

INVESTIGATION OF *IN VITRO* ANTI INFLAMMATORY AND COX-2 INHIBITORY ACTIVITY OF ROOT EXTRACTS OF *Berberis aristata*

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ABSTRACT

The study was aimed to investigate *in vitro* anti-inflammatory and COX-2 inhibitory activities of dried root extracts of *Berberis aristata*. For the present investigation, extracts were obtained by successive soxhlet extraction. Petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts at a concentration of 300µg/ml and 500µg/ml were used. HRBC membrane stabilization method was used to study *in vitro* anti-inflammatory activity. Diclofenac sodium was used as standard to compare the membrane stabilisation of the test extracts. Enzyme immunoassay method was used to evaluate *in vitro* COX-2 inhibitory activity. Aqueous extract at a concentration of 500µg/ml showed significant membrane stabilization of 83.67% and 78.12 % of COX-2 inhibition in comparison to other extracts studied and was comparable to standard drug, Diclofenac sodium. The results observed thus, suggest that the aqueous extract of dried roots of *Berberis aristata* possesses significant *in vitro* anti-inflammatory and COX-2 inhibitory activity.

KEY WORDS

Berberis aristata; COX-2; Enzyme immunoassay; Membrane stabilization.

INTRODUCTION

Berberis aristata commonly called barberry belongs to family berberidaceae [1]. Roots of barberry have been used in folk medicine for treatment of diarrhoea, improper appetite, reduce fever, promote vigor, in wound healing, ENT infections, all types of inflammation and immunopotentiating effect [2]. Extracts from the roots have been used to treat rheumatism and other chronic inflammatory disorders in eastern and Bulgarian folk medicine and Ayurveda in India, in Chinese medicine [3, 4]. Chemical constituents obtained from stem, root bark, fruit include isoquinoline alkaloids like protoberberines including berberine, berbamine, jaterrhizine, palmatine, oxycanthine, berberine being the chief constituent [5, 6]. Berberine and Oxycanthine obtained from total ethanolic extract was effective in carrageenan and Zymosan induced paw edema and in adjuvant arthritis [3, 7]. Berberine was concluded to be the active anti-inflammatory constituent of berberis which acts by reducing PGE₂, COX-2 and TNF-

α [2]. It has been used in various conditions as an antipyretic, analgesic, antiarrhythmic, antidiarrhoeal, anticonvulsant, anti-inflammatory, immunostimulant, diuretic, in dysmenorrhea, antimalarial, antirheumatic, antiseptic, sedative, purgative [5]. Dried root extract cured ophthalmic conditions upon external application on eyelids [8]. Berbamine also helped to reduce inflammation by inhibiting IL – 1, TNF – α, platelet activating factor [9]. Though few studies reported involvement of barberry as an antiinflammatory agent, no source reported the mechanism of inflammatory response. Hence an attempt was made to explore the inflammatory activity and specific COX-2 inhibitory activity by *in vitro* methods.

MATERIAL AND METHODS

Collection and extraction of plant material

For the present investigation, Barberry roots were obtained from Yucca Enterprises, Mumbai. The roots were checked for any foreign matter and shade dried.

After complete drying the drug was powdered by using a laboratory grinder and sieved. 50 g of powder was extracted by successive soxhlation with petroleum ether (60 - 80 ° C), chloroform, ethyl acetate, methanol and water for 8 hours. The solvent extracts obtained were further concentrated in vacuo by using rotary vacuum evaporator and then, dried in a desiccator.

Chemicals and reagents

Diclofenac sodium was obtained from Mangalam Drugs and Pharmaceuticals Ltd, Wapi, Gujarat. All the solvents were procured from E. Merck, Mumbai. The colorimetric human COX-2 inhibitor screening kit (Item No. 560131) used for *in vitro* COX-2 inhibitory activity was manufactured by Cayman Chemical, USA. The contents of the kit includes, PG screening EIA antiserum, PG screening AChE tracer, PG screening EIA standard, EIA buffer concentrate, wash buffer concentrate, polysorbate 20, mouse antirabbit IgG coated plate, 96 well cover sheet, Ellman's reagent, reaction buffer, COX-1 (ovine), COX-2 (human recombinant), heme, arachidonic acid (substrate), potassium hydroxide, hydrochloric acid, stannous chloride.

