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Research Article

In vivo anti-inflammatory characterization of crude extract and solvent fractions of *Viola serpens*

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Abstract

The aim of this research activity was the *in vivo* evaluation of anti-inflammatory effect and acute toxicity of the crude methanol extract of *Viola serpens* and its various fractions. For acute toxicity test a dose of 2 g/Kg was found to be safe. The Wistar albino rats were used for the models of Carrageenan-induced paw edema (CIPE), histamine induced paw edema (HIPE) and xylene induced ear edema (XIEE). In CIPE and HIPE models all the fractions and the crude extract mostly showed more significant responses in all the three test doses from the 2nd till the 5th hour. These effects may be the results of certain compounds which may resists or inhibit the histamine, prostaglandins or mediators of the mast cells. Similarly, in model of XIEE both the crude extract and *n*-hexane fraction showed significant inhibition ($P < 0.05$) in a dose dependent way. Whereas, the other tested fractions showed topically subsided effect at 300 mg/kg, suggesting that fractions act by inhibiting the enzyme phospholipase A2 (PLA2).

Keywords: Anti-inflammatory; Acute toxicity; Carrageenan; Histamine and xylene

Introduction

Viola serpens Wall (*V. serpens)* belongs to the family Violaceae which consists of about twenty-three genera and 930 species **[1]**. There are total 500 reported species of viola, out of which 17 are found in Pakistan **[2]**. *V. serpens* commonly known as Gul-e-banafsha **[3]** is found mostly in mountains at an elevation of around 800-3000 m **[4]**. Various species Viola genus are distributed in Pakistan, Kashmir, India, Afghanistan, Bhutan, Malaysia, Indonesia, Thailand, Australia, Sri Lanka, China, Myanmar and Nepal **[5]**. In folk medicine it is used as an antipyretic, laxative, emollient, expectorant, purgative, anti-asthmatic, anti-cancer, against jaundice, hepatitis, skin diseases and for management of constipation **[5-7]**. It is also used for treatment of headache, cough, cold, dermatitis, diseases of kidneys, liver, lungs and urinary infections **[8-10]**. Phytochemical investigations showed the

existence of glycosides, flavonoids, alkaloids, saponins and tannins along with methyl salicylate, mucilage, sugars and violin gum in the plant [6, 7, 11]. Its constituents responsible for the antioxidant activity are ascorbic acid, peroxidase, ascorbate oxidase and catalase [12].

The present study was conducted to prove the anti-inflammatory potentials of *Viola serpens* as the same activity has already been reported from the other species such as *Viola betinocifolia* [13].

Material and methods

Plant collection

The plant collection was done from District Shangla (Village, Puran), Khyber Pukhtunkhwa, Pakistan, (April, 2011). Dr. Mohammad Ibrar, Professor Department of Botany, UOP identified the plant specimen. A specimen with voucher # Bot.20158 (PUP) was deposited in department's herbarium. The whole plant collected was weighted before and after drying at ambient temperature.

Extraction and fractionation

The shade dried powdered plant 10 kg was macerated with 25 L methanol at room temperature for 10 days with vigorous stirring on daily basis. Whatmann filter paper was used to obtain the residue soluble in methanol which was first filtered through a colorless thin cloth layer in the separating funnel was collected and dried (706 g). Chloroform was then added with the layer separated from *n*-hexane layer and shaken vigorously. Chloroform being a dense. Filtrates were dried by a rotary evaporator (R-210, Buchi, Switzerland) at 40-45^{o}C and fitted with a re-circulating chiller (NESLAB instruments). Crude methanol extract was obtained from the procedure (1.57 kg) which was treated with various solvents (based on polarity) for fractionation. A 5L separating funnel was used for fractionation of the crude methanol extract of the plant (1.32 kg). 1L distilled water along with 1.5L *n*-hexane was used in a separating funnel (shacked vigorously) and fixed on stand till the immiscible layers appearance. *n*-Hexane accumulated as an upper solvent was collected as a lower layer with dried mass of 17 g. The same process was followed for ethyl acetate and *n*-butanol obtaining masses of 22.7 g and 35 g respectively. The finally left fraction was recovered and concentrated as an aqueous fraction (45 g).

Experimental animals

Albino mice (20.0-25.0 g) were obtained from NIH (National Institute of Health Islamabad). Standard laboratory conditions and required formulated diet were provided to the animals with open access to fresh water.

Acute toxicity

The crude methanol extract of the whole plant at three different doses ranging from 1g/kg to 2 g/kg were used for determination of the acute toxicity. The mice were uniformly grouped into three, comprising six mice each. Treated the negative controlled group with distilled water (10 ml/kg) whereas rest of the two groups with the crude methanol extracts (1 mg/kg and 2 mg/kg). Animals' observation was done in 24 hrs after the administration of the test doses. The initial 4 hrs observations of the animals were for the acute toxicity effect. After 24 hrs the no of death if any were identified [13].

