Anti-inflammatory activity of the topical preparation of *Valeriana wallichii* and *Achyranthes aspera* leaves

Fazli Khuda[1]*, Zafar Iqbal[1], Ayub Khan[2], Zakiullah[1], Fazli Nasir[1] and Yasar Shah[1]
[1]Department of Pharmacy, University of Peshawar, Peshawar, Pakistan
[2]Department of Chemistry, University of Peshawar, Peshawar, Pakistan

Abstract: *In vivo* and *in vitro* screening of anti inflammatory activity of *Valeriana wallichii* and *Achyranthes aspera* leaves crude extract was performed, using standardized procedures. Methanolic crude extract topical formulation (cream) of *Valeriana wallichii* and *Achyranthes aspera* leaves (Family *Valerianaceae* and *Amaranthaceae* respectively), were screened for their anti-inflammatory activity, through "Carrageenan induced hind paw edema" test, for their effect on the acute and chronic phase inflammation models in male Wistar rats. Methanolic extract and its fractions were also evaluated for their *in vitro* anti-inflammatory activity using lipoxygenase inhibition assay. Leaves of *Valeriana wallichii* showed significant ($p<0.001$), dose dependant anti inflammatory activity, comparable with that of the standard, in animal model. The ethyl acetate fraction of *Valeriana wallichii* also showed considerable (IC 50=73±0.36) *in vitro* anti-inflammatory activity as compared to standard (6.11±0.02). Similarly *Achyranthes aspera* leaves showed relatively weak ($p>0.05$) *in vivo* anti- inflammatory activity. However, its activity was comparable with that of standard at 10% concentration after 5 hrs of carrageenan injection. This activity was present in ethyl acetate fraction during *in vitro* screening (IC 50=76±0.14) as compared to that of standard (IC 50=6.11±0.02). The combined *in vitro* and *in vivo* Anti-inflammatory screening shows that the ethyl acetate fraction of the crude extract of *Valeriana wallichii* and *Achyranthes aspera* can be used for the isolation of new Anti-inflammatory lead compounds.

Keywords: Anti-inflammatory activity, *Valeriana wallichii* and *Achyranthes aspera.*

INTRODUCTION

Inflammation is a 'protective response of vascularized tissues to injuries that leads to the local accumulation of pus and other dead cells' (Sosa *et al*., 2002). Primarily it is a defensive mechanism, but it can also induce and maintain many diseases because it involves complex events and various mediators (Yonathan *et al*., 2006; Hajhashemi *et al*., 2010). Therefore, the use of anti-inflammatory agents is helpful in the treatment of such disease. For this reason medicinal plants are widely used in folk medicine to treat various inflammatory conditions especially skin inflammations (Cuellar *et al*., 2001). Dermatological inflammatory conditions are much common but are usually transitory and less harmful.

However, some of them are chronic and can cause damage to patient's life. Conditions like atopic dermatitis, psoriasis and eczema are very harmful and affect around 2% of the world population (Gomig *et al*., 2008). Drugs are available for the treatment of these diseases either topically or systemically. Systemic therapeutics are usually aggressive and has many side effects as compared to topical preparations (Maldini *et al*., 2009; Ouedraogo *et al*., 2011). In traditional Chinese medicine *V. wallichii* and *A. aspera* extract are used for different skin infections (Khuda *et al*., 2012; Sharma, 2003; Gilani *et al*., 2005).Therefore the purpose of the present study was to validate the anti-inflammatory potentials of topically formulated *V. wallichii* and *A. aspera* leaf extracts. The data obtained can help in developing a new plant based anti-inflammatory topical formulation.

MATERIAL AND METHODS

Plant material

Leaves of *V. wallichii* and *A. aspera* were collected from Bara Gali (Hazara Division), and Charsadda (Peshawar Division), Khyber Pakhtunkhwa, Pakistan in August 2009, and were authenticated by Prof. Dr. Muhammad Ibrar of the Department of Botany, University of Peshawar, Pakistan. A voucher specimen with catalogue No: 9526 (BOT) and 8708-1 (BOT) were deposited in the herbarium of Botany Department.

Plant extraction and fractionation

Air dried, powdered leaves were extracted using methanol at room temperature for three days. After filtration the dark green extract was concentrated to dryness under vacuum at low temperature (40°C) using rotary evaporator (10), until 25 g of the crude extract was obtained. The extract was then dissolved in distilled water and sequentially partitioned with various solvents to obtain n-hexane, Chloroform, ethyl acetate, n-butanol and aqueous fractions (Khan and Gul, 2007).

Formulation of topical preparation

Cream was prepared by the following procedure (table 1).

