Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(12):745-749

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Invitro anti-inflammatory activity of *Cassia kleinii*

Vivek D.[1], Molly Mathew[2], Sarala Devi A.[3], Bijesh Vatakkeel[1], Shahin Mohammed T. K.[1], Sajith Kumar P. N.[1] and Sreeraj K.[1]

[1]*Academy of Pharmaceutical Science, Academy of Medical Sciences, Pariyaram, Kannur, Kerala, India*
[2]*Malik Deenar College of Pharmacy, Seethangoli, Bela, Kerala*
[3]*Academy of Medical Sciences, Pariyaram, Kannur, Kerala, India*

ABSTRACT

The in vitro anti-inflammatory activity of Cassia Kleini by HRBC lysis and protein denaturation method. The extract at different concentrations was incubated with HRBC and egg albumin in controlled experimental conditions and subjected to determination of absorbance to assess the anti-inflammatory property. Diclofenac sodium was used as the reference drug. The present findings exhibited a concentration dependent inhibition of lysis of HRBC and protein (albumin) Denaturation by the C.kleinii extract. The fraction of petroleum ether namely CKPEECF had anti-inflammatory activity comparable to diclofenac sodium. From the present study it can be concluded that C. kleinii possess marked in vitro anti-inflammatory effect against the HRBC lysis and denaturation of protein. The effect might be possibly due to the kleinii oxanthrones, phytosterols and triterpenes contents of C. Kleinii.

Key words: *C.kleinii*, anti-inflammatory, stabilization, protein denaturation.

INTRODUCTION

Inflammation is defined as local response of living mammalian tissue to injury due to any agent. It is the body defense reaction in order to eliminate or limit the spread of injurious agent as well as to remove the necrosed cells and tissue[1].A variety of molecules are released from cells and plasma proteins during acute inflammation whose net overall effect is to increase vascular permeability, resulting in tissue edema. The released molecules include histamine, PGs, eicosanoids, PAF, Bradykinin and serotonin[2].The most commonly used drug for management of inflammatory conditions are non steroidal anti-inflammatory drugs (NSAIDs), which have several adverse effects especially gastric irritation leading to formation of gastric ulcers[3]. For this reason, in recent time, more interest is shown in alternative and natural drugs for treatment of various diseases, but there is lack of scientific evidence.

C. Kleinii (Fabaceae) is a prostrate herb, leaves upto 2 cm, 8-10 leaflets, oblong elliptic, 5-9x 3mm, gland 1, stipulate, peltate, at the base of the petiole, Recemes 2 fld, fls 1cm across, petals yellow. ovate=- oblong, stamens 10, all atheriferous, pod short-stipulate 3 x 0.4 cm warty, compressed downy-tomentose, obtuse, seeds c 10, flat, ovoid, longitudinal Melagins 1000-1400m, among grass on bare slopes and Cappler hill on the coast[4].

EXPERIMENTAL SECTION

1. Plant material

The plant *C.Kleinii*, was collected during October 2012 from Kanakamala, Thalassery, Kannur, Kerala, India. The plant material was taxonomically identified by Dr. P.G Radha, Associate Professor, HOD, Department of Botany, Government Brennen College, Thalassery, Kannur, Kerala, India. Herbarium specimen bearing voucher number A.P.S.C 569 have been deposited in the Department of Pharmacology, Academy of Pharmaceutical Sciences, Pariyaram, Kannur, Kerala, India for future reference. The plant material was shade-dried with occasional shifting and then powdered with mechanical grinder, passing through sieve no. 40, and stored in an airtight container.