Anti-inflammatory activity

Crude methanol extract as well as other fractions were tested for anti-inflammatory effect. The activity of crude extract and different fractions were determined by three different protocols in order to make clear the mechanism involved in the anti-inflammatory potentials of the plant.

Carrageenan induced paw edema (CIPE)

Crude methanol extract along with its different fractions were tested for occurrence of anti-inflammatory potentials. BALB/C mice (either sex, 25-30g) were

selected**.** They were divided into fourteen groups. Each group included 6 mice (n=6). Group I (negative control) was given normal saline 10 ml/kg, while Group II (positive control) was given diclofenac sodium at a dose of 10 mg/kg. The crude methanol extract and various fractions were given to rest of the groups, III-XIV at a dose of 100, 200 and 300 mg/kg respectively. After 30 min of the test samples administration each mouse was injected Carrageenan (1%) in sub-planter tissue of right hind paw. Plethysmometer (LE 7500 plan lab S.L) was used for measurement of anti-inflammatory potentials for a total duration of 5 hrs (0, 1, 2, 3, 4 and 5 hrs) **[13]**. Percent inhibition of edema was calculated by using the formula:

% Inhibition = A-B/B X100

Where A and B represents edema volume of negative control, paw edema of tested groups.

Histamine-induced paw edema (HIPE)

The HIPE trial was adopted according to the authentic protocol **[14]**. The test sample includes the oral administration of indomethacin (10 mg/kg) and distilled water 10ml/kg. Histamine (0.1 ml) was administered in sub-plantar injection to the tissue of right hand paw after one hour of test samples administration. After histamine injection, paw thickness was noted (at 30 min interval) for 3hrs. The % inhibition was calculated by using the formula:

% Inhibition = A-B/B X100

Where A and B represents edema volume of negative control, paw edema of tested groups.

Xylene induced ear edema (XIEE)

Ear edema test induced by xylene was conducted as per the authentic protocol **[15, 16]**. The test samples include oral administration of Ibuprofen (100mg/kg, positive- control) to test mice (BALB/C mice, 25-30g). Plant crude extract and fractions, 100, 200 and 300 g/kg was used (p.o). The test animal after an hour, received 20 µl (0.02 ml) of xylene on the right ear lobe at both the posterior as well on the anterior surfaces. Left ear lobe was taken as control. An hour after the application of xylene, sacrificed the treated mice and via cork borer (3 mm diameter), took circular sections of the ear and weighed. Calculated the weight in percent of ear edema by comparing with the weight of untreated left ear.

Results and discussion

Anti-inflammatory effects of the crude extract/fractions of *V. serpens* in CIPE in mice.

The activity of crude extract of *V. serpens* at various doses during different assessment times is shown in Table 1-5 and Figure 1. It exhibited significant inhibitory activity of CIPE only at 3rd h at 100 mg/kg i.p. However, it showed marked anti-inflammatory effect after 2nd h of injection and remained significant till 5th h at 200 and 300 mg/kg i.p. The crude extract was then fractionated into various fractions which showed different anti-inflammatory effects at different doses. The Hex fraction showed maximum effect against the CIPE at a dose of 100 to 300 mg/kg i.p in 2nd and 3rdh. Whereas, the effectiveness of the carageenan induced anti-inflammatory effect remained till the 5th hour of injection. The chloroform and aqueous fractions showed significant effect only at 200 and 300 mg/kg in the 3rd h of induced inflammation. Ethyl acetate fraction showed anti-inflammatory potentials at 300 mg/kg in the 2nd h. Moreover, in the 3rd h both the afore mentioned doses were significant.

