ORIGINAL RESEARCH

ANTI-HYALURONIDASES ACTIVITY OF CRUDE EXTRACTS OF *PULSATILLA NIGRICANS*

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ABSTRACT:

Pulsatila nigrican belong to the family Ranunculaceae. The dried aerial part of plant was extracted with different solvents viz. Petroleum ether, chloroform, ethyl acetate and methanol. The methanolic extract of *Pulsatilla nigrican* showed potent hyaluronidase inhibition (IC_{50} 77.90µg/ml) which is compable to strandard Indomethacin(IC_{50} 68.09µg). The hyaluronidase inhibition (IC_{50}) of other aerial part extracts (ethyl acetate, chloroform, petroleum ether) was found within the range of 99.98 to 189.50µg/ml.

KEY WORDS: *Pulsatilla nigricans,* Hyaluronidases, Hyaluronan

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1. INTRODUCTION:

Pulsatilla nigricans had been used in nervousness, sadness, mild restlessness and mental unrest[1]. The plant had been used as a remedy for ovaritis, ovaralgia, pain associated with debility and due to acute inflammation, epididymitis, and orchitis. It also increases sexual power, but lessens morbid sexual excitement. *P. nigricans* relieves urethral irritation, consequent spermatorrhoea and prostatorrhoea, amaurosis, cataract and opacity of the cornea. *P. nigricans* has been used in uterine affections, dyspepsia, coryza, otitis, rhinitis, conjuctivitis, coughs, cutaneous affections, acute meningitis, and as taeniafuge [2]. *P. nigricans* roots have been used for blood-cooling and detoxifying effects in traditional system of Chinese medicine [3].

Pulsatilla nigricans belongs to family Ranunculaceae. *P. nigricans* Storck (Synonym *P. pratensis* Mill.) [4] is a perennial plant; stem simple, erect, rounded, 3-5 inches high; leaves radical, pinnatifid, downy, the segments many-parted, with linear lobes; flowers solitary, terminal, pendulous, deep-purple or violet-brown, somewhat narrow, pointed, reflected at the point, erect and converging at the base; sepals 6; stalked glands or sterile stamens are found between the fertile stamens and sepals, the proximity of the involucre is such that it has a calyx like appearance[1,5].

Hyaluronan is a major constituent of the extracellular matrix, for example in the vitreous humor of the human eye (0.1-0.4 mg/g wet weight), in the synovial joint fluid (3-4 mg/ml), in the matrix produced by the cumulus cells around the oocyte Hyaluronan is present in all vertebrates and also in the capsule of some *Streptococci* strains. As hyaluronan serves as an essential structural element in the matrix, it plays an important role for tissue architecture. Hyaluronan is important for cell proliferation, cell migration and cell growth as well as the metastasis of tumour cells. Morphogenesis, embryonic development, wound healing and inflammation is associated with an increase of hyaluronan production [1,6]. Hyaluronic acid interacts with a variety of receptors and binding proteins on the surface of cells[1]. The most common hyaluronan receptor and the most studied to date is CD44 (lymphocyte homing receptor).

Hyaluronidases (HAases) are a family of enzymes that depolymerizes the polysaccharide hyaluronic acid (HA) in the extracellular matrix of connective tissues. The enzyme is known to be involved in allergic effects [8], migration of cancer[9] and in inflammation. Potent Hyaluronidases inhibitors might have anti allergic and anti inflammatory activities Thus our present study is aimed to investigate the Hyaluronidases inhibitory activity of *Pulsatilla nigricans.*

2. MATERIAL AND METHODS:

2.1 Collection and Authentication of Plant Material:

Pulsatilla nigricans aerial parts were procured from KR Indo German, American Trading Company, Kurukshetra (Haryana). Identity of the plant was confirmed through Department of pharmacognosy, Adesh Institute of Pharmacy and Biomedical Science, Bathinda, Punjab. In India, *P. nigricans* has not been reported from wild sources. *Pulsatilla Nigrican stock* is also present in Phanerogams herbarium in hungery and specimen no Cat. P00040545. This report was seen in at the Central National Herbarium of the Botanical Survey of India, Kolkata.

2.2 Plant Extraction:

The arial parts of *Pulsatilla nigrican* were ground to half dust (each 1 kg) and soaked in petroleum

ether (60-80 °C) for 72 h at room temperature with occasional shaking. The extract was filtered and the filtrate evaporated to dryness under reduced pressure. The residue was soaked with the fresh solvent (3x1Lit). The entire procedure repeated twice more to get maximum extract of constituents. The residue was extracted in the same way with chloroform (3x1Lit.), ethyl acetate (3x1Lit.), and methanol (3x1Lit.). The extracts of *Pulsatilla nigrican* were collected and the solvents were evaporated using rotary vacuum at 40º C to get various fractions given All the crude extracts were stored at 4ºC[10] before performing biological activities and isolation.

Table 1: Extracted amount of the different extracts of *Pulsatilla nigrican*

Plant	Extract	Amount in grams
Pulsatilla nigrican	ME	5
	ET	6.4
	CH	4.1
	PE	9

Notes: [a] ME: Methanol Extract; ET: Ethyl acetate Extract; Petroleum ether Extract; CH: Chloroform Extract.

