ANTI-INFLAMMATORY ACTIONS OF *BERBERIS LYCIUM* (WHOLE PLANT) IN ACUTE AND CHRONIC MODELS OF INFLAMMATION IN MICE

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ABSTRACT

Berberis lycium Royle (Berberidaceae) is used in the traditional system of medicine for the treatment of rheumatism. The purpose of the current study was to evaluate the anti-inflammatory activity of *Berberis lycium*, to explore its medicinal use in inflammatory ailments. *Berberis lycium* crude extract (Bl.Cr) was investigated in acute (carrageenan and xylene mediated) and chronic (formalin evoked) models of inflammation in mice. Bl.Cr tested positive for alkaloids, carbohydrates, flavonoids, glycosides, phenols, proteins, saponins, steroids and tannins. Bl.Cr caused a dose-dependent (10, 30 and 50 mg/kg i.p) reduction against carrageenan-induce paw edema ($P < 0.001$ vs. saline group). Bl.Cr (10, 30 and 50 mg/kg i.p) decreases xylene-mediated ear edema (30.64, 54.83 and 72.58 % respectively; $P < 0.001$ vs. saline group). In chronic inflammation, Bl.Cr (10, 30 and 50 mg/kg i.p) exhibited a dose-dependent reduction in inflammation ($P < 0.05$, $P < 0.01$, $P < 0.001$ vs. saline group). Diclofenac sodium (20 mg/kg), a standard drug also showed similar effects ($P < 0.01$ vs. saline group). These results indicate that *Berberis lycium* possesses significant anti-inflammatory activity, thus providing scientific evidence for its folkloric use in the treatment of rheumatism.

Key words: *Berberis lycium* whole plant extract, inflammation models in mice, anti-inflammatory activity, diclofenac sodium, rheumatism.

INTRODUCTION

Rheumatism includes a wide range of disorders marked by inflammation, degeneration and pain, affecting connective tissues structures, specially joints and related structures (Dorland, 2007). Inflammatory cytokines IL-1, IL-2, leukotriene-B_4 and TNF-α have major role in the pathogenesis (Davidson *et al*., 1983; Rang *et al*., 2007). Other mediators involved in inflammation includes prostaglandin, nitric oxide, serotonin, bradykinin, leukotrienes and histamine (Banasik, 2000; Chandrasoma and Taylor, 2005). Uncontrolled and persistent inflammation contributes to the progression of many chronic diseases such as multiple sclerosis, rheumatoid arthritis, atherosclerosis, psoriasis and inflammatory bowel disease (Talwar *et al*., 2011). The conventional therapy used for the treatment of inflammation and rheumatoid disorders includes non-steriodal anti-inflammatory drugs and disease modifying anti-rheumatic drugs. However, these drugs produces undesirable side effects such as bleeding, peptic ulcer formation and perforation of the gastric mucosa (Silverstein *et al.,* 1995). There is still need of drugs which produce less side effects and are more effective than the currently available drug.

Berberis lycium commonly known as Barberry is the member of the genus Berberis belongs to family Berberidaceae (Sabir *et al*., 2013). It is native to Pakistan, India and whole regions of Himalayas (Shabbir *et al*., 2012). Traditionally, *Berberis lycium* is used against diarrhea, intestinal colic, piles, jaundice, internal wounds, rheumatism, diabetes, ophthalmic, gingivitis, throat pain, backache, scabies, bone fracture, sun blindness, pustules, menorrhagia, fever, expectorant and as diaphoretic (Jabeen *et al*., 2015). Plant of the genus Berberis, *Berberis vulgaris* and *Berberis aristata* have been examined for anti-inflammatory activity (Ivanovska and Philipov, 1996; Potdar *et al.,* 2012). This plant is rich in medicinally important phytochemical constituents like berberine (Ali and Khan, 1978), umbellatine (Baquar, 1989), plamitine (Gosh *et al*., 1990), baluchistanamine, karakoramine, gilgitine, jhelumine, punjabine, sindamine, chinabine (Manske, 1998), berbamine (Khare, 2004), β-sitosterol, 4-4,dimethylhexadeca-3-ol, Butyl-3-hydroxypropylphthalate, 3-(4'-(6-methylbutyl) phenyl) propan-1-ol (Sabir *et al*., 2013). *Berberis lycium* has been reported to possess pesticidal (Tewary *et al*., 2005), anti-bacterial, anti-fungal (Singh *et al*., 2007), anti-diabetic (Gulfraz *et al*., 2007), anti-hyperlipidemic (Chand *et al*., 2007), wound healing (Asif *et al*., 2007), anti-coccidial, immunomodulatory (Nidaullah *et al*., 2010), anti-mutagenic (Khan *et al*., 2010) and anti-oxidant (Mashwani *et al*., 2013) properties. In this investigation, we evaluated the anti-inflammatory activity of *Berberis*