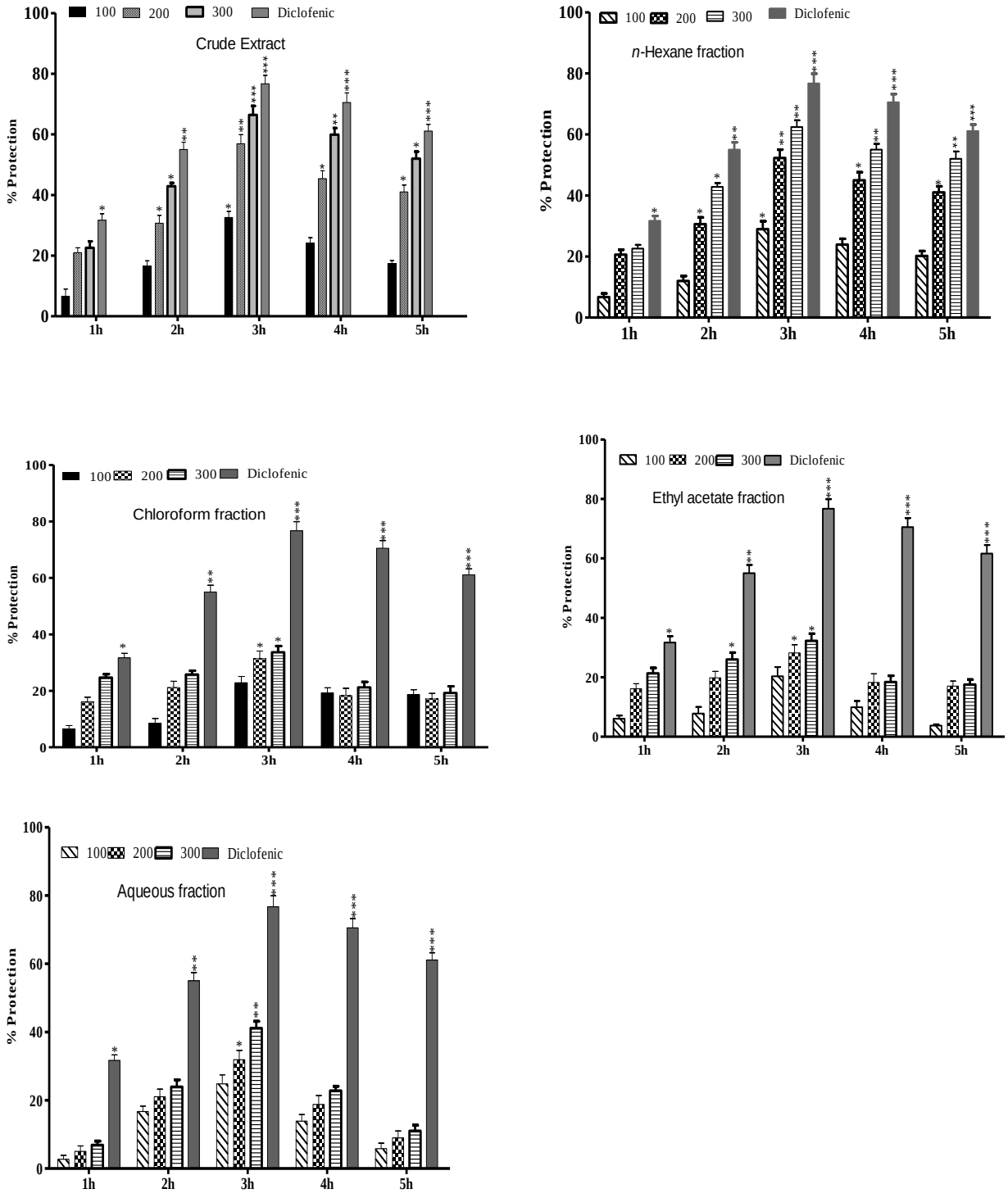

Figure 1. Anti-inflammatory effect of the crude extract/ fractions of *V. serpens* on carageenan induced inflammation

Table 1. Anti-inflammatory effect against CIPE and HIPE in mice for *V. serpens* crude extract

Treatment	Dose mg/kg	NPS	0h	1h	2h	3h	4h	5h
Saline	10ml	0.0950 ± 0.10	0.2150 ± 0.20	0.2160 ± 0.15	0.2160 ± 0.10	0.2090 ± 0.20	0.2070 ± 0.10	0.2092 ± 0.14
Diclofenac	10mg	0.0910 ± 0.25	0.2130 ± 0.15	0.1475* ± 0.05	0.0970** ± 0.05	0.0486** ± 0.07	0.0610** ± 0.05	0.0811** ± 0.12
Anti-inflammatory effect against CIPE								
	100	0.0915 ± 0.10	0.2134 ± 0.10	0.2018 ± 0.15	0.1803 ± 0.10	0.1408* ± 0.10	0.1572 ± 0.15	0.1590 ± 0.20
Crude extract	200	0.0965 ± 0.13	0.2050 ± 0.11	0.1700* ± 0.19	0.1495* ± 0.21	0.0901** ± 0.17	0.1130* ± 0.19	0.1230* ± 0.13
	300	0.0970 ± 0.11	0.2178 ± 0.05	0.1670* ± 0.18	0.1233* ± 0.22	0.0702** ± 0.18	0.0830** ± 0.25	0.1003** ± 0.19
Anti-inflammatory effect against HIPE								
	100	0.0970±0.05	0.2001± 0.05	0.2005± 0.19	0.1966± 0.25	0.1850± 0.25	0.1960± 0.05	0.1990± 0.35
Crude extract	200	0.0955 ± 0.10	0.2055 ± 0.20	0.1614* ± 0.15	0.1510* ± 0.20	0.0990** ± 0.23	0.1083* ± 0.25	0.11087* ± 0.10
	300	0.0962 ± 0.15	0.2087 ± 0.30	0.1180* ± 0.55	0.1150* ± 0.10	0.0803** ± 0.20	0.0921** ± 0.30	0.1231** ± 0.30

Values are reported as mean ±SEM for group of six mice each for carrageenan and Histamine by applying ANOVA followed by Dunnett tests for data analysis. Significant and satisfactory values are represented by asterisks from the control. *P<0.05 or **P<0.01

Table 2. Anti-inflammatory effect against CIPE and HIPE in mice for *V. serpens* *n*-hexane fraction

