Evaluation of anti-inflammatory activity of selected medicinal plants of Khyber Pakhtunkhwa, Pakistan

Fazli Khuda[1]*, Zafar Iqbal[1], Ayub Khan[2], Zakiullah[1], Yasar Shah[1], Lateef Ahmad[1], Fazli Nasir[1], Muhammad Hassan[3], Ismail[1] and Waheed Ali Shah[1]

[1]Department of Pharmacy, University of Peshawar, Peshawar, Pakistan
[2]Department of Chemistry, University of Peshawar, Peshawar, Pakistan
[3]Gandhara College of Pharmacy, Gandhara University, Peshawar

Abstract: In present study, the anti-inflammatory potential of three medicinal plants, *Xanthium strumarium, Achyranthes aspera* and *Duchesnea indica* were evaluated, using both *in vitro* and *in vivo* assays. Carrageenan induced hind paw edema model was used to carry out the *in vivo* anti-inflammatory activity, while for *in vitro* screening lipoxygenase inhibition assay was used. Crude extract of all the selected plants depicted significant ($p \leq 0.001$) anti-inflammatory activity, at late phase of inflammation. *Achyranthes aspera* also showed considerable anti-inflammatory activity (47%) at relatively lower concentration (200 mg/ml), at the initial phase of inflammation. Similarly the ethyl acetate fraction of all the selected plants showed significant lipoxygenase inhibition activity when compared with the standard drug (Baicalein). The results obtained from both *in vitro* and *in vivo* anti-inflammatory activity suggest that the ethyl acetate fraction of the crude extract of all the selected plants can be used for the isolation of new lead compounds with better anti-inflammatory activity.

Keywords: Anti-inflammatory activity, *Xanthium strumarium, Achyranthes aspera, Duchesnea indica.*

INTRODUCTION

Inflammation is a protective response of the body towards various injurious stimuli like infections and trauma (Vijayalakshmi *et al*., 2011; Yonathan *et al*., 2006). At the same time it is accompanied with pain, redness, swelling and malfunctioning of the affected part of the body (Amira *et al*., 2012). Inflammation is accompanied by the release of various chemical mediators that are responsible for signs and symptoms associated with such conditions. To alleviate the pain and other associated symptoms various anti-inflammatory agents are used, most of which are synthetic drugs, associated with various side effects such as peptic ulcer and bleeding etc. (Dharmasiri *et al*., 2003; Bepary *et al*., 2008). Based on ethno-pharmacological uses, many medicinal plants have attracted considerable interest, particularly in the treatment of various medical conditions including chronic inflammatory diseases (Moro *et al*., 2012; Yu-Cui *et al*., 2011). The screening of these medicinal plants for lead anti-inflammatory compounds may guide to the discovery of more safer and effective compounds.

Xanthium strumarium (Compositae) is a common weed found in India and Pakistan (Fazli *et al*., 2012). It has been used for various inflammatory conditions like arthritis, urticaria, sinusitis and headache (Han *et al*., 2007; Qin *et al*., 2006; Yoon *et al*., 2003). *Achyranthes aspera* (Amaranthaceae) is another herb found in the same region and is reportedly used in various inflammatory conditions in Ayurveda medicine. (Paul *et al*., 2006; Chakraborty *et al*., 2002; Rao *et al.,* 2006; Gokhale *et al*., 2002). Similarly *Duchesnea indica* (Rosaceae), a perennial herb, commonly occur on shady, grassy slopes (up to 2400 meters) in Pakistan, India and China (Qiao *et al.,* 2009), has also been used for the same conditions (Lee *et al.,* 2008; Zuoa *et al.,* 2008; Peng *et al.,* 2008).

Based on the above-mentioned facts the stated plants were therefore screened for anti-inflammatory activity using both *in vivo* and *in vitro* models.