2.3. Hyaluronidase Inhibition Activity:

2.3.1. Chemicals:

Hyaluronidase, hyalurnic acid,(Sigma Aldrich) Sodium chloride, Sodium actetate, acetic acid, DMSO, Potasioum chloride, Disodium hydrogen phosphate, Potasium Dihyrogen phosphate and the remaining chemicals used in this study were of analytical grade and were obtained from Merck Specialties Pvt. Ltd, Mumbai, India

2.3.2. Hyaluronidase Inhibition Assay

The assay medium consisting of 3 - 5U hyaluronidase (from Sigma –Aldrich, Bangalore) in 100µl of 20mM sodium phosphate buffer (pH 7.0) with 77mM sodium chloride, 0.01% BSA was preincubated with different concentrations (5µg, 50µg and 100 µg) of crude extracts for 15 min at 37 °C. The assay was commenced by adding 100µl hyaluronic acid, 0.03% in 300mM sodium phosphate, pH 5.35) to the incubation mixture and incubated for a further 45 min at 37 °C. The undigested hyaluronic acid was precipitated with 1ml acid albumin solution made up of 0.1% bovine serum albumin in 24mM sodium acetate and 79mM acetic acid, (pH 3.75). After standing at room temperature for 10 min, the absorbance of the reaction mixture was measured at 600 nm. The absorbance in the absence of enzyme was used as the reference value for maximum inhibition. The inhibitory activity of crude extractswere calculated as the percentage ratio of the absorbance in the presence of crude extract *vs.* absorbance in the absence of enzyme. The enzyme activity was checked by control experiment run simultaneously, in which the enzyme was pre incubated with 5µl DMSO instead, and followed by the assay procedures described above.

3. STATISTICAL ANALYSIS

All data are expressed as means_SEMs. The statistical analysis of all the observations was carried out using one-way ANOVA followed by the multiple comparison test of Tukey–Kramer, where necessary. Values of p50.05 areconsidered as significant compared with the control.

4. RESULTS AND DISCUSSION:.

It is a well known fact that the demand for plant drugs for treatment of various ailments is

increasing and plant drugs from the ayurvedic or homeopathy system are being explored more, not only in India but also globally. Crude plant extracts were tested for different biological activities by various bioassays. Bioassays offer a special advantage in the standardization and quality control of heterogeneous plant product. In our research work, bioassay is performed to show the biological avtivites (Hyaluronidase inhibitory assay).

Hyaluronidase is a mucolytic enzyme found in the testes, in snake venom, and in hemolytic streptococci and certain other bacteria, that decreases the viscosity of the intercellular matrix by breaking down hyaluronic acid, thereby increasing tissue permeability. Hyaluronidase hydrolyzes hyaluronan, in the extracellular matrix during tissue remodeling. Hyaluronidase activity increases in chronic inflammatory conditions like inflammatory joint disease

Enzymes belonging to this class mainly decompose hyaluronic acid. Because of the striking physic chemical properties of hyaluronan solutions, various physiological functions have been assigned to it, including lubrication, water homeostasis, filterin effects and regulation of plasma protein

distribution. In animals and man, the half-life of hyaluronan in tissues ranges from less than 1 to several days. It is catabolized by receptor-mediated endocytosis ad from blood, with a half-life of 2–5 min, mainly by the endothelial cells of the liver sinuoids.

In our study hyaluronidase inhibition assay showed that the methanolic extract of *Pulsatilla nigrican* showed potent hyaluronidase inhibition with IC_{50} 77.90µg/ml which is compable to strandard Indomethacin IC_{50} 68.09µg. The hyaluronidase inhibition (IC_{50}) of other aerial part extracts (ethyl acetate, chloroform, petroleum ether) was found within the range of 99.98 to 189.50µg/ml. (Table 3.)

Table 2: Hyaluronidase Inhibition Activity *Pulsatilla nigrican*

Plants	Extracts	IC_{50} (µg/ml)
P.nigrican	Me	77.90±0.66
	ET	99.98±0.36
	CH	145.62±0.50
	PE	189.50±0.31
Standard	Indomethacin	68.09±0.32

Notes: ME: Methanol Extract; ET: Ethyl acétate Extract;PE: Petroleum ether Extract CH: Chloroform Extract. Values represent means ± SEM of three different experiments.

5. CONCLUSION:

We have investigated the plant chosen for this study is *Pulsatila nigrican* a which belong to the family Ranunculaceae. The dried aerial part of plant was extracted with different solvents viz. Petroleum ether, chloroform, ethyl acetate and methanol. The methanolic extract of *Pulsatilla nigrican* showed potent hyaluronidase inhibition with IC_{50} 77.90µg/ml. The hyaluronidase inhibition (IC_{50}) of other aerial part extracts was found within the range of 99.98 to 189.50µg/ml. Thus, the present study clearly suggests that the biological

activities exhibited by the folklore plant may be due to synergic effects of the presence of bioactive compounds in the crude extracts.

6. ACKNOWLEDGEMENTS

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