Treatment	Dose mg/kg	NPS	0h	1h	2h	3h	4h	5h
Saline	10ml	0.0950 ± 0.10	0.2150 ± 0.20	0.2160 ± 0.15	0.2160 ± 0.10	0.2090 ± 0.20	0.2070 ± 0.10	0.2092 ± 0.14
Diclofenac	10mg	0.0910 ± 0.25	0.2130 ± 0.15	0.1475* ± 0.05	0.0970** ± 0.05	0.0486** ± 0.07	0.0610** ± 0.05	0.0811** ± 0.12
Anti-inflammatory effect against CIPE								
n-Hexane	100	0.0910 ± 0.20	0.2130 ± 0.10	0.2015 ± 0.05	0.1900 ± 0.20	0.1485* ± 0.20	0.1575 ± 0.10	0.1670 ± 0.15
	200	0.0970 ± 0.15	0.2053 ± 0.10	0.1715* ± 0.20	0.1500* ± 0.24	0.0985** ± 0.19	0.1135* ± 0.20	0.1232* ±0.15
	300	0.0970 ± 0.10	0.2180 ± 0.02	0.1672* ± 0.25	0.1235* ± 0.20	0.0785** ± 0.20	0.0930** ± 0.25	0.1003**±0.20
Anti-inflammatory effect against HIPE.								
	100	0.0982± 0.03	0.2091± 0.15	0.2100± 0.18	0.1963± 0.19	0.1547± 0.25	0.1700± 0.12	0.1960± 0.27
n-Hexane	200	0.0895 ± 0.11	0.2119 ± 0.22	0.2021* ± 0.16	0.1506* ± 0.22	0.1052** ± 0.24	0.1285* ± 0.25	0.1330* ± 0.12
	300	0.0901 ± 0.14	0.2067 ± 0.24	0.178`5* ± 0.25	0.1612* ± 0.11	0.1078** ± 0.15	0.1338** ± 0.30	0.1421** ± 0.26

Values are reported as mean ±SEM for group of six mice each for carrageenan and Histamine by applying ANOVA followed by Dunnett tests for data analysis. Significant and satisfactory values are represented by asterisks from the control. *P<0.05 or **P<0.01

Table 3. Anti-inflammatory effect of chloroform fraction of *V. serpens* in CIPE and HIPE in mice

Treatment	Dose mg/kg	NPS	0h	1h	2h	3h	4h	5h
Saline	10ml	0.0950± 0.10	0.2150± 0.20	0.2160± 0.15	0.2160± 0.10	0.2090± 0.20	0.2070± 0.10	0.2090± 0.14
Diclofenac	10mg	0.0910± 0.25	0.2130± 0.15	0.1475* ± 0.05	0.0970** ± 0.05	0.0486** ± 0.07	0.0610** ± 0.05	0.0811** ± 0.12
Anti-inflammatory effect against CIPE								
Chloroform	100	0.0917± 0.11	0.2135± 0.15	0.2020± 0.13	0.1976± 0.17	0.1610*± 0.11	0.1669 ± 0.15	0.1699± 0.15
	200	0.0972± 0.10	0.2001± 0.20	0.1811* ± 0.21	0.1702*± 0.15	0.1433** ± 0.12	0.1692* ± 0.19	0.1734*± 0.17
	300	0.0999± 0.21	0.1788± 0.23	0.1627* ± 0.14	0.1601*± 0.20	0.1386** ± 0.19	0.1630** ± 0.19	0.1688** ± 0.21
Anti-inflammatory effect against HIPE								
Chloroform	100	0.0964±0. 05	0.2112 ± 0.05	0.2120± 0.19	0.1989± 0.25	0.16702 ± 0.25	0.1761± 0.05	0.1805± 0.35
	200	0.0955± 0.10	0.2050± 0.20	0.2003* ± 0.15	0.1770*± 0.20	0.1483** ± 0.23	0.1709*± 0.25	0.1790*± 0.10
	300	0.0962± 0.15	0.2085± 0.30	0.1831* ± 0.55	0.1697*± 0.10	0.1432** ± 0.20	0.1670** ± 0.30	0.1699** ± 0.30

Values are reported as mean ±SEM for group of six mice each for carrageenan and Histamine by applying ANOVA followed by Dunnett tests for data analysis. Significant and satisfactory values are represented by asterisks from the control. *P<0.05 or **P<0.01

Table 4. Anti-inflammatory effect of Ethyl acetate fraction of *V. serpens* in CIPE and HIPE in mice