lycium with aim to rationalize the traditional use in rheumatism.

MATERIALS AND METHODS

Plant material and extraction: The whole plant of *Berberis lycium* was collected from Kashmir, Pakistan in the July-August, 2015 and was identified by Dr. Mushtaq Ahmad taxonomist at Quaid-e-Azam University, Islamabad. The sample specimen bearing voucher # 98730 was deposited to herbarium of the Plant Sciences Department, Quaid-e-Azam University. Plant was thoroughly washed, dried at room temperature and coarsely ground. The grinded powder (5 kg) was extracted for seven days with 70% methanol with mixing at regular interval. The macerated plant material was filtered (Williamson *et al*., 1998). The filtrate was concentrated on a rotary evaporator, to obtain the thick semi-solid paste under reduced pressure i.e. *Berberis lycium* crude extract (Bl.Cr), yielding 6.75 % (w/w).

Chemicals: Carrageenan (SigmaChemicals Co, St Louis, MO, USA), diclofenac sodium (Olive Laboratories National Industrial Zone Rawat, Islamabad), ethanol, formalin (37% v/v), and xylene (Merck, Darmstadt, Germany).

Animals: All the experiments performed on Balb-C mice (25-35 g), divided in different groups, 5 in each group. Animals were kept at the animal house of Riphah Institute of Pharmaceutical Sciences. They were housed at 23-25^{o}C with 12 h light and dark cycle and received standard diet and water *ad libitium*. All experiments performed were in accordance with the guidelines of Institute of Laboratory Animal Resources, Commission on Life Sciences (NRC, 1996) and approved by Ethical Committee of Riphah Institute of Pharmaceutical Sciences, Riphah International University (Ref.#: REC/RIPS/2015/001).

Phytochemical analysis: The *Berberis lycium* crude extract was tested, for various chemical constituents according to established protocols. Plant material when treated with sodium hydroxide, appearance of yellow color indicates the presence of flavonoids and tannins were positive, when cream yellow color was produced with lead acetate (Edeoga *et al*., 2005). Proteins were detected using ninhydrin reagent, blue color indicated presence of proteins. Steroids were considered positive, if plant material treated with chloroform and sulfuric acid subsequently produced red coloration. Dragendroff's reagent was used for detection of alkaloids, formation of red precipitates is considered positive (Harborne, 1984). Saponins were positive, when froth formation of diluted sample occurs, on vigorous shaking. When extract treated with ferric chloride, appearance of black color indicates the positive result for phenols (Yadav *et al*., 2011). Glycosides were detected using keller-killiani test, brown ring formation at the interface was considered positive. Carbohydrates were detected using Molisch's test, violet ring formation indicates the presence of reducing sugars (Evans, 1996).

Carrageenan-induced hind paw edema: Overnight fasted mice were used in the study. The paw volume displacement was determined before administration of any drug using Plethysmometer, Ugo Basile, Italy (Padilha *et al*., 2010). Mice with similar paw volume displacement were placed in one group (n=5, each group). Acute inflammation was induced by 0.1 mL (1% w/v solution in normal saline) of carrageenan sub-plantar injection. Half an hour prior to carrageenan injection, Normal saline (10 mL/kg) was given to first group, served as control. Bl.Cr (10, 30 and 50 mg/kg i.p) were given to second, third and fourth group and diclofenac sodium (20 mg/kg) to fifth group. The paw volume displacements were measured with one hour interval, up to 5 hour following carrageenan injection.