MATERIAL AND METHODS

Plant material

Leaves of *Xanthium strumarium* and *Achyranthes aspera* were collected from Charsadda (Peshawar Division), while roots of *Duchesnea indica* were collected from 'Bara Gali (Hazara Division), Khyber Pakhtunkhwa, Pakistan'. Plant material was identified by 'Prof. Dr. Muhammad Ibrar of the Department of Botany, University of Peshawar, Pakistan'. Voucher specimens bearing catalogue No: 8708 (BOT), 8708-1 (BOT) and10708 (BOT) were deposited in the herbarium of the same Department for *Xanthium strumarium, Achyranthes aspera* and *Duchesnea indica*, respectively.

Plant extraction and fractionation

Shade dried leaves and roots of the mentioned plants were powdered and separately extracted using methanol as extraction solvent. The plant extracts were then filtered and dried under vacuum. Dried extracts were dissolved in

**Corresponding author:* e-mail: fazlikhuda2012@upesh.edu.pk

distilled water and successively partitioned with different solvents to obtain chloroform, ethyl acetate, *n*-hexane, *n*-butanol and aqueous fractions (Fazli *et al*., 2012).

Animals

Male Wistar rats (120-170g each) obtained from the Laboratory Animal House, HEJ Research Institute, University of Karachi, Pakistan were used in the assay. The animals were kept in a well-ventilated environment and had free access to food and water ad libitum. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at the HEJ Research Institute and conducted according to IACUC guidelines. The sample size of 6 animals for each test group was used in this study.

Anti-inflammatory models used

Rate paw edema

The anti-inflammatory activity of the test compound was investigated using the carrageenan induced hind paw edema model in rats employing 1.0% carrageenan solution as the phlogistic agent (Winter *et al*., 1962). The test compounds were injected intra peritoneally, 30 min before the injection of 0.1 ml carrageenan (1% *w/v* in normal saline), at dose level of 100, 200 and 400 mg/kg body weight. Diclofenac sodium was used as a standard at a dose level of 5 mg/kg body weight. Dimethylsulphoxide (DMSO, 0.4%) served as a control. The volume of the paw edema was measured by water plethysmometer (model 7150, Ugo Basile, Italy) before, and 1.0, 3.0 and 5.0 h after the injection of carrageenan. The results are summarized in table 2.

In vitro anti-inflammatory activity

A mixture of lipoxygenase solution (20 µl), sodium phosphate buffer (160 µl, 0.1 mM, pH 7.0), along with sample extract (10 ml) were incubated at 25ºC for 5 min and the reaction was initiated using linoleic acid (10 µl) substrate solution. The formation of '(9Z, 11E)-13S)-13-hydroperoxyoctadeca-9, 11-dienoate' was monitored by

Table 1: Lipoxygenase inhibition activities (%) of crude extract and various fractions of *Xanthium strumarium, Achyranthes aspera* and *Duchesnea indica*

Lipoxygenase Inhibition (%), IC_{50} ± SEM			
Drug/Fractions(µg/ml)	*Xanthium strumarium*	*Achyranthes aspera*	*Duchesnea indica*
Crude extract	87 ± 0.27 (59)[a]	129 ± 0.24 (41)	59 ± 0.19 (73)
Chloroform	109 ± 0.34 (44)	105 ± 0.16 (48)	97 ± 0.14 (52)
n-Hexane	134 ± 0.18 (37)	89 ± 0.11 (60)	108 ± 0.26 (43)
n-Butanol	76 ± 0.41 (61)	141 ± 0.27 (33)	138 ± 0.34 (31)
Ethyl acetate	81 ± 0.16 (63)	76 ± 0.14 (70)	44 ± 0.26 (75)
Aqueous	239 ± 0.17 (31)	194 ± 0.26 (21)	112 ± 0.28 (39)
Baicalein	6.11 ± 0.02 (83)		

Baicalein: Standard inhibitor of lipoxygenase. [a]Each value in parentheses indicates the percentage inhibition rate.