Treatment	Dose mg/kg	NPS	0h	1h	2h	3h	4h	5h
Saline	10ml	0.0950 ± 0.10	0.2150 ± 0.20	0.2160 ± 0.15	0.2160 ± 0.10	0.2090 ± 0.20	0.2070± 0.10	0.2090± 0.14
Diclofenac	10mg	0.0910 ± 0.25	0.2130 ± 0.15	0.1475* ± 0.05	0.0970** ± 0.05	0.0486** ± 0.07	0.0610** ± 0.05	0.0811** ± 0.12
Anti-inflammatory effect against CIPE								
Ethyl Acetate	100	0.0888 ± 0.21	0.2100 ± 0.19	0.2029 ± 0.11	0.1991 ± 0.12	0.1665* ± 0.20	0.1865± 0.11	0.2011± 0.19
	200	0.0972 ± 0.13	0.2021 ± 0.19	0.1811* ± 0.22	0.1732* ± 0.20	0.1500** ± 0.16	0.1692*± 0.13	0.1734*± 0.12
	300	0.0892 ± 0.18	0.1868 ± 0.19	0.1699* ± 0.22	0.1598* ± 0.23	0.1416** ± 0.16	0.1689** ± 0.14	0.1723** ± 0.17
Anti-inflammatory effect against HIPE								
Ethyl Acetate	100	0.0932± 0.02	0.2109± 0.10	0.2021± 0.20	0.1980± 0.22	0.1773± 0.10	0.1859± 0.12	0.1970± 0.33
	200	0.09032 ± 0.13	0.2067 ± 0.18	0.1810*± 0.22	0.1751*± 0.25	0.1642± 0.18	0.1752± 0.22	0.1820± 0.09
	300	0.09319 ± 0.11	0.1893 ± 0.26	0.1779*± 0.36	0.1706*± 0.03	0.1609 ± 0.16	0.1709± 0.25	0.1801± 0.28

Values are reported as mean ±SEM for group of six mice each for carrageenan and Histamine by applying ANOVA followed by Dunnett tests for data analysis. Significant and satisfactory values are represented by asterisks from the control. *P<0.05 or **P<0.01

Table 5. Anti-inflammatory effect against CIPE and HIPE in mice for *V. serpens* aqueous fraction

Treatment	Dose mg/kg	NPS	0h	1h	2h	3h	4h	5h
Saline	10ml	0.0950 ± 0.10	0.2150 ± 0.20	0.2160 ± 0.15	0.2160 ± 0.10	0.2090 ± 0.20	0.2070 ± 0.10	0.2090 ± 0.14
Diclofenac	10mg	0.0910 ± 0.25	0.2130 ± 0.15	0.1475* ± 0.05	0.0970** ± 0.05	0.0486** ± 0.07	0.0610** ± 0.05	0.0811** ± 0.12
Anti-inflammatory effect against CIPE								
	100	0.0864 ± 0.03	0.2092 ± 0.19	0.2101 ± 0.20	0.1833 ± 0.13	0.1571* ± 0.22	0.1782 ± 0.10	0.1968 ± 0.11
Aqueous	200	0.0921 ± 0.12	0.2090 ± 0.21	0.2152* ± 0.12	0.1705* ± 0.24	0.1423** ± 0.14	0.1680* ± 023	0.1902* ± 0.16
	300	0.0906 ± 0.15	0.2099 ± 0.17	0.2012* ± 0.19	0.1643* ± 0.16	0.1230** ± 0.13	0.1598** ± 0.15	0.1860** ± 0.14
Anti-inflammatory effect against HIPE								
	100	0.0961± 0.02	0.2009± 0.02	0.2013± 0.19	0.1993± 0.15	0.1603± 0.25	0.1803± 0.05	0.1995± 0.30
Aqueous	200	0.0894 ± 0.13	0.2003 ± 0.23	0.1959 ± 0.15	0.1823± 0.21	0.1530 ± 0.23	0.1721 ± 0.21	0.1920 ± 0.20
	300	0.0905 ± 0.13	0.2011 ± 0.32 (6.4%)	0.1901 ± 0.55 (12%)	0.1603 ± 0.14 (25%)	0.1413 ± 0.20 (34.6%)	0.1550 ± 0.24 (25.12%)	0.1828 ± 0.28 (12.6%)

Anti-inflammatory effects of crude extract/ fractions of *V. serpens* on (HIPE) in mice

The effect of the crude extract of *V. serpens* in HIPE at various doses (100, 200 and 300 mg/kg i.p) in various durations (1-5 h) is presented in the table 1-5 and figures 2. The crude extract at a dose of 200 and 300 mg/kg showed more pronounced anti-inflammatory effects on the 2nd to the 5th hour of histamine induced edema injection. The significance level reached the maximum in the 3rd hour and then decreased slowly till the 5th hour. The hydro-methanol extract was then subjected to various fractions, exhibiting different inhibitory effects. In fractions maximum anti-inflammatory activity against the HIPE was produced by the *n*-hexane fraction at 200 mg/kg in the 3rd h of HIPE with percent inhibition 46.70%. The significant anti-inflammatory effect started from the 2nd h and lasted till 5th h of edema induction. On other hand chloroform and aqueous fractions showed significant activity at 200 and 300 mg/kg on the 3rd h of histamine induced edema with percent inhibition values of 31.48 and 34.60 % respectively. Whereas, the ethyl acetate fraction was non-significant in the three test doses in all the 5 mentioned tested hours.

