Physical Education, Health and Social Sciences

https://journal-of-social-education.org E-ISSN: 2958-5996

P-ISSN: 2958-5988

Investigation of Anti-Inflammatory Potential of Tamaricaria Elegans Royle with Evidence Based Mechanism of Action

Salimullah Khan[1*], Salar Muhammad[1], Mian Inaam Zeb[2], Zia-ul Islam[3], Raham Sher Khan[3], Fawad Ahmad[1], Izhar Ahmad[1]

[1] Department of Pharmacy, Abdul Wali Khan University, Mardan, Pakistan
[2] Department of Pharmacy, Bacha Khan University, Charsadda
[3] Department of Biotechnology, Abdul Wali Khan University Mardan
***Corresponding author:** Salimullah Khan, Department of Pharmacy, Abdul Wali Khan University Mardan, Pakistan. E-mail: drsalimkhan@awkum.edu.pk

DOI: https://doi.org/10.63163/jpehss.v3i2.691

Abstract

This study is focused to investigate macronutrients and in-vivo anti-inflammatory activity of Tamaricaria Tamaricaria Elegans Royle (Tamaricaceae) using rat paw edema model. Shade dried plant was exhaustively extracted by maceration with methanol (80%) to get crude methanol extract (a), followed by fractionation with different organic solvents to get pet-ether (b), chloroform (c), ethyl acetate (d) and butanol (e) fractions. Fraction (c) was found to be the most potent inhibitor of carragenin induced edema with 67.65% inhibition during 1 h at a dose of 100 mg/kg followed by 66.67% and 62.50% at 3 and 5 h, respectively. Like aspirin -a COX inhibitor, the plant has also demonstrated sustained inhibition of edema both at early and later phase of the experiment. Such aspirin like sustained effect is more likely due to COX inhibition, which may be attributed to triterpenes content of the plant.

Key words: Tamaricaria, Edema, Inflammation, Aspirin, COX

Introduction

Upon injury whether caused by trauma, heat, chemicals, bacteria or any other phenomenon, the injured tissues release multiple substances and cause dramatic successive changes in the surrounding uninjured tissues. This entire complex of tissue change is called inflammation. Vasodilatation of blood vessel at local site, increased permeability of the capillaries, often associated with clotting of the fluid in interstitial space, migration in greater number of granulocytes and monocytes to the tissues and swelling of the tissue cells are the common outcomes of inflammation (Guyton and Hall, 2006). The outcome of the immune response may be beneficial for the host by neutralizing the invading organisms, but it may be deleterious if it leads to chronic inflammation, which may cause shortening of life (Wagner et al., 2004). Keeping in view the importance of natural products for human health care and folkloric use of Tamaricaria Elegans Royle (Tamaricaceae) the plant was selected for anti-inflammatory study. T. Tamaricaria Elegans is classified under shrub having reddish brown to black brown bark growing to about 4 m height with straight slender stem (Hooker, 1982). It grows in stony slopes at an altitude of about 3000 – 4300 m (Daoyuan et al., 2006) and is found in East Asia, West Himalaya and Tibet (Kirtikar and Basu, 1981). In Pakistan, the plant has been reported from Chitral, Gilgit, Swat, Kashmir,

Baltistan and other northern areas connected to China and India (Qaiser, 1982). Generally used as firewood and fodder (Hussain et al., 2007), leaves are applied in India as poultice to bruises (Chopra et al., 1956) and the twigs are browsed by goats and sheep in Ladakh (Watt, 1972). The plant has been reported to possess some antibacterial and antinociceptive activities (Ahmad et al., 2008; Khan et al., 2010).

Material And Methods

Plant material

Fresh plant was collected in July 2002 from the bank of River Swat in Swat hills of Pakistan and the species was identified by Mehboobur Rahman, Department of Botany, Jehanzeb College Saidu Sharif, Swat. A voucher specimen (EM-004) was properly deposited with the Botany Department of the same college for future reference.

Extraction and isolation

Aerial parts of the plant (5 kg) were shade dried, powdered and exhaustively extracted with 80% MeOH by maceration at r.t. The combined extract was dried in vacuo at ambient temperature to afford 560 g of crude MeOH extract (a). After removing a quantity, the rest of the extract (482.7 g) was subjected to fractionation with different organic solvents to obtain pet. ether fraction (b) 55.2 g, chloroform fraction (c) 17.3 g, ethyl acetate fraction (d) 8.11 g and butanol (e) 52.5 g. The presence of various groups of organic compounds was assessed through gross phytochemical screening using reported methods (Nayak et al., 2007).