**Corresponding author:* e-mail: fazli_khuda2008@yahoo.com

Span 80 and propyl paraben were dissolved in liquid paraffin, whereas tween 80 and methyl paraben were dissolved in water. Both the oily and aqueous phases were heated separately to 75°C and mixed thoroughly (290 rev. /min) for 30 min., using double blade mixer. The mixture was neutralized by Sodium Hydroxide (pH 6.2) and then mixed (290 rev./min) for 30 min.. The preparation was stirred until congealed at room temperature. Cream base was partitioned in to two samples, one of which represents the control and the other was functionalized with crude extract (2.5, 5 and 10% crude extract of each plant), in propylene glycol and then added to the cream base after neutralization (Calvo, 2006).

Animals

Male Wistar rats (120 -170 g each) maintained at standard environmental conditions and fed with standard food and water ad libitum, were used. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at the HEJ Research Institute, University of Karachi, Pakistan and conducted according to IACUC guidelines. The sample size of 6 animals for each test group was used in this study.

Carrageenan induced hind paw edema in rats

'Male Wistar rats' (120 - 170 g) kept at the laboratory Animal house of the HEJ Research Institute, University of Karachi, Pakistan were used. Standard environmental conditions and free access to standard diet and water were provided to the animals. Carrageenan induced rat paw oedema test was used to determine the Anti- inflammatory activity. Cream (0.2 g) of strength 2.5%, 5% & 10% of crude methanolic extract were applied with the index finger smoothly rubbing 50 times to the plantar surface of the hind paw. The control group received only cream base. Piroxicam gel (0.5%) was used as reference. Preparation containing plant extract under test were applied an hour before the carrageenan injection. Linear paw circumference was measured by water plethysmo-meter (model 7150, Ugo Basile, Italy) before, and 60, 180 and 300 min after the injection of carrageenan in to the plantar region of the right hind paw. Anti-inflammatory activity was determined as percent inhibition in edema level in comparison with reference drug (Piroxicam gel 0.5%), relative to control (Niemegeer *et al*., 1964).

In vitro anti-inflammatory activity (in vitro lipoxygenase inhibition assay)

The following assay was performed for investigation of *in vitro* anti-inflammatory activity. A mixture of sodium phosphate buffer (160 µl, 0.1 mM, pH 7.0), lipoxygenase solution (20 µl) and sample extract (10 ml) were incubated at 25°C for 5 min. linoleic acid (10 µl) substrate solution was used to initiate the reaction. The absorption changes were monitored with the formation of (9Z, 11E)-13S)-13-hydroperoxyoctadeca-9,11-dienoate. The test sample (extract) and the control (Baicalein) were dissolved in ethanol (5%) and experiment was performed in triplicate. For lipoxygenase inhibition baicalein was used as positive control. Using the EZ-Fit Enzyme Kinetics program the IC_{50} values were calculated (Lap-chak *et al*., 2007).

STATISTICAL ANALYSIS

Results are expressed as Mean±S.E.M. Statistical significance of the data was analyzed by one way analysis of variance (Anova). $P<0.001$ was considered as significant.

RESULTS

Valeriana wallichii and *Achyranthes aspera* Anti-inflammatory activity were screened by *in vitro* Lipoxygenase enzyme inhibition assay. Various fractions of the crude extract of both plants were compared for Anti-inflammatory activity with standard (Baicalein). Ethyl acetate fraction showed comparable Anti-inflammatory activity (69% and 70% inhibition for *V. wallichii* and *A. aspera*, respectively) with that of the standard (83%) (table 2).

In vivo screening for Anti-inflammatory activity of topical cream of crude extract of *V. wallichii* and *A. aspera* were also performed by comparing its activity with that of standard (Piroxicam gel) and cream base at 1, 3 and 5 hrs after the injection of carrageenan in the plantar surface of the right hind paw of rate. The activity of 2.5%, 5%, and 10% cream of *V. wallichii* was significantly different ($p <0.001$) from that of cream base, while it was comparable ($p >0.05$) with that of standard at 10% concentration after 3 and 5 hrs (Table 3).

Thus it can be concluded that the leaves of *V. wallichii* posses sustained dose dependent Anti-inflammatory activity. The activity of 2.5%, 5%, and 10% cream of *A. aspera* was significantly different ($p<0.001$) from that of cream base, while it was also significantly different from that of the standard ($p<0.001$); and comparable ($p>0.05$) with that of the standard at 10% concentration after 5 hrs only (table 4).