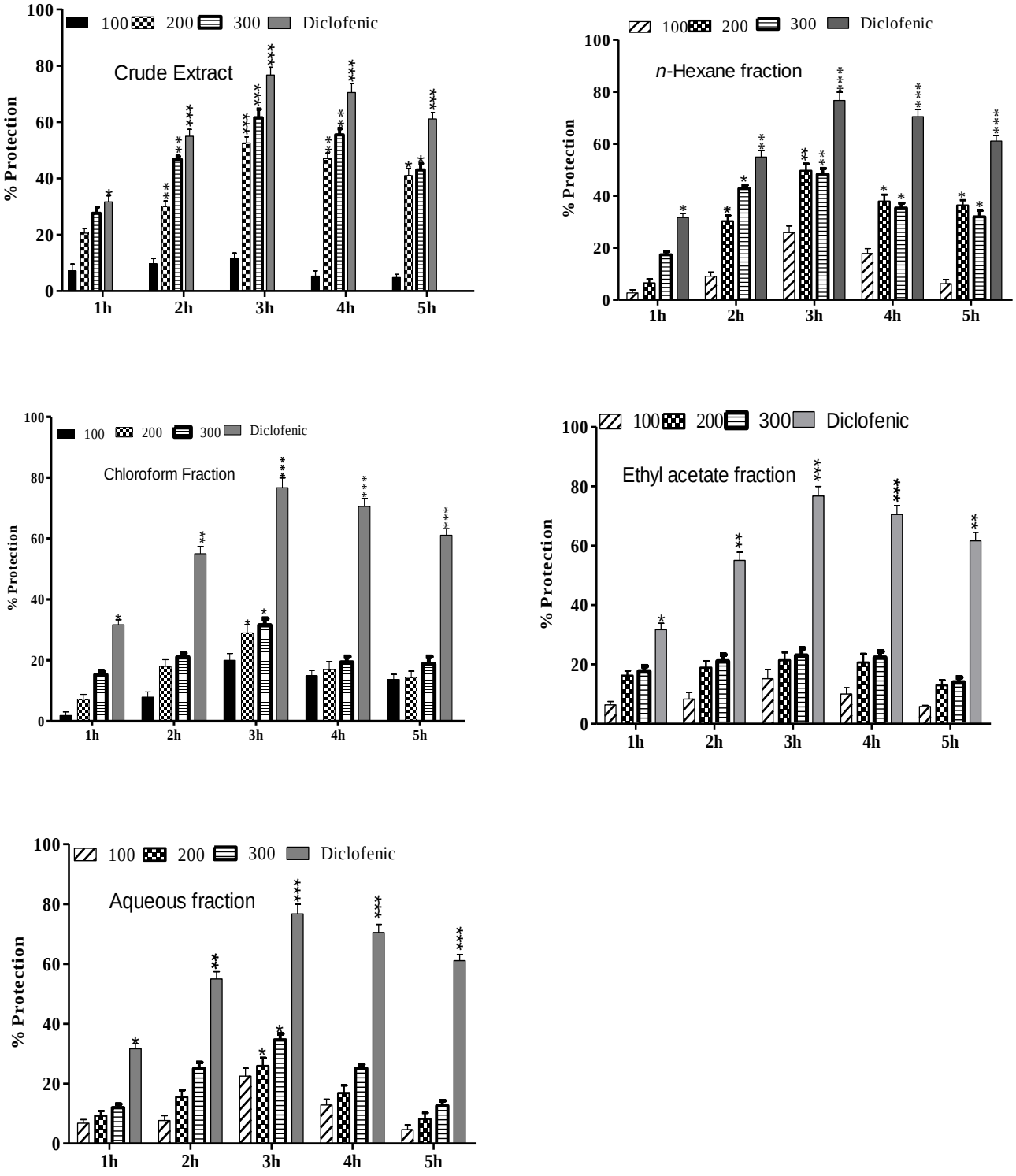

Figure 2. Anti-inflammatory effect of the crude extract/fractions on HIPE in mice

Anti-inflammatory effects of the crude extract/ fractions of *V. serpens* on Xylene induced ear edema: (XIEE) in mice

Results of the above mentioned activity are presented in the table 6 and the percent inhibition in the figures 3. The crude

extract/subsequent fractions of *V. serpens* were subjected for the anti-inflammatory effect by using XIEE protocol. Three test doses were selected (300, 200 and 100 mg/kg oral administration) for anti-inflammatory effective results determination. The crude extract showed maximum inhibitory effect (57.6 %) at 300 mg/kg. The activity of crude extract was significant in a dose dependent way. Upon treatment with different solvents the fractions obtained showed different anti-inflammatory effects. The most effective and significant fraction considered was the n-hexane which also showed significance in a dose dependent way with the maximum percent inhibition value of 55 % at 300 mg/kg. This was followed by the chloroform and ethyl acetate fractions and then by the aqueous fraction whose considerable effects were shown at 200 and 300 mg/kg with a maximum inhibition values of 51, 49 and 48.5 % respectively. Inflammation being a complex process has direct association with pain which may involve increase in: vascular permeability, cells migration (mononuclear and granulocytes) and proliferation of granulomatous tissue. Anti-inflammatory compounds act through different mechanisms. Either by blocking the pro-inflammatory mediators (directly via enzyme like COX-2 inhibition) or enzyme expression is decreased such as anti-inflammatory steroidal compounds or substrate levels are decreased like reduction in the release of arachidonic acid. The release of stored mediators/blockage of interaction of receptors mediators (histamine antagonists receptor). Immuno-stimulation is also one of the mechanism i.e phagocytosis activation as well as maturation of myeloid cells which ultimately response to the challenge of allergen **[17]**. The plant extract/ fractions of *V. serpens* demonstrated its effectiveness against the induced inflammation protocols that are CPE, HPE and XEE.