Animals. Male rats of Sprauge-Dawley types weighing 100-120 g were used for the study. All animals were kept in a place maintained under environmentally controlled conditions of 24 ± 1 ºC and 12 h light-12 h dark cycle. Free access to both water and food was provided. All experiments performed during the study complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences (National Research Council, 1986).

Carragenin-induced Rat Paw Edema

To test the crude and sub-crude fractions on acute inflammation, the method described by Winter et al., (1962) was employed. A freshly prepared carragenin suspension 0.1 ml (1% in normal saline) was injected under the sub-plantar region in the right hind paw of the rats. Paw thickness as function of edema volume, was determined prior to and 1, 3 and 5 h after carragenin administration. Test samples were prepared by suspending the sample in normal saline, given orally 1 h prior to carragenin injection in 50, 75 and 100 mg/kg doses. Negative control received vehicle only, while positive control was given aspirin as standard in a dose of 100 mg/kg body weight.

Toxicity study

LD_{50} values were determined for crude extract and all sub-crude fractions using reported method (Nayak et al., 2004).

Statistical Analysis

The inhibition activity of orally administered samples on carragenin-induced rat paw edema was determined and reported as mean ± S.E.M. Statistical significance was deduced using the Student's t-test (Olajide et al., 2004), values with $p< 0.01$ and $p< 0.001$ were considered significantly different from control.

Results

Gross phytochemical screening of crude extract gave strongly positive test for triterpenes. The inhibitory effect on carragenin-induced rat paw edema caused by the oral administration of standard, crude extract and various fractions of Tamaricaria Elegans at 1, 3 and 5 h after carragenin injection is presented in Table 1, where the effect of increasing dose of all samples on edema formation is shown in Figure 1. Aspirin a known cyclooxygenase inhibitor exhibited excellent edema inhibition in a dose of 100 mg/kg at all observed durations. Among the samples fraction (c) was found to be the most potent inhibitor (67.65%) of edema formation in a dose of 100 mg/kg during 1 h followed by 66.67% and 62.50% inhibition in 3 and 5 h, respectively. Other doses of the same fraction also showed good inhibitory activity. Fraction (d) was also found potent enough to inhibit inflammatory response effectively in all doses used. The same fraction showed maximum inhibition (61.76%) of edema during 1 h at a dose of 100 mg/kg. Crude extract (a) also showed good inhibitory activity on carragenin induced edema in higher dose used. Other fractions of the plant showed poor or no effect on post carragenin edema formation. LD_{50} was found to be greater than 3.0 g/kg orally for all samples tested.

Table I - Effect of Crude Extract and Different Fractions of Tamaricaria Tamaricaria Elegans on Edema

Group	Dose (mg/kg)	Paw volume increase (ml)		
		1h	3h	5h
Control	-	0.34 ± 0.03	0.66 ± 0.03	0.72 ± 0.04
Aspirin	100	0.11 ± 0.04***	0.20 ± 0.06***	0.26 ± 0.04***
(a)	50	0.28 ± 0.03	0.42 ± 0.04***	0.51 ± 0.04**
	75	0.22 ± 0.04***	0.33 ± 0.06***	0.46 ± 0.04***
	100	0.19 ± 0.04**	0.28 ± 0.03***	0.39 ± 0.05***
(b)	50	0.30 ± 0.06	0.62 ± 0.05	0.68 ± 0.12
	75	0.28 ± 0.07	0.60 ± 0.11	0.71 ± 0.05
	100	0.25 ± 0.05	0.60 ± 0.04	0.69 ± 0.10
(c)	50	0.15 ± 0.04***	0.37 ± 0.02***	0.39 ± 0.04***
	75	0.14 ± 0.03***	0.29 ± 0.02***	0.33 ± 0.03***
	100	0.11 ± 0.03***	0.22 ± 0.03***	0.27 ± 0.03***
(d)	50	0.17 ± 0.04**	0.49 ± 0.04**	0.52 ± 0.03***
	75	0.15 ± 0.06**	0.40 ± 0.03***	0.47 ± 0.04***
	100	0.13 ± 0.03***	0.32 ± 0.02***	0.36 ± 0.04***
(e)	50	0.33 ± 0.03	0.68 ± 0.04	0.73 ± 0.04
	75	0.34 ± 0.04	0.65 ± 0.07	0.73 ± 0.05
	100	0.32 ± 0.03	0.66 ± 0.04	0.75 ± 0.04

Values are mean ± SEM (n=6)

Significantly different from control group represents in **$p<0.01$, ***$p<0.001$