Evaluation of *In Vitro* Anti-inflammatory activity:

In vitro antiinflammatory activity of the extracts was performed by the method described by Mongeli *et al* 1997 [10]. The method involves stabilisation of human red blood cells which is the measure of antiinflammatory response, as HRBC membrane is

similar to that of lysosomal membrane [11]. Thus, stabilisation of lysosomal membrane prevents the release of lysosomal enzymes responsible for inflammation.

Preparation of HRBCs (human red blood cells)

Blood (5 ml) was collected from healthy human donors and centrifuged. The supernatant was then carefully pipetted with sterile pipettes. The packed cells were resuspended in an equal volume of isosaline and centrifuged. The process was repeated 4 times until the supernatants were clear. A 10% HRBC suspension was then prepared with normal saline and kept at 4 ° C until use.

Effect of plant extracts on HRBC system

The reaction mixture (4.5 ml) consisted of 2ml hyposaline (0.25% w/v NaCl), 1 ml of isosaline buffer solution, pH 7.4 (6.0 g TRIS, 5.8g NaCl, HCl to regulate the pH and water to make 1000 ml) and varying volumes of the extract solution in isotonic buffer (concentration = 10mg/ ml) to make the volume to 4.0 ml. Then 0.5 ml of 10% HRBC in normal saline was added. Two controls were performed. One with 1.0 ml of isosaline buffer instead of extract (control 1) and the other one with 1 ml of extract solution and without red blood cells (control 2). The mixture was incubated at 56°C for 30 min. The tubes were cooled under running water for 20 min. The mixture was centrifuged, and the absorbance of the supernatant was read at 560 nm. The percentage of membrane stabilization was determined using the formula

$$100 - \frac{(\text{Extract absorbance value} - \text{control 1 absorbance value})}{\text{Control 2 absorbance value}} \times 100$$

The control 1 represents 100% HRBC lysis. The HRBC membrane stabilizing standard drug used was diclofenac sodium.

Evaluation of *in vitro* COX-2 inhibitory activity

In vitro COX-2 inhibition was evaluated by the method described by Pradelles *et al*, 1985 [12]. The ability of the test compound to inhibit COX-2 (human recombinant) was determined by using enzymes immunoassay (EIA) kit (Catalogue No.560131, Cayman Chemical, Ann Arbor, MI, USA) according the Manufacturer's instructions. Cyclooxygenase catalyzes the first step in the biosynthesis of the arachidonic acid (AA) to PGH₂. PGF₂α produced from the PGH₂ by

reduction with stannous chloride, was measured by enzymes immunoassay (EIA). The test compound was dissolved in DMSO, and the solution was made at the final concentration of 10 μM. Reaction buffer solution (960μl, 0.1M Tris-HCL, pH-8 containing 5mM EDTA and 2 mM phenol) containing COX- 2 enzymes (10 μl) in the presence of heme (10 μl) was added with 10 μl of 10 μM test drug solution. These solutions were incubated for a period of 10 min at 37°C after then 10 μl of AA solution was added followed by stopping the