Carrageenan being a choice of phlogistic agent is used for anti-inflammatory drugs testing and having an extensive measurement of reproducibility **[18]**. It is a biphasic model, with the early phase including 1–2 h, mediated mostly by the release of serotonin, histamine and prostaglandins increased level. The late phase includes the release of prostaglandin whereas kinine release in between the two phases **[19, 20]**. The enzyme cyclooxygenase (COX) catalyse the biosynthesis of Prostaglandins (PGs) metabolites of (metabolite of arachidonic acid) in the early phase **[21]**. COX-1 (constitutive form of COX) is involved in cellular function housekeeping **[22]**. COX-2 (inducible isoform) increases response to various tissue inflammatory stimuli **[21]**. COX-3 is determined in the heart tissue and brain cortex **[23]**. In CIPE protocol the crude extract and n-hexane fractions were effective against the inflammation challenge from the 2^{nd} till the 5^{th} h at 200 and 300 mg/kg. whereas the chloroform, aqueous and ethyl acetate fractions also showed significant effects and reduced paw edema in the 3^{rd} h at 300 mg/kg i.p. The crude extract and the n-hexane fraction of *V.serpens* are effective in both the phases whereas the rest of the fractions showed significant effets only in the late phase.

Histamine being a fundamental amine and mediator that is associated with inflammation and allergic reactions that causes both increase in the vascular permeability and vasodilatation **[24, 25]**. The lipoxygenase and cyclooxygenase pathways are followed by the arachidonic acid metabolites. PG, PGE2 is mainly involved in the cause or enhancement of the signs of cardinal inflammation. The mentioned enzymes of the arachidonic acid provoke the inflammatory response **[26, 27]**.

The XIEE in mouse is a testing and investigating procedure for acute anti-inflammatory activity response, resulting in sever vasodilation and skin oedem (ear) [28-30]. Xylene tropical application on ear leads to an immediate mouse ear irritation resulting in the fluid accumulation (edema formation) and acute response of inflammation [31]. Anti-inflammatory steroidal and non-steroidal anti-phlogistic agents are evaluation by this method especially the ones inhibiting phospholipase A2 [32]. The results obtained from the study showed that the ear edema in the crude extract as well in the fractions subsided in a dose dependent manner (crude extract and n-hexane). Whereas, in the other fractions significance is found only at high dose in 300 mg/kg i.p. Thus, the effectiveness of *V. serpens* in the model suggests that the plant extract and its fractions possibly acts by inhibiting the enzyme phospholipase A2 (PLA2) [28]. Phytochemically, different groups of compounds are reported to be present in viola species including triterpenoids, cyclotide, alkaloids and flavonoids [33]. Triterpenoids are one of the important contributors of anti-inflammatory activity [17, 34]. Along with this the presence of inflammation sites in high concentration oxidant and free radicals also contributes to the anti-inflammatory process and play an important role in avoiding the process of inflammation [35]. *V. serpens* also contain various phenolic compounds [36] and possess antioxidant activity along with the triterpenes (anti-inflammatory compounds) which may be the major contributors for its anti-inflammatory activity. Flavonoid and glycosides also contribute significantly in the analgesic as well as anti-inflammatory action [37]. In the present study the isolated flavonoids 1-6 from the chloroform fraction of the plant also showed marked scavenging effect against DPPH so this may also give a solid scientific background to the plant as a strong anti-inflammatory agent. Moreover, further work in future is required to be focused on this plant as an anti-inflammatory agent to make its use more authentic and more common with the scientific knowledge.

Table 6. Effect of the crude extract along with the subsequent fractions of *V. serpens* on XIEE in mice

Group	Doses (mg/kg)	Ear weight (mg)	Inhibition (%)
Control		185	
Ibuprofen	100	57.32±3.51	69.0
Crude extract	100	129.5±2.34	30.0
	200	101.9±1.91	45.4
	300	78.3±2.09	57.7
n-Hexane	100	135±2.56	27.0
	200	105±4.01	43.0
	300	83±2.11	55.0
Chloroform	100	137±4.12	25.9
	200	112±2.78	39.5
	300	90.5±3.11	51.0
Ethyl acetate	100	133±2.34	28.0
	200	117±1.77	36.8
	300	93.2±2.05	49.6
Aqueous	100	132±3.00	28.6
	200	107±2.13	42.0
	300	95.3±2.34	48.5