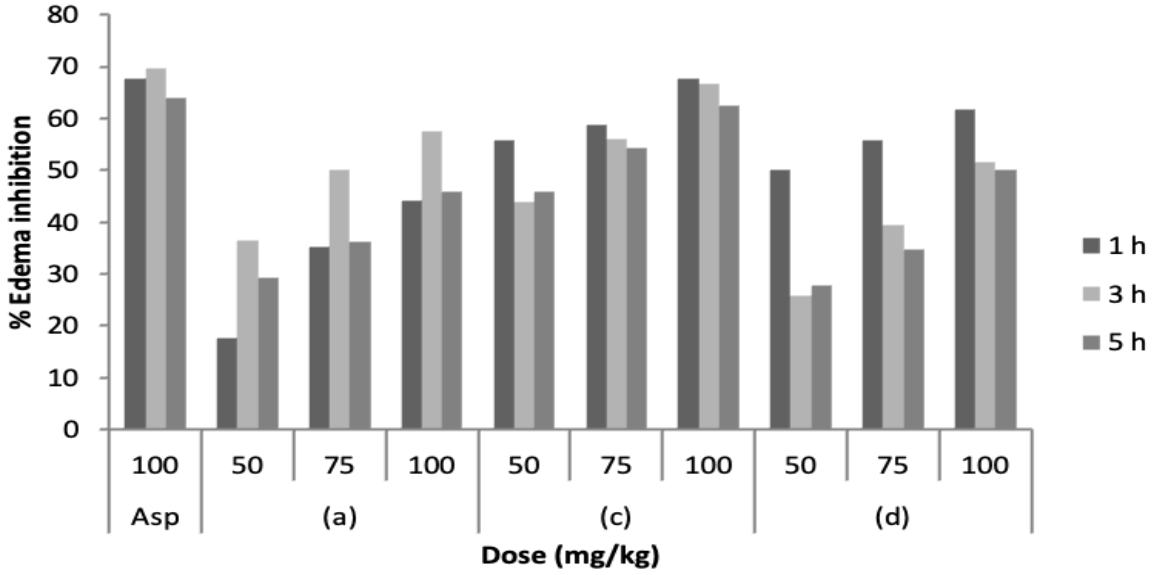

FIGURE 1 -Anti-inflammatory effect of increasing dose of active fractions of T. Tamaricaria Elegans

Discussion

Carragenin-induced rat paw edema is provoked by an early event involving the release of histamine and serotonin in high concentration (Vinegar et al., 1969). The late phase of the inflammatory process is characterized by the presence of bradykinin, prostaglandins (PGI_2) and maximal edema volume (Di Rosa et al., 1971; Gilman, 1985). All these mediators of inflammation and pain are the end product of arachidonic acid metabolism which is induced in response to tissue injury associated with trauma, bacteria, chemicals or any other stimuli. A number of triterpenes are well documented anti-inflammatory agents (Karachurina et al.,2002; Baricevic et al., 2001; Puerta et al., 2000). Similarly crude plant extracts with terpenes and terpenoids like compounds have also been reported to exhibit significant anti-inflammatory activities (Rahman et al., 2010; Okokon and Paul, 2010). Most anti-inflammatory triterpenes have been reported to inhibit synthesis of prostaglandins (PGs) and different mediators of inflammation by interfering with Cyclooxygenase (COX) pathway of arachidonic acid metabolism (Zhang et al., 2005).

Fraction (c) has exerted a potential inhibitory effect on edema formation during the 1 h and the same was maintained till end of the experiment comparable to aspirin.

Presence of triterpenes demonstrated by strongly positive test and the sustained nature of observed inhibitory effect on carragenin induced edema suggested inhibition of different mediators of inflammation. Since the plant has also been reported to exhibit antinociceptive property associated with inhibition of PGs synthesis, it may be suggested that Tamaricaria Eleganse interfere with arachidonic acid metabolism more likely due to COX inhibition.

Conclusion

On the basis of this study and already reported antinociceptive properties of the plant it may be concluded that Tamaricaria Eleganse inhibit the synthesis of different mediators of inflammation and pain by interfering with arachidonic acid metabolism more likely due to COX inhibition.