Table 2: The effects (Mean ± SEM) of methanolic extracts of *Xanthium strumarium*, *Achyranthes aspera* and *Duchesnea indica* against carrageenan-induced paw edema (ml) in rats

Extract/Compound	Dose (mg/kg)	Edema rate (%) after injection		
		1h	3h	5h
Xanthium strumarium				
Crude extract	100	0.28 ± 0.05[ns] (22)[d]	0.26 ± 0.03[b] (49)	0.24 ± 0.04[b] (59)
	200	0.23 ± 0.05[ns] (36)	0.21 ± 0.04[b] (56)	0.20 ± 0.09[b] (66)
	400	0.20 ± 0.07[ns] (44)	0.19 ± 0.01[b] (62)	0.18 ± 0.03[b] (69)
Achyranthes aspera				
	100	0.25 ± 0.07[ns] (30)	0.24 ± 0.05[a] (52)	0.24 ± 0.03[b] (56)
	200	0.19 ± 0.09[ns] (47)	0.21 ± 0.04[a] (58)	0.20 ± 0.08[b] (63)
	300	0.17 ± 0.04[ns] (52)	0.19 ± 0.06[a] (62)	0.18 ± 0.06[b] (67)
Duchesnea indica				
	100	0.32 ± 0.04[ns] (11)	0.31 ± 0.08[c] (39)	0.30 ± 0.04[b] (49)
	200	0.27 ± 0.05[ns] (25)	0.26 ± 0.04[c] (49)	0.25 ± 0.09[b] (57)
	400	0.20 ± 0.08[ns] (44)	0.20 ± 0.06[a] (60)	0.19 ± 0.03[b] (67)
Diclofenac	5.0	0.16 ± 0.03[c] (55)	0.17 ± 0.04[a] (66)	0.23 ± 0.02[b] (61)
Control	-	0.36 ± 0.01	0.51 ± 0.08	0.59 ± 0.01

Values are Mean ± SEM (n=6), [a]$p \leq 0.01$, [b]$p \leq 0.001$, [c]$p \leq 0.05$.
[d]Each value in parentheses indicates the percentage inhibition rate; [ns]Not significant

observing changes in absorption. For *in vitro* lipoxygenase inhibition assay, baicalein was used as a standard. IC_{50} values were calculated using the 'EZ-Fit Enzyme Kinetics program' (Lapchak *et al*., 2007). The results of the *in vitro* assay are presented in table 1.

STATISTICAL ANALYSIS

The data obtained was expressed as Mean ± S.E.M. Analysis of variance (ANOVA) was performed to determine statistical significance. $P<0.001$ was considered as significant.

RESULTS

The results of *in vitro* anti-inflammatory activity of both crude extract and its different fractions, of the selected plants in comparison to Baicalein (standard drug) are presented in table 1. Among the crude extracts tested, *Duchesnea indica* displayed highest inhibition (73%), as compared to that of standard (83%). Regarding different fractions, ethyl acetate fraction of all the tested plants showed highest lipoxygenase inhibition (63%, 70% and 75% for *Xanthium strumarium, Achyranthes aspera* and *Duchesnea indica*, respectively) when compared with standard. The *n*- butanol fraction of *Xanthium strumarium* also showed significant activity (61%).

Results of the *in vivo* anti inflammatory activity of crude extracts, administered at dose of 100, 200 and 400 mg/kg are reported in table 2. At 1h post-carrageenan, no significant anti-inflammatory activity was observed at any doses of crude extracts except *Achyranthes aspera* which showed considerable activity (47%) at relatively lower concentration (200 mg/ml). At 3 and 5h post-carrageenan, all of the doses of crude extracts depicted significant ($p \leq 0.001$) anti-inflammatory activity as compared to the standard drug

DISCUSSION

For the investigation of anti-inflammatory activity of the selected plants, the commonly used *in vivo* model, the carrageenan-induced hind paw edema model, was used (Winter *et al.,* 1962).