2. Preparation of plant extracts

The dried powdered material was successively extracted with petroleum ether, chloroform, methanol and water by cold maceration process. The solvent was distilled off in reduced pressure using rotary flash evaporator to yield a solid residue[5]. The preliminary phytochemical analysis was performed for all four extracts to identify the phytoconstituents present in the extracts[6].The most active extracts namely petroleum ether extract and ethanol extract were further fractionated to determine their activity. The petroleum ether extract was fractionated to 1-chlorobutane fraction (CKPEECF), butanol fraction (CKPEEBF) and aqueous fraction (CKPEEAF). The ethanol extract was further fractionated to ethyl acetate fraction (CKEEEAF), ethyl methyl ketone fraction (CKEEEMKF) and aqueous fraction (CKEEAF). The aqueous fraction from both the extracts was omitted for further studies.

3. Chemical and Instruments

All chemicals used in the estimation were of analytical grade. Shimadzu 1700 UV visible spectrophotometer, Weiber-Acmas Technocracy BOD incubator, Superfit rotavapour and Remi mechanical stirrer was used for the in vitro study.

4. Screening of Anti-inflammatory Activity

a)HRBC Method

The human red blood cell (HRBC) membrane stabilization method was used as one of the methods for the determination of anti-inflammatory activity *invitro*[7]).The blood was collected from healthy human volunteer who was not taken any NSAIDS for two weeks prior to the experiment and mixed with equal volume of Alsever solution and centrifuged at 3,000 rpm and the packed cells were separated. The packed cells were washed with isosaline and a 10% suspension was made. This HRBC suspension was used for the estimation of anti-inflammatory property. Different concentration of extract, reference sample and control were separately mixed with 1ml of phosphate buffer, 2ml hypo saline and 0.5 ml of HRBC suspension were added. All the assay mixtures were incubated at 37^0C for 30 min in a BOD incubator and sufficiently centrifuged at 3,000-rpm. The supernatant liquid was decanted and the hemoglobin content was estimated spectrophotometrically at 560 nm. The percentage hemolysis was estimated by spectrometrically at 560 nm. The percentage hemolysis was estimated by assuming the hemolysis produced in the control as 100%.

Percentage protection = (1-(OD sample/OD control) x 100

b) Protein denaturation method

The protein denaturation method is the other method of assay for the assessment of anti-inflammatory activity *invitro* (Azeem AK, 2014). The reaction mixture (5ml) consisted of 0.2 ml of egg albumin, 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2ml of varying concentrations of the test extract so that final concentration become 31.25, 62.5, 125, 250, 500, 1000 μg/ml. similar volume of double-distilled water served as control. The mixtures were incubated at $37\pm2\ ^0$C in a BOD incubator for 15 minutes and then heated at for 5 minutes. After cooling their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at the final concentration of (31.25, 62.5, 125, 250, 500, 1000 μg/ml) was used a reference drug and treated similarly for determination of absorbance of test extract. The percentage inhibition of protein denaturation was calculated by using the following formula:

% inhibition = 100x [Vt/Vc-1]

Where Vt= absorbance of test sample, Vc= absorbance of control.

The extract/drug concentration for 50% inhibition (IC_{50}) was determined form the dose response curve by plotting percentage inhibition with respect to control.

5. Statistical Analysis
The Statistical analysis was done with one way analysis of variance followed by Dunnets multiple comparison test .P value<0.05 was considered as significant.

RESULTS

1 Phytochemical analysis
The qualitative chemical screening for the identification of various classes of active chemical constituents of petroleum ether extract and ethanol were carried out. The petroleum ether extract showed the presence of fats, oils, phytosterols, oxanthrones and triterpenes. The ethanol extract showed the presence of flavonoids, coumarins, polyphenolic compounds and tannins.

2 Anti-inflammatory activity by HRBC method
The whole plant extracts of *C.Kleinii*at different concentration showed significant stabilization towards HRBC membranes. The percentage protection of petroleum ether extract showed significant anti-inflammatory activity in a concentration dependent manner. The results are tabulated in Table 1 and 2.

