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Evaluation of *in vitro* anti-inflammatory activity in *Berberis lyceum* Royle of Shivalik range of Himalaya

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Abstract
Berberis lyceum Royle is a traditional medicinal plant of the Shivalik range of Himalaya, India, and reported first time *in vitro* anti-inflammatory potentials in the root, stem, and leaves against denaturation of protein. The different concentration of *B. lyceum* Royle extract, pure berberine, and diclofenac as standard was incubated with albumin in controlled experimental conditions and subjected to determine the absorbance and to measure their viscosity. The assay is based on the denaturation of protein upon the heat treatment as the anti-inflammatory agent will stabilize or prevent the protein from denaturation. A concentration-dependent inhibition of protein (albumin) denaturation by the *B. lyceum* Royle and berberine was found in both. However, hydroethanolic extract *B. lyceum* Royle was found to more effective when compared to pure berberine and diclofenac. High anti-inflammatory effect of hydroethanolic extract due to the synergistic effect of other phytoconstituents present in *B. lycium* extract along with berberine. Therefore from the present study, we can conclude that both berberine and *B. lyceum* Royle possess anti-inflammatory activity.

Keywords: Anti-inflammatory, *Berberine lyceum* Royle, diclofenac, Berberine, protein denaturation, viscosity

Introduction
Inflammation is a pathophysiological response of the defense mechanism of the living system which is characterized by redness, edema, fever, pain, and loss of function as a result of any tissue damage, injury, or pathogen. This is a mechanism of defense however loss of regulatory mechanism i.e. when the inflammation is allowed to continued and unchecks it results in auto-immune or auto-inflammatory disorders, neurodegenerative disease, or cancer [1]. Steroidal anti-inflammatory drugs (SAID) and non-steroidal anti-inflammatory drugs (NSAID) are being used to treat acute inflammatory disease, but the success of these drugs is limited in case of chronic inflammatory disorders such as rheumatoid arthritis, atherosclerosis, type 2 diabetes, Alzheimer's disease, and atopic dermatitis. Also, the long-term use of these drugs can have an adverse effect too i.e., gastric ulcers, therefore there is a need for new and safe anti-inflammatory agents and plant-derived medicine are one of the ongoing research candidates. The advantages of plant-based medicines are their perceived efficacy, low cost, and less incidence of serious adverse effects [2, 3].
Berberis lyceum Royle belongs to the family of *Berberidaceae*, often used as a traditional medicine plant by the local people of the Himalayan region. It is an evergreen shrub commonly named Kilmoda or Daruhaldi because of the presence of yellow color pigment i.e. Berberine in the roots of the plant. The other chemical constituents found in *B. lycium* are berbamine, chinabine, karakoramine, palmatine, sindamine etc. however the berberine is the major bioactive compound that is mainly found in the roots of the plant, and an appreciable amount found in the leaves, stems, and berries [4] It is found to be effective for eye infection, diabetes, obesity, hemorrhoids, wound healing, dysentery, skin disease, uterine and vaginal disorder [5-7]. Berberine (5,6-dihydro-9,10-dimethoxybenzo(g)-1,3-benzodioxolo(5,6-a)quinolizinium) is an isoquionide alkaloid found in plants belong *Berberidaceae* family. It is a yellow-colored powder, odorless with a characteristic alkolodial taste [8]. It is a relevant molecule in pharmacology and medicinal chemistry. Berberine is now manufactured by chemical synthesis also. For clinical purposes, the sulfate or chloride salt of berberine is generally used. Berberine is also used for the synthesis of several bioactive derivatives by the means of condensation, modification, and substitution of functional groups in strategic positions for the design of new, selective, and powerful drugs [9, 10]. Various clinical and pre-clinical studies on berberine clinical show the curative and protective effect against many

disorders such as metabolic, neurological, and cardiological [11]. It also shows therapeutic implications in the treatment of rheumatoid arthritis through its anti-proliferative effect [12]. Therefore based on previous research on berberine, which is a major bioactive compound found in *B. lyceum* Royle it might be expected that the *B. lycium* has some anti-inflammatory properties. The present study was conducted to investigate the possible *in-vitro* anti-inflammatory effect of *B. lyceum* Royle and its comparative study with pure berberine against the denaturation of protein. This can provide us a natural source of anti-inflammatory agents.

Material and Methods

Chemicals and plant materials

B. lyceum Royle roots, stems, and leaves were collected from a village Narendra Nagar at 1020m altitude in height of Shivalik range of Himalaya, Uttrakhand. Berberine chloride was purchased from Sigma-Aldrich and Albumin was purchased from Sisco Research Laboratories Pvt Ltd. Diclofenac salt was purchased from Novartis pharmaceuticals. All the other chemicals were of analytical grade obtained commercially.

Preparation of extracts

Hydro-ethanolic extracts of the collected plant roots, stems, and leaves were prepared by the cold maceration method. Roots were ground into a fine powder and twenty-five grams of each plant was taken in 250ml of hydro-ethanol (1:1) and kept for 48 hrs at 37°C in a rotatory shaker. The extract was repeatedly filtered 2-3 times with muslin cloth and whattman filter paper then the sample was kept in a water bath at 40 °C for the evaporation of hydro-ethanol until a dark semisolid extract was prepared.