It has been reported that inflammation occurs in two phases. The first phase begins immediately after the injection of carrageenan and diminishes after 1 h. This phase of inflammation is accompanied by the release of serotonin and histamine while the second phase begins at the end of first phase and persisted for at least 5 h. This phase is mediated by several agents e.g., bradykinin, prostaglandin and lysosome (Vijayalakshmi *et al*., 2011). The later phase of inflammation is reportedly, sensitive to most of the currently available drugs (NSAIDs). No doses of the crude extracts of all the selected plants showed significant anti-inflammatory effect at I h, and significant edema inhibitory response started at 3 and 5 h after following carrageenan injection as diclofenac, a well known cyclooxygenase inhibitor. It is well known that both the cyclooxygenase and lipoxygenase pathways are involved in the inflammatory process however, cyclooxygenase inhibitors are more effective in inhibiting carrageenan-induced inflammation (Nurcan *et al*., 2012).

Cyclooxygenase pathway is involved in the release of several mediators particularly prostaglandins, bradykinin and lysosomes thereby, the edema inhibition by crude extracts at effective doses may be due to the inhibition of these mediators. It has been reported that leaves and roots of the mentioned plants contain biologically active compounds such as glycosides, flavonoids, alkaloids and tannins (Sharma, 2003; Han *et al*., 2007; Qiao *et al.,* 2009). In addition, another report has suggested the involvement of these compounds in anti-inflammatory activities (Nurcan *et al*., 2012). Similarly, the use of these plants in alleviating inflammatory disorders has been mentioned in ayurvedic medicine (Sharma, 2003). On the basis of these reports it is possible to speculate that these compounds might be responsible for the observed anti-inflammatory activities of *Xanthium strumarium, Achyranthes aspera* and *Duchesnea indica.* The results from the *in vivo* anti-inflammatory activity are further supported by the *in vitro* lipoxygenase inhibitory activity, which reveal that the ethyl acetate fractions of all the plants posses strong anti-inflammatory activity as compared to standard drug (Baicalein).

CONCLUSION

The present study investigated the anti-inflammatory activities of crude extracts of the leaves and roots of *Xanthium strumarium, Achyranthes aspera* and *Duchesnea indica,* respectively. It was concluded that the crude extract of all selected plants posses significant anti-inflammatory effect at later phase of inflammation and one of the action mechanism may be the inhibition of prostaglandin synthesis. The ethyl acetate fraction exhibit more potent anti-inflammatory activity so we believe that this fraction can be used for the activity guided isolation of the specific compound (s) responsible for anti-inflammatory activity. However, the mechanism (s) of anti-inflammatory activities remains unclear and need to further investigation.

REFERENCES

Amira S, Dad M, Schinell G and Jose-Luis R (2012). Anti-inflammatory, anti-oxidant and apoptotic activities of four plant species used in folk medicine in the Mediterranean basin. *Pak. J. Pharm. Sci*., **25**: 65-72.

Bepary S, Biplab KD, Sitesh CB, Joydev KK, Abu SSR and Bidyut KD (2008). Anti-inflammatory activity of indanyltetrazole derivatives. *Pak. J. Pharm. Sci*., **21**: 295-298.

Chakrabortya A, Brantnera A, Mukainakab T, Nobukunib

Y, Kuchideb M, Konoshimac T, Tokudab H and Nishino H (2002). Cancer chemopreventive activity of Achyranthes aspera leaves on Epstein Barr virus activation and two-stage mouse skin Carcinogenesis. *Cancer Lett*., **177**: 1-5.

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.

Gokhale AB, Damre AS, Kulkarni KR and Saraf MN (2002). Preliminary evaluation of anti inflammatory and anti-arthritic activity of *S. lappa, A. speciosa* and *A. aspera*. *Phytomedicine*., **9**: 433-437.

Han T, Li HL, Zhang QY, Haa P, Zheng HC, Rahman K and Qin LP (2007). Bioactivity guided fractionation for anti-inflammatory and analgesic properties and constituents of *Xanthium strumarium* L. *Phytomed*., **14**: 825-829.