Table.1: ***In vitro*** **anti-inflammatory activity of** ***C. kleinii*** **activity by HRBC method**

Concentration µg/ml	Diclofenac sodium	Petroleum ether extract	Chloroform extract	Ethanol extract	Aqueous extract
31.25	43.54±0.43	33.63±0.58	13.28±0.87	23.33±0.56	13.13±0.74
62.50	46.48±0.28	35.94±0.63	15.68±0.62	25.24±0.38	15.66±0.23
125	53.86±0.52	36.76±0.33	18.66±0.88	26.34±0.39	16.69±0.48
250	58.86±0.44	39.68±0.74	20.58±0.64	29.46±0.49	19.84±0.58
500	68.28±0.46	51.73±0.41	23.75±0.38	32.64±0.64	22.84±0.49
1000	74.86±0.39	58.86±0.82	30.57±0.84	38.53±0.84	28.74±0.82

Values are expressed as mean ± S.D (n=6)

Table.2: ***In vitro*** **anti-inflammatory activity of** ***C kleinii*** **fractionsby HRBC method**

Concentration µg/ml	Diclofenac sodium	CKPEECF	CKPEEBF	CKEEEAF	CKEEEMKF
31.25	43.42±0.07	42.73±0.38	13.84±0.31	19.36±0.46	12.34±0.36
62.50	46.78±0.22	44.46±0.37	14.68±0.15	20.28±0.27	14.14±0.27
125	53.41±0.28	49.58±0.46	16.70±0.28	21.64±0.43	17.82±0.26
250	58.69±0.26	54.61±0.27	18.87±0.46	24.30±0.39	19.62±0.38
500	68.85±0.46	59.72±0.17	19.12±0.16	27.47±0.48	22.36±0.41
1000	74.48±0.14	65.43±0.36	22.65±0.41	33.38±0.35	27.30±0.46

Values are expressed as mean ± S.D (n=6)

3 Anti-inflammatory activity by protein denaturation method
The denaturation of proteins is a well-documented cause of inflammation. Phenylbutazone, Salicylic acid, flufenamic acid etc., have shown dose dependent ability to thermally induced protein denaturation. As a part of the investigation on the mechanism of the anti-inflammatory activity, ability of extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation at different concentrations as shown in Table3 and 4.

Table.3. ***In vitro*** **anti-inflammatory activity of** ***C. kleinii*** **activity by protein denaturation method**

Concentration µg/ml	Diclofenac sodium	Petroleum ether extract	Chloroform extract	Ethanol extract	Aqueous extract
31.25	57.34±0.34	46.38±0.56	15.86±0.66	22.41±0.22	15.41±0.22
65.50	58.65±0.58	47.62±0.73	16.75±0.55	23.58±0.34	16.58±0.34
125	61.87±0.67	49.42±0.32	19.66±0.44	26.36±0.52	19.36±0.52
250	65.68±0.69	51.80±0.48	22.46±0.47	29.83±0.98	20.63±0.98
500	70.66±0.53	54.56±0.77	26.23±0.76	31.87±0.59	21.87±0.59
1000	81.83±0.49	60.88±0.83	29.65±0.78	36.84±0.68	23.84±0.68

Percentage protection is a mean of six readings ± S.D (n=6)

Table.4. ***In vitro*** **anti-inflammatory activity of** ***C. kleinii*** **fractions by protein denaturation method**

Concentration µg/ml	Diclofenac sodium	CKPEECF	CKPEEBF	CKEEEAF	CKEEEMKF
32.25	58.06±0.12	53.02±0.38	13.28±0.26	14.56±0.51	11.28±0.29
65.50	59.85±0.49	54.98±0.34	14.58±0.46	16. 09±0.38	13.56±0.33
125	62.36±0.87	57.84±0.36	17.04± 0.26	19.87±0.39	16.74±0.53
250	64.68±0.42	60.23±0.43	20.39 ±0.09	25.68±0.27	19.84±0.15
500	72.64±0.32	65.92 ±0.28	23.62±0.21	29.37±0.35	23.77±0.38
1000	82.35±0.82	74.79±0.44	28.37±0.22	38.08±0.26	27.36±0.42

Percentage protection is a mean of six readings ± S.D (n=6)