References

1. Ahmad M, Ahmad W, Khan S, Zeeshan M, Obaidullah, Nisar M, Shaheen F, Ahmad M (2008). New antibacterial pentacyclic triterpenes from Tamaricaria Tamaricaria Elegans Royle. Enz. Inhib. Med. Chem., 23(6): 1023-1 027.
2. Baricevic D, Sosa S, Loggia RD, Tubaro A, Simonovska B, Krasna A, Zupancic AA (2001). Topical anti-inflammatory activity of Salvia officinalis L. leaves: the relevance of ursolic acid. Ethnopharmacol., 75(2-3) 125-132.
3. Chopra RN, Nayar SL, Chopra IC (1956) Glossary of Indian Medicinal Plants. Council of Scientific and Industrial Research. New Delhi, India, p.170.
4. Daoyuan Z, Yuan Z, Gaskin JF, Zhiduan C (2006). Systematic position of Myrtama Ovcz & Kinz based on morphological and nrDNA ITS sequence evidence. Chin. Sci. Bull., 51(A01): 117-123.
5. Di Rosa M, Papadimitrion JP, Willoughby DA (1971). Study of the mediators of the inflammatory response induced in rats in different sites by carrageenin and turpentine. Pathol., 104(1): 15-29.
6. Gilman AG (1985). The Pharmacological Basic of Therapeutics. Flower BA, Moncada S, Vane JR. ed. Macmillan Publishing, New York, p. 674.
7. Guyton C, Hall JE (2006). A Text Book of Medical Physiology. 11th edn. Elsevier Saunders, Philadelphia, p. 429.
8. Hooker JD (1982). Flora of British India VI. Bishen Singh Mahendra Pal Singh, New Connaught Dehra Dhun, p. 249.
9. Hussain F, Shah SM, Sher H (2007). Traditional resource evaluation of some plants of Mastuj, district Chitral Pakistan. Pak. J. Bot., 39(2): 339-354.
10. Karachurina LT, sapozhnikova TA, Zarudii F, Flekhter OB, Galin FZ (2002). Anti-inflammatory and antiulcer properties of betulin bis-hemiphthalate. Pharm. Chem., 36: 432-433.
11. Khan S, Nisar M, Khan R, Ahmad W, Nasir F (2010). Evaluation of chemical constituents and antinociceptive properties of Tamaricaria Tamaricaria Elegans Royle. Chem. Biodiv., 7(12): 2897-2900.
12. Kirtikar ID, Basu BD (1981). Indian medicinal plants. Sudhindra Nath Basu Bahadur Gunj, Allahabad, p.140.
13. National Research Council (1986). Guide for the Care and use of laboratory animals. National Academic Press, Washington DC, p.1.
14. Nayak BS, Isitor G, Davis EM, Pillai GK (2007). The evidence based wound healing activity of Lawsonia inermis Linn. Phytother. Res., 21(9): 827-831.
15. Nayak SS, Ghosh AK, Debnath B, Vishnoi SP, Jha T (2004). Synergistic effect of Methanol extract of *Abies webbiana* leaves on sleeping time induced by standard sedatives in mice and anti-inflammatory activity of extracts in rats. Ethnopharmacol., 93(2-3): 397-402.
16. Okoko JE, Paul A (2010). Anti-inflammatory, analgesic and antipyretic activities of ethanolic root extract of Croton zambesicus. Pak. J. Pharm. Sci. 23(4): 385-392.
17. Olajide OA, Kolawole OT, Fagbohun TR, Ajayi FF (2004). Evaluation of the anti-inflammatory properties of Chlorophora excelsa stem bark extract. Pharm. Biol., 43(9): 746-748.
18. Puerta DLR, Dominguez EM, Gutierrez RV (2000). Effect of minor components of virgin olive oil on topical anti-inflammatory assays. Z. Naturforsch C., 55(9-10): 814-819.
19. Qaiser M (1982). Flora of Pakistan 141, Shamim Printing Press, Lahore, p. 43.

20. Rahman MA, Bachar SC, Rahmatullah M (2010). Analgesic and anti-inflammatory activity of methanolic extract of Acalypha indica Linn. Pak. J. Pharm. Sci. 23(3): 256-258.
21. Vinegar R, Schreiber W, Hugo R (1969). Biphasic development of carrageenin-edema in rats. Pharmacol. Exp. Ther. 166(1): 96-103.
22. Wagner W, Khanna P, Furst DE (2004). Basic and Clinical Pharmacology. Katzungb G, ed. McGraw-Hill Companies, Singapore, p. 576.
23. Watt G (1972). The Dictionary of Economic Products of India V(1). Cosmo Publications, New Delhi, p. 310.
24. Winter CA, Risley EA, Nuss CW (1962). Carragenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med., 111: 544-547.
25. Zhang Y, Dewitt DL, Murugesan S, Nair MG (2005). Cyclooxygenase-2 enzyme inhibitory triterpenoids from Picrorhiza kurroa seeds. Life. Sci. 77(25): 3222-3230.