In-vitro anti-inflammatory assay

This assay is based on the denaturation of protein (Albumin) upon the heat treatment as the anti-inflammatory agent will prevent the protein from denaturation. In the present study, diclofenac is used as a standard drug with the concentration of 1000µg/ml, 500µg/ml, 250µg/ml, 100µg/ml, and 50µg/ml. The plant hydroethanolic extracts were reconstituted in distilled water and 1000µg/ml, 500µg/ml, 250µg/ml, 100µg/ml and 50µg/ml. Similar concentrations of pure berberine were prepared in warm distilled water. Albumin (0.1% w/v) and phosphate buffer of pH 6.4 were freshly prepared. The reaction mixture (5ml) consisted of 02.ml of egg albumin and 2.8ml of phosphate buffer saline and 2ml of varying concentrations of hydroethanolic extracts of *B. lyceum* Royle and berberine. A similar volume of distilled water served as control. The tubes were incubated at 37 °C in a BOD incubator for 15min and then heated at 70 °C for 10 minutes in a water bath. After cooling, their absorbance was measured at 660nm. (Chandra *et al*. 2012)

The percentage inhibition of protein denaturation was calculated by using the following formula.

$$\% \text{ inhibition} = 100 \times \frac{V1}{V2} 1$$

Where,
V1 = absorbance of the test sample,
V2 =absorbance of control.

Viscosity was measured in Ostwald viscometer by the following formula

$$\text{Viscosity} = \frac{\rho 1 t 1}{\rho 2 t 2} \times 1$$

Where, ρ1= specific density of test, ρ2 specific density of water, t1= flow time of test
t2= flow time of water

Inhibitory concentration IC50 value was calculated using the formula.

$$IC_{50} = \sum C / \sum L \times 50$$

$\sum \mathbf{C}$ is the sum of extract concentration used for testing and $\sum$L is the sum of the percentage of inhibition at different concentrations [14].

Results and Discussions

In the present study, *in vitro* anti-inflammatory effect of *B. lycium* root, stems and leaves extracts and pure berberine was evaluated against the heat denaturation of albumin. All three HE extract of *B. lycium* and pure berberine showed inhibition in a concentration-dependent of protein denaturation from concentration 50µg/ml to 1000µg/ml. The standard drug diclofenac also exhibited protein denaturation in an almost similar concentration-dependent manner. The highest percentage of protein denaturation inhibition was observed in berberine 99% with the concentration of 1000µg/ml and 98% in root and stem extracts *B. lycium* Royale extract at 1000µg/ml. However, in comparison with diclofenac, the hydroethanolic extract of *B. lyceum Royle* and berberine was found to more effective than even in the lower concentration i.e. 50µg/ml, 100µg/ml and150µg/ml. This is further supported by comparing the IC 50 values (Table 2). The lowest IC 50 (197µg/ml) value was found in the root extract of *B. lycium* Royale while the highest value is of diclofenac 287µg/ml. This may be attributed due to the synergistic effect of other constituents present in the extract along with berberine. The change in the viscosity due to denaturation of protein is also reported as a part of anti-inflammatory studies. The denaturation causes the increase in viscosities of protein solution. Chandra *et al*. (2012) also reported that the anti-denaturation effect was also determined by the change in viscosity of the protein-containing sample after the heat treatment. In the present study, it was observed that the viscosity of the control was highest among all the test and standard samples which suggest the prevention of heat denaturation of protein by the presence of extract and drug in the protein solution. However, the changes in viscosity were also found affected with respect to the dilution of the diclofenac and extracts. Therefore it requires further studies on the viscosity behavior of denatured protein during *in-vitro* studies with respect to the concentration effect of the test.

The stabilization and prevention of denaturation of albumin on heat treatment at the physiological pH are reported as one of the features of several non-steroidal anti-inflammatory drugs [15]. Muzushima and Kobayashi, [16] reported that NSAID influences the conformational changes suffered by some proteins on heating. Various studies on the plant extract also reported their anti-inflammatory activity *in vitro* and *in vivo* although the exact mechanism of their action is not fully understood. One of the important mechanisms is inhibition of eicosanoid generating enzymes including phospholipase A2, cyclooxygenases, and lipoxygenases, thereby reducing the concentrations of prostanoids and leukotrienes which plays

important role in the inflammatory response. The plant flavonoids express their anti-inflammatory activity by modulation of pro-inflammatory gene expressions such as cyclooxygenase-2, inducible nitric oxide synthase, and several pivotal cytokines [17].

Table 1: Effect of diclofenac, hydro-ethanolic extract of *B. lycium* Royle root (BLR), stems (BLS), leaves (BLL) and Berberine (BBR) on protein denaturation on the protein denaturation

Concentration (µg/ml)	% inhibition	Viscosity (cp)
Control	-	1.10
DC 1000	91	1.08
DC 500	80	1.06
DC 250	72	1.01
DC 100	53	1.03
DC 50	34	0.97
BBR 1000	99	1.03
BBR 500	98	0.99
BBR 250	93	0.98
BBR 100	88	0.98
BBR 50	78	0.96
BLL 1000	97	1.03
BLL 500	91	0.98
BLL 250	86	0.98
BLL 100	81	0.98
BLL 50	67	0.96
BLS 1000	98	1.01
BLS 500	98	0.99
BLS 250	93	0.98
BLS 100	91	0.94
BLS 50	87	0.96
BRR 1000	98	1.02
BRR 500	98	1.01
BRR 250	97	0.98
BRR 100	95	0.96
BRR 50	92	0.97

Table 2: IC_{50} Value of hydroethanolic extract of *B. lycium* Royle and diclofenac for denaturation of protein. BBR: Berberine, BLR: root, BLS: stems, BLL leaves

Treatments	IC 50 Values (µg/ml)
Diclofenac	287
BBR	208
BLR	197
BLS	203
BLL	225

Conclusions

The present study concluded that the hydroethanolic extract and the berberine both possess the anti-inflammatory effect against the denaturation of albumin. *B.* lyceum Royle has shown to be more effective as compared to pure berberine. This may be attributed to the synergistic effect of other phytoconstituents present in the extract along with berberine. Since this is a preliminary study that needs to be more investigated in both *in-vivo* and *in vitro* to elucidate the mechanism involved in it.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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