DISCUSSION

The *C.Kleinii* of the family Fabacea is commonly found in the hill top of flora of Kerala, India. The plant is used in folk medicine and also believed to have active components that help to treat and manage various diseases. The hypoglycemic activity of the plant is scientifically evaluated[9,10]. It has also been reported for the presence of Kleinii oxanthrone esters namely kleinii oxanthrone 1, 2, 3 and 4 from the aerial parts and roots of *Cassia kleinii*[11,12].Chemical evaluation of the whole plant of petroleum ether extract showed the presence of fats, oils, phytosterols, oxanthrones and triterpenes. The phytochemicals like phytosterols and triterpenes have already been proved forpreventing inflammatory process[13,14].In HRBC method the erythrocytic membrane is similar to lysosomal membrane and its stabilization entails that the CKPEECF may stabilize lysosomal membranes. This stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents, which causes further tissue inflammation and damage upon extracellular release[15]. The result indicated that the CKPEECF had significant anti-inflammatory activity comparable to diclofenac sodium. Denaturation of proteins is of the causes for the production of auto antigens in certain rheumatic diseases invivo. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphidebonding. The present study reveals that *C.kleinii*might be capable of controlling the production of autoantigens due to denaturation of proteins and stabilize the lysosomal membranes *invivo*[16].

Acknowledgement
The authors wish to acknowledge the Academy of Medical Sciences, Pariyaram, Kannur, Kerala, India for the facilities extended to complete this research work.

REFERENCES

[1]Harsh Mohan. Text book of pathology, 5thEdn, New Delhi; Jaypee Publications, **2002**, 114-60.
[2]HardmannJA, Limbard LE, Goodmann, APharmacological basis therapeutics. US: Mc Graw-Hill Inc., **1998**, 1465.
[3]Rang HP, Dale MM, Ritter JM Flower RJ, Henderson G. Rang and Dales Pharmacology, 7thEdn , Elsevier London, **2011**, 350-67
[4]Mathew KM. An excursion flora of Central Tamilnadu, India, New Delhi: Oxford & IBH Pub.Co., **1991**.162
[5]Saroj SV, Archana RJ, Mukta PB, and Parikshit RJ. *Ind J Pharmacol*. **2012**; Nov-Dec; 44(6): 788-91.
[6]Harbone JB. Phytochemical methods a guide to modern techniques of plant analysis, Edn. 7 Chapman and Hall, London, **1998**, 16-30
[7]Vivek D, Nimisha G, Anju MP, Bijesh Vatakkeel, Siju EN. Aiswarya AG. *Int. JToxicol. & Pharmacol. Res* **2015** 7(1) 57-9.
[8]Azeem AK, Dilip C, Prasanth SS, Junaise V, Hanan S, Sajeev K, Naseera C. *Asia Pac. J Public Health* **2010**; 3(10): 794-6
[9]Babu V, Gangadevi T, Subramonium A. *Ind J pharmacol*. **2002**; 34: 409-15.
[10]Babu V, Gangadevi T, Subramonium A. *Ind J pharmacol*. **2003**; 35:290-6.
[11]Anu SJ, Rao JM. *Phytochemistry*, **2001**; 57:583-6.
[12]Anu SJ, Rao JM. *Phytochemistry*, **2002**; 59: 425-7.
[13]Akihisa T, Yasukawa K, Oinuma H, Kasahara Y, Yamanouchi S, Takido M, Kumaki K, and Tamura T. *Phytochemistry*, Dec **1996**; 43(6): 1255-60.
[14]Garcia MD, Saenz MT, Gomez MA and Fernandez MA. Topical anti-inflammatory activity of phytosterols isolated from *Eryngium foetidium* on chronic and acute inflammation models. Feb **1999**; 13 (1): 78-80
[15]Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Saraf VO. *Fitoterapia* **1999**; 70(2): 251-7.

[16]Grant NH. Alburn HE, Kryzanauskas C. *Biochem. Pharmacol*. **1970**; 19:715-22.