Fazli K, Iqbal Z and Khan A (2012). Pakistani Medicinal Plants-A potential source for new lead compounds. Lambert Academic Publishing, p.19.

Fazli K, Iqbal Z, Zakiullah, Khan A and Nasir F (2012). Antimicrobial and anti-inflammatory activities of leaf extract of *Valeriana wallichii* DC. *Pak. J. Pharm. Sci*., **25**: 715-719.

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

Lee S, Xiao C and Pei S (2008). Ethnobotanical survey of medicinal plants at periodic markets of Honghe Prefecture in Yunnan Province, SW China. *Ethnopharmacol*., **117**: 362-377.

Moro C, Irene P, Miguel L, Matilde DA, Eva G, Ana V, Jose AM and Garcia-Lafuente A (2012). Anti-inflammatory activity of methanolic extracts from edible mushrooms in LPS activated RAW 264.7 macrophages. *Food Chem*., **130**: 350-355.

Nurcan B, Rana A, Fatih G, Nese K and Yusuf O (2012). Investigation for anti-inflammatory and anti-thromboticactivities of methanol extract of *Capparis ovata* buds and fruits. *Ethnopharmacol*., **142**: 48-52.

Paul D, Bera S, Jana D, Maiti R and Ghosh TD (2006). *In vitro* determination of the contraceptive spermicidal activity of a composite extract of *Achyranthes aspera* and *Stephania hernandifolia* on human semen. *Contraception*., **73**: 284-288.

Peng B, Chang Q, Wang L, Hu Q, Wang Y, Tang J and Liu X (2008). Suppression of human ovarian SKOV-3 cancer cell growth by Duchesnea phenolic fraction is associated with cell cycle arrest and poptosis. *Gynecologic Oncology*, **108**: 173-181.

Qiao W, Yao Z, Zhang W and Duan HQ (2009). Two new triterpenes from *Duchesnea indica*. *Chinese Chem. Lett*., **20**: 572-575.

Qin L, Han T, Li H, Zhang Q and Zheng H (2006). A new thiazinedione from *Xanthium strumarium*. *Fitoterapia*., **77**: 245-246.

Rao YV, Das BK, Jyotyrmayee P and Chakrabarti R (2006). Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila. Fish Shell Fish Immun*., **20**: 263-273.

Sharma R (2003). Medicinal Plants of India. Daya Publishing House, Dehli, India, pp.5-6.

Vijayalakshmi A, Ravichandiran V, Velraj M, Hemalatha S, Sudharani G and Jayakumari S (2011). Anti-anaphylactic and anti-inflammatory activities of a bioactive alkaloid from the root bark of *Plumeria acutifolia* Poir. *Asian Pac. J. Trop Biomed*., **10**: 401-405.

Winter CA, Risley EA and Nuss GW (1962). Carrageenin-induced edema in hind paw of the rat as assay for anti-inflammatory drug. *Proc. Soc. for Exp. Biol. Med*., **111**: 544-554.

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.

Yoon JH, Lim HJ, Lee HJ, Kim HD, Jeon R and Ryu JH (2003). Inhibition of lipopolysaccharide-induced inducible nitric oxide synthase and cyclooxygenase-2 expression by xanthanolides isolated from *Xanthium strumarium*. *Bioorg. Med. Chem. Lett*., **18**: 2179-2182.

Yu-Cui L, Yan-Fang X, Siu-Po I, Zi-Ren S, Ji-Yan S, Jing-Jin H, Qing-Feng X, Xiao-Ping L and Zhi-Xiu L (2011). Anti-inflammatory activity of patchouli alcohol isolated from *Pogostemonis Herba* in animal models. *Fitoterapia*., **82**: 1295-1301.

Zuoa GY, Wanga GC, Zhaoa YB, Xua GL, Haob XY, Hanc J and Zhaoc Q (2008). Screening of Chinese medicinal plants for inhibition against clinical isolates of methicillin resistant *Staphylococcus aureus* (MRSA). *Ethnopharmacol*., **120**: 287-290.