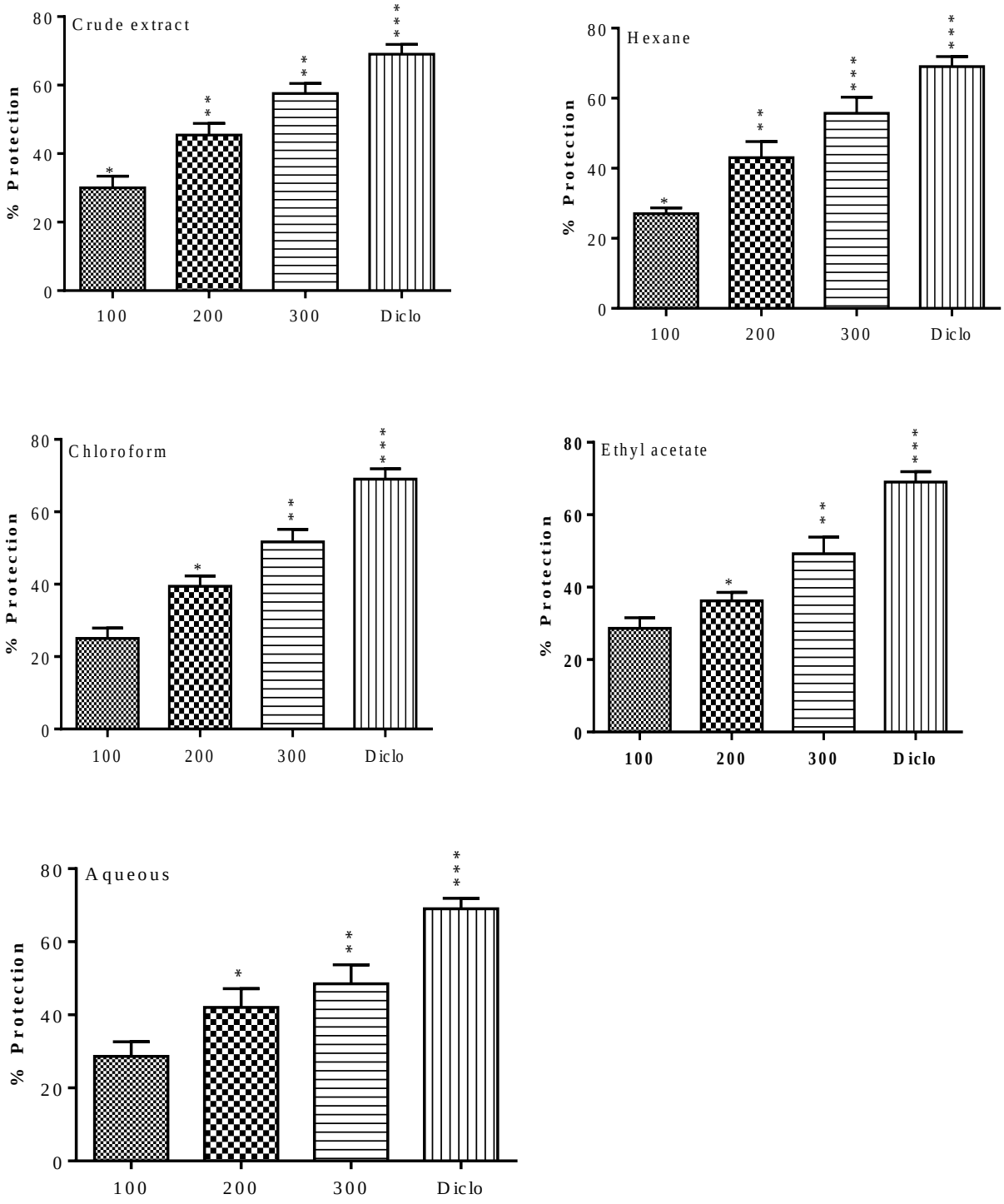

Figure 3. Anti-inflammatory effect of the crude extract/ fractions of *V.serpens* on XIEE

Conclusion

It is concluded from the results that the crude extract and the different fractions of *V. serpens* contain anti-inflammatory activity in a dose-dependent manner. This provides a solid background to the traditional uses of

the drug as an effective anti-inflammatory drug. The presence of certain compounds in the plant are responsible for resisting/ inhibiting the release of histamine, prostaglandins or mediators of the mast cells (histamine, PG and 5-HT) and enzyme phospholipase A2 (PLA2). Further study of compounds isolation can authenticate more the use of the plant in the clinical side.

Authors' contributions

Conceived and designed the experiments: R Ghaffar & M Saeed, Performed the experiments: R Ghaffar, Analyzed the data: R Ghaffar, H Khan & S. Ahmad, Contributed reagents/ materials/ analysis tools: R Ghaffar, M Saeed & M Ahmad, Wrote the paper: R Ghaffar, M Saeed, M Ahmad, H Khan & S Ahmad.

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