COX reaction by addition of 50 μ l of 1 M HCL. The $\text{PGF}_{2\alpha}$ produced from the PGH_2 by reduction with stannous chloride (100 μ l), was measured by enzyme immunoassay. This was based on the competition between PGs and PG-acetyl cholinesterase conjugation (PG tracer) for the limited amount of PG antiserum. The amount of PG tracer that is able to bind to the PG antiserum is inversely proportional to the concentration of PGs in the well since the concentration of PGs tracer is held constant while the concentration of PGs varies. This antibodies–PG complex bind to mouse anti-rabbit monoclonal antibodies that has been previously attached to the well. The plate is washed to remove any unbound reagents and then Ellman's reagents, which contain the substrate to acetylcholine esterase, are added to the well. The product of this enzymatic reaction produced a distinct yellow colour, determined by spectrophotometrically (Micro titre Plate reader) at 412 nm, is proportional to the amount of PG tracer bound to the well, which is inversely proportional to the amount of free PGs present in the well during the incubation:

$$\text{Absorbance} \propto [\text{Bound PG tracer}] \propto 1/\text{PGs}.$$

Percentage inhibition was calculated by the comparison of compound treated by control incubations.

Statistical analysis

Data were expressed as mean \pm SEM, where, $n = 3$, $p \leq 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSIONS

The membrane of lysosomal enzymes released during inflammation is similar to HRBC membrane, the protection of HRBC membrane from lysis induced by hypotonicity is the measure of anti-inflammatory

activity of the test drug. The present study has revealed that the aqueous extracts have capability of stabilizing red blood cell membrane lysis leading to anti-inflammatory effect. By performing *in vitro* anti-inflammatory studies by HRBC membrane stabilization method the percentage of membrane stabilization for aqueous extracts of barberry at a concentration of 500 μ g/ml was significant when compared to other extracts studied. The percentage protection of aqueous extracts at a concentration of 500 μ g/ml was comparable to 500 μ g of standard drug diclofenac. The percentage of membrane stabilisation of various extracts are represented in table 1. To determine the mechanism involved in the anti-inflammatory effect the extracts were studied for their *in vitro* COX-2 antiinflammatory by enzyme immunoassay method. The test extracts were studied by *in vitro* method using COX catalysed prostaglandin biosynthesis assay for COX-2 inhibitory capacity. Among all the herbal extracts studied for *in vitro* COX-2 inhibitory effect, the aqueous extract at a concentration of 500 μ g/ml was found to show promising COX-2 inhibitory response in comparative with other extracts. Results for COX-2 antiinflammatory activity are shown in table. 2.

CONCLUSION

The significant percentage protection of HRBC membrane indicated the anti-inflammatory capacity of the aqueous extracts of Barberry. From the results pertaining to COX-2 inhibitory response, it can be concluded that the mechanism involved for this anti-inflammatory activity of the aqueous extract of barberry roots may be by COX-2 inhibition. However, isolation of the active constituents from the crude extracts showing significant anti-inflammatory response and their detailed study may be helpful to develop potent COX-2 inhibitors with fewer adverse effects in comparison to modern anti-inflammatory drugs.

Table 1 Percentage of membrane stabilisation of various extracts.

Extract	% protection Concentration ($\mu\text{g/ml}$)	
	300	500
PEE	46.26 \pm 0.02	51.12 \pm 0.04
CE	35.56 \pm 0.03	39.48 \pm 0.04
EA	49.54 \pm 0.01	54.52 \pm 0.03
ME	59.91 \pm 0.02	62.56 \pm 0.02
AE	79.24 \pm 0.01	83.67 \pm 0.02
Diclofenac sodium (Standard)	84.73 \pm 0.01	88.45 \pm 0.01

PEE – petroleum ether extract. CE – chloroform extract, EAE – ethyl acetate extract, ME – methanolic extract, AE – aqueous extract.

Table 2 Percentage COX-2 inhibition of various extracts

Extract	% COX-2 inhibition Concentration ($\mu\text{g/ml}$)	
	300	500
PEE	56.11 \pm 0.03	60.43 \pm 0.02
CE	36.38 \pm 0.02	40.42 \pm 0.04
EA	44.65 \pm 0.03	50.28 \pm 0.02
ME	55.66 \pm 0.04	61.62 \pm 0.03
AE	73.66 \pm 0.02	78.12 \pm 0.01

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