Xylene-induced ear edema: Mice were divided in to different groups. Normal saline (10 mL/kg) was administered to 1st group served as control, Bl.Cr (10, 30 and 50 mg/kg i.p) given to 2nd, 3rd and 4th group and diclofenac sodium (20 mg/kg) to 5th group. Half an hour after administration of extract and standard drug, acute inflammation induced by applying 30 µL of xylene to right ear (Atta and Alkofahi, 1998). The left ear of the same mouse served as control. Two hour later, mice were sacrificed by cervical dislocation and both ears were excised. Circular sections cut with the help of cork borer and weighed. The ear weight changes produced by irritant was calculated as the difference of ear weight of the right and left ear (treated vs. untreated) and % inhibition was calculated as follows:

$$\% \text{ Inhibition} = [(W_c - W_t)/W_c] \times 100$$

Where, W_c = Ear weight difference of control, W_t = Ear weight difference of treated groups.

Formalin-induced arthritis inflammation: The anti-inflammatory activity against chronic inflammation was determined by formaldehyde-induced arthritic inflammation (Cho *et al*., 2011). Inflammation induced by sub-aponeurotic injection of 0.1 mL of formalin (2% v/v formaldehyde) in left hind paw on 1st and 3rd day. Treatment with the drug and extract started on the 1st day and continues for 10 days. 1st group treated with normal saline (10 mL/kg), served as negative control, group II – IV were treated with Bl.Cr (10, 30 and 50 mg/kg) and diclofenac sodium (20 mg/kg) was given to 5th group. The changes in paw sizes were measured on daily basis and continue up to 10 days (Hosseinzadeh *et al*., 2000).

Acute toxicity test: Mice divided in three groups. Saline (10 mL/kg) was given to 1st group, served as negative control. Plant extract in increasing doses were

administered (10 mL/kg volume) to 2nd and 3rd group. The mice were given standard diet and water *ad libitum* and observed for 24 hrs. The number of deaths was counted (Sanmugapriya and Venkataraman, 2006).

Statistical analysis: Data expressed are mean ± standard error of mean (SEM). All the results were analyzed by one-way analysis of variance (ANOVA) with Tukey post-hoc test and considered statistically different at $P < 0.05$. The bar-graphs were generated by Graphpad prism (GraphPAD, San Diego, CA, USA).

RESULTS

Preliminary phytochemical screening: Bl.Cr was tested positive for presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols, proteins, saponins, steroids and tannins.

Effect on carrageenan-induced paw edema: The subplantar injection of carrageenan produce edema which progressively increases with time in the saline treated control group. Peak edema development 3 hour (0.42 ± 0.01). Treatrment of animal with Bl.Cr at the dose of 10, 30 and 50 mg/kg reduced carrageenan-induced paw edema in a dose dependent manner ($P < 0.001$ vs. saline group). Similarly, diclofenac sodium (20 mg/kg) reduced the carrageenan-induced paw edema (Figure 1).

Effect on xylene-induced ear edema: Bl.Cr dose-dependently (10-50 mg/kg) caused reduction of xylene-induced ear edema. Bl.Cr at dose of 10, 30 and 50 mg/kg produced 42, 57.14 and 71.42% inhibition of ear edema, respectively. Diclofenac sodium (20 mg/kg) caused 74.19 % inhibition of ear edema (Table 1).

Effect on formalin-induced arthritis inflammation: A significant increase in the left hind paw thickness was observed in the saline treated control group after formalin injection. Continuous treatment with Bl.Cr (10-50 mg/kg) and diclofenac sodium (20 mg/kg) remarkably reduces the paw edema. The reduction in paw thickness was observed from the day 1st and throughout the period of study (10 days) compared with saline treated group ($P < 0.05$, $P < 0.01$, $P < 0.001$ vs. saline group) as shown in Figure 2.

Acute toxicity test: The two groups of mice were administered Bl.Cr in graded doses of 100 and 300 mg/kg respectively and animals were observed for mortality up to 24 hrs. No mortality observed at the dose of 100 mg/kg while it caused 50 % deaths at dose of 300 mg/kg dose.

