine as Antiinflammatory and Analgesic in rats and mice. Folia Med Indonesiana. 2005; 41:190-4.
8. Meier R, Schuler W, Desaulles P: Zur Frage des Mechanismus der Hemmung des Bindegeweb swachstmus durch Cortisone Experientia 1950;6:469-471
9. Penn GB, Ashford A. The inflammatory response to implantation of cotton pellets in rats. J Pharm Pharmacol 1963; 15:798-803.
10. Pearson CM, Wood FD. Studies on polyarthritis and other lesions induced in rats by injection of mycobacterium adjuvant. I. General clinic and pathological characteristics and some modifying factors. Arthur Rheum 1959; 2:440-459
11. Randall LO, Sellitto JJ. A method for measurement of analgesic activity on inflamed tissue. Arch Int Pharmacodyn 1957; 111:1170-1174
12. Boissier JR, Simon P. Lutilisation du test de la traction (Test de JULOU-CURVOISIER) pour l. etude des psycholeptiques. Therapie 1960; 15: 1170-1174.
13. Joosten LAB, Lubberts E, Helsen MMA, Van den Berg WB. Dual role of IL-12 in early and late stages of murine collagen type II arthritis. J Immunol 1997 :159:4094-4102
14. Joosten LAB, Helsen MMA, Van de Loo FAJ, Van den Berg WB, Anticytokine treatment of established collagen type-II arthritis in DBA/1 mice: a comparative study using anti-TNFα,β and ILRa Arthitis Rheum,1996:39:797-809
15. Taniguchi K, Kohsaka H,Incue N,Terada Y, Ito H , Hirokawa K,Miyasaka N,induction of p16INK4a senescense gene as a new therapeutic strategy for treatment of rheumatoid arthritis,Nat Med 1999; 5:760-767.
16. Robert H Shimerling *et al*: The American Journal of Medicine1991,91:528-534.
17. Vnegar R, Schreiber W and W and Hugo R (1969) Biphasic development of carrageenan in rats. Journal of pharmacology and experimental Therapeutics 166:96-103.
18. Brito ARMS, Antonio MA. Oral anti-inflammatory andantiulcerogenic activities of a hydroalcoholic extract and partitioned fractions of Tumera ulmifolia (Turnaraceae). J. Ethnopharmacology 1998; 61:215-228.
19. Davis -RH, Leittner –MG: Russo-JM:Bryne-ME:Aloe vera and gibberellin Anti-inflammatory activity of aloe vera against a spectrum of irritants.J Am Podiatr Med Assoc 1989 Jun :79(6):263-76.ISSN:8750-7315.
20. Geboes L, Klerck BD, Balen MV, Kelchtermans H, Mitera T, Boon L, *et al*. Freund's Complete Adjuvant Induces Arthritis in Mice Lacking a Functional Interferon- Receptor by Triggering Tumor Necrosis Factor-Driven Osteoclastogenesis. Arthritis and Rheumatism. 2007;56(8):2595-607.
21. Kshirsagar AD, Panchal PV, Harle UN, Nanda RK, Shaikh HM. Anti-Inflammatory and Antiarthritic Activity of Anthraquinone Derivatives in Rodents. International Journal of Inflammation. 2014:1-12.

22. Egesie UG, Chima KE, Galam NZ. Anti-inflammatory and Analgesic Effects of AEAV [Aloe barbadensis] in Rats. Afr. J. Biomed. Res. 2011; 14:209-12.
23. Bhattacharjee S, Paul S, Dutta S, Chaudhuri TK. Antiinflammatory and protective properties of aloe vera leaf crude gel in carrageenan induced acute inflammatory rat models. Int J Pharm Sci. 2014;(9):368-71.
24. Devaraj A, Karpagam T. Evaluation of antiinflammatory activity and analgesic effect of Aloe Vera leaf extract in rats. Int Res J of Pha. 2011;2(3):103-10.
25. Lowry OH, Rosenbrogh NJ, Far AL, Randall RJ. Protein Measurement With The Folin Phenol Reagent J.Biol Chem 1958; 193:265-275.
26. Castro J. Saseme H, Sussman H, Bullette P. Diverse effect of SKF 52 and antioxidants on CCL4 induced changes in liver microsomal P450 content and ethylmorphine etabolism Life Sci 1968;7:129-136.
27. Hart *et al*. Antiinflammatory activity of Aloe vera Plysachharides on Cotton pellet granuloma model: Journal of Immunol,1990; 39.
28. Colpert KM Evidence that adjuvant arthitisin the ratsis associated with chronic pain,1987:28:201-222.
29. D Sarkar *et al*., Effect of Aloe vera on Nitric oxide production by macrophages during inflammation, IJP, 2005;37: Dec: 371-375