DISCUSSION

The Inflammatory reactions are biphasic in nature. The initial phase occurs within 2 h of the Injection of pathologic substance. It has been reported that during first phase serotonin and histamine are released while bradykinin is released 2 h after the carrageenan injection (Patil *et al*., 2011). In about 3 h the edema volume reaches its maximum and then begins to decline. Over production of prostaglandins are involved in the late phase and may continue until 5 h post carrageenan injection. The secondary phase response is reportedly affected by most

Table 1: Composition of cream (g/100g)

Formulation (%)	Oily phase					Aqueous phase		Plant Extract
	Cetylstearyl alcohol	Span 80	Propyl paraben	Liquid paraffin	Propylene glycol	Tween 80	Methyl paraben	*V. wallichii* and *A. aspera*
2.5	7	0.9	0.1	12.0	8.0	2.1	0.1	2.5
5	7	0.9	0.1	12.0	8.0	2.1	0.1	5.0
10	7	0.9	0.1	12.0	8.0	2.1	0.1	10

Table 2: Lipoxygenase inhibition activities (%) of crude extract and various fractions of *Valeriana wallichii* and *Achyranthes aspera*

Lipoxygenase Inhibition (%), IC_{50} ± SEM (µg/ml)		
Drug / Fractions	*Valeriana wallichii*	*Achyranthes aspera*
Crude extract	69 ± 0.41 (67)[a]	129 ± 0.24 (41)
Chloroform	127 ± 0.26 (41)	105 ± 0.16 (48)
n-Hexane	93 ± 0.31 (54)	89 ± 0.11 (60)
n-Butanol	69 ± 0.17 (66)	141 ± 0.27 (33)
Ethyl acetate	73 ± 0.36 (69)	76 ± 0.14 (70)
Aqueous	114 ± 0.72 (39)	194 ± 0.26 (21)
Baicalein	6.11 ± 0.02 (83)	6.11 ± 0.02 (83)

Baicalein: Standard inhibitor of α-glucosidase, [a]Each value in parentheses indicates the percentage inhibition rate

Table 3: Effect of topical administration of crude methanolic extract formulation (cream) of *Valeriana wallichii* on carrageenan induced paw edema in rats

Extract/Compound	Dose (%)	Edema rate (%) after injection		
		1h	3h	5h
Base	-	1.65 ± 0.02	1.78 ± 0.04	1.89 ± 0.03
Cream	2.5	0.98 ± 0.03[a](40.61)[b]	0.78 ± 0.05[a](48.32)	0.74 ± 0.02[a](53.44)
	5	0.84 ± 0.02[a](49.10)	0.67 ± 0.01[a](56.18)	0.60 ± 0.05[a](60.85)
	10	0.76 ± 0.03[a](53.94)	0.37 ± 0.04[a](62.36)	0.40 ± 0.04[a](68.26)
Piroxicam gel	0.5	0.48 ± 0.01[a](70.91)	0.37 ± 0.04[a](79.22)	0.40 ± 0.04[a](78.84)

Values are Mean ± SEM (n = 6), [a]$P \leq 0.001$, [b]Each value in parentheses indicates the percentage inhibition rate

Table 4: Effect of topical administration of crude methanolic extract formulation (cream) of *Achyranthes aspera* on carrageenan-induced paw edema in rats

Extract/Compound	Dose (%)	Edema rate (%) after injection		
		1h	3h	5h
Base	-	1.65 ± 0.02	1.78 ± 0.04	1.89 ± 0.03
Cream	2.5	1.10 ± 0.01[a](33.34)[b]	1.01 ± 0.03[a](43.26)	0.81 ± 0.02[a](51.33)
	5	0.98 ± 0.03[a](40.61)	0.89 ± 0.05[a](50.00)	0.66 ± 0.05[a](57.15)
	10	0.84 ± 0.02[a](49.10)	0.72 ± 0.01[a](59.56)	0.40 ± 0.04[a](65.08)
Piroxicam gel	0.5	0.48 ± 0.01[a](70.91)	0.37 ± 0.04[a](79.22)	0.40 ± 0.04[a](78.84)

Values are mean ± SEM (n = 6), [a]$P < 0.001$, [b]Each value in parentheses indicates the percentage inhibition rate

of the currently available anti-inflammatory drugs (Dharmasiri *et al*., 2003; Chattopadhyay *et al*., 2002). The crude methanolic leaf extract of *V. wallichii* and *A. aspera* showed significant anti-inflammatory effect in late stage of inflammation as compared with the standard NSAID. Regarding the *in vitro* anti-inflammatory data, ethyl acetate fraction showed considerable inhibitory activity when compared with the standard drug (Baicalein).

CONCLUSION

It can be concluded from the present data, that the leaves

of *V. wallichii and A. aspera* posses weak Anti-inflammatory activity as compared to standard at initial stages, however it shows comparable effect with that of standard at later stages of inflammation. It indicates that certain species may be there that posses specific inhibitory effect on certain late stage mediators of inflammation. However, the specific compound and its mechanism of action is yet to be determined to have some fruitful results.