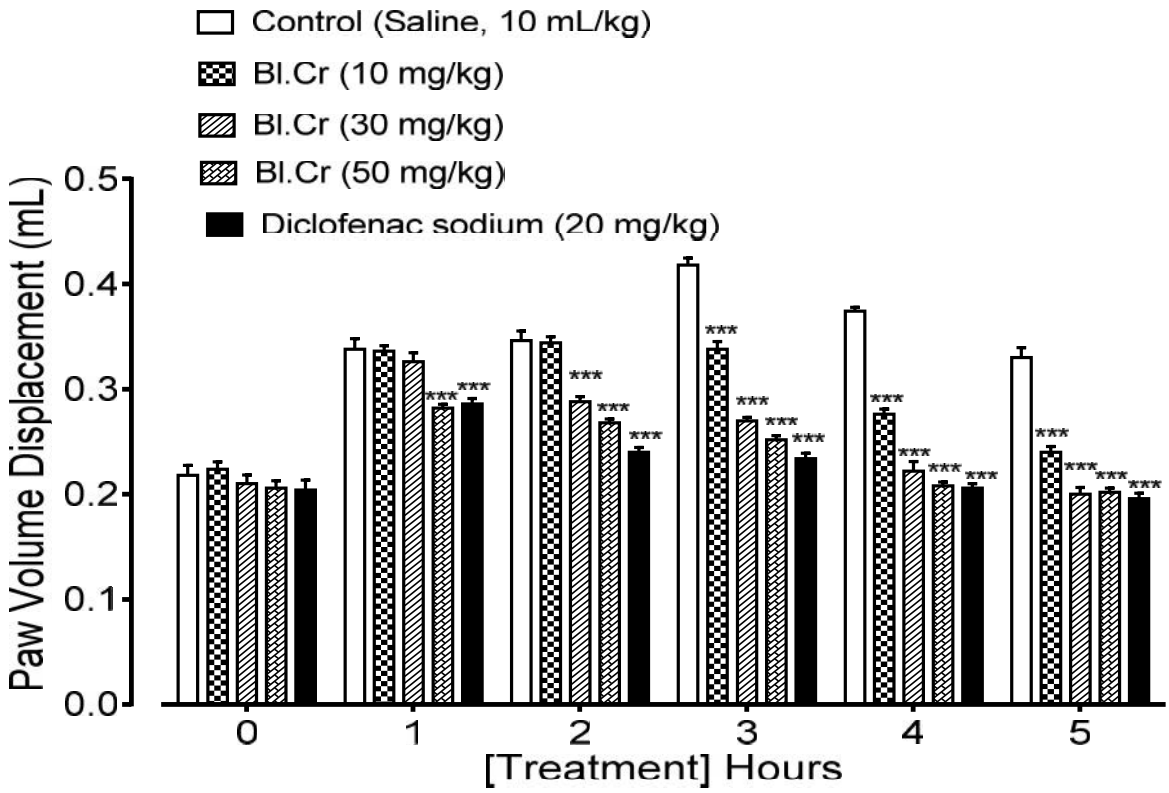

Figure 1. Effect of *Berberis lycium* crude extract (Bl.Cr) and diclofenac sodium on carrageenan- induced paw edema in mice. Values shown are mean ± SEM, n=5. ***P < 0.001 vs. saline group, one-way analysis of variance with post-hoc Tukey test.

Table 1. Effect of *Berberis lycium* (Bl.Cr) and diclofenac sodium on xylene-induced ear edema in mice.

Treatment	Weight of right ear (g)	Weight of left ear (g)	Differences (g)	Inhibition (%)
Control (Saline, 10 mL/kg)	0.12 ± 0.01	0.05 ± 0.00	0.07 ± 0.00	-
Bl.Cr (10 mg/kg)	0.13 ± 0.003	0.09 ± 0.00	0.04 ± 0.00***	42
Bl.Cr (30 mg/kg)	0.11 ± 0.01	0.08 ± 0.01	0.03 ± 0.00***	57.14
Bl.Cr (50 mg/kg)	0.11 ± 0.005	0.09 ± 0.01	0.02 ± 0.00***	71.42
Diclofenac sodium (20 mg/kg)	0.10 ± 0.00	0.08 ± 0.00	0.02 ± 0.00***	71.42

Values are expressed as mean± SEM, n=5. ***P < 0.001 vs. saline group, one way analysis of variance with post-hoc Tukey test.