REFERENCES

Calvo MI (2006). Anti-inflammatory and analgesic activity of the topical preparation of Verbena of cinalis L. *Ethnopharmacol.*, **107**: 380-382.

Chattopadhyay D, Arunachalam G, Mandal AB, Sur TK, Maandal SC and Bhattacharya SK (2002). Anti-microbial and anti-inflammatory activity of folklore: Mallotus peltatus leaf extract. *Ethnopharmacol.*, **82**: 229-237.

Cuellar MJ, Giner RM, Recio MC, Manez S and Ryos JL (2001). Topical anti inflammatory activity of some Asian medicinal plants used in dermatological disorders. *Fitoterapia,* **72**: 221-229.

Dharmasiri MG, Jayakody JRAC, Galhena G, Liyanage SSP and Ratnasooriya WD (2003). Anti inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo. Ethnopharmacol.,* **87**: 199-206.

Khuda F, Iqbal Z, Zakiullah1, Khan A, Nasir F, Muhammad N, Khan JA and Khan MS (2012). Metal analysis, phytotoxic, insecticidal and cytotoxic activities of selected medicinal plants of Khyber Pakhtunkhwa. *Pak. J. Pharm. Sci*., **25**: 51-58.

Khuda F, Iqbal Z and Khan A (2012). Pakistani Medicinal Plants-A Potential source for new lead compounds. Lambert academic publishing, p. 22.

Gilani AH, Khan AU, Jabeen Q, Subhan F and Ghafar RJ (2005**)**. Antispasmodic and blood pressure lowering effects of *Valeriana wallichii* are mediated through K^+ channel activation. *Ethnopharmacol.*, **100**: 347-352.

Gomig F, Pietrovski EF, Guedes A, Dalmarco EM, Calderari MT, Guimaraes CL, Pinheiro RM, Cabrini DA and Otuki MF (2008). Topical anti-inflammatory activity of *Serjania erecta* Radlk (Sapindaceae) extracts. *Ethnopharmacol*., **118**: 220-224.

Hajhashemi V, Ghannadi A and Hajiloo M (2010). Analgesic and anti-inflammatory effects of *Rosa damascene* hydroalcoholic extract and its essential oil in animal models. *Iranian J. Pharma Research*, **9**: 163-168.

Khan S and Gul MK (2007). *In vitro* antifungal activity of *Rhazya stricta. Pak. J. Pharm. Sci*., **20**: 274-279.

Lapchak PA, Maher P, Schubert D and Zivin JA (2007). Baicalein, an antioxidant 12/15 lipoxygenase inhibitor improves clinical rating scores following multiple infarct embolic strokes. *Neuroscience*, **150**: 585-591.

Maldini M, Sosa S, Montoro P, Giangaspero A, Balick MJ, Pizza C and Loggia RD (2009). Screening of the topical anti-inflammatory activity of the bark of *Acacia cornigera* Willdenow, *Byrsonima crassifolia* Kunth, *Sweetia panamensis* Yakovlev and the leaves of *Sphagneticola trilobata* Hitchcock. *Ethnopharmacol*., **122**: 430-433.

Niemegeer CJE Verbruggen FJ and Janssen PAJ (1964). Effect of various drugs in Carrageenan induced oedema in the rat hind paw. *Pharm. Parmacol*., **16**: 810-816.

Ouedraogo N, Tibiri A, Sawadogo RW, Lompo M, Hay AE, Koudou J, Dijoux MG and Guissou IP (2011). Antioxidant anti-inflammatory and analgesic activities of aqueous extract from stem bark of *Pterocarpus erinaceus* Poir, (Fabaceae). *Med. Plant. Research*, **5**: 2047-2053.

Patil S, Anarthe S, Jadhav R and Surana S (2011). *In vitro* antioxidant activity of indian mistletoe, the Hemiparasite *Dendrophthoe falcata* L.F (Loranthaceae). *Iranian J. Pharma. Research*, **10**: 253-259.

Sharma R (2003). Medicinal Plants of India, Daya Publishing House, p.5.

Sosa S, Balick MJ, Arvigo R, Esposito RG, Pizza C, Altinier G and Tubaro A (2002). Screening of the topical anti-inflammatory activity of some Central American plants. *Ethnopharmacol*., **81**: 211-215.

Yonathan M, Asres K, Assefa A and Bucar F (2006). *In vivo* anti-inflammatory and anti-nociceptive activities of *Cheilanthes farinose. Ethnopharmacol*., **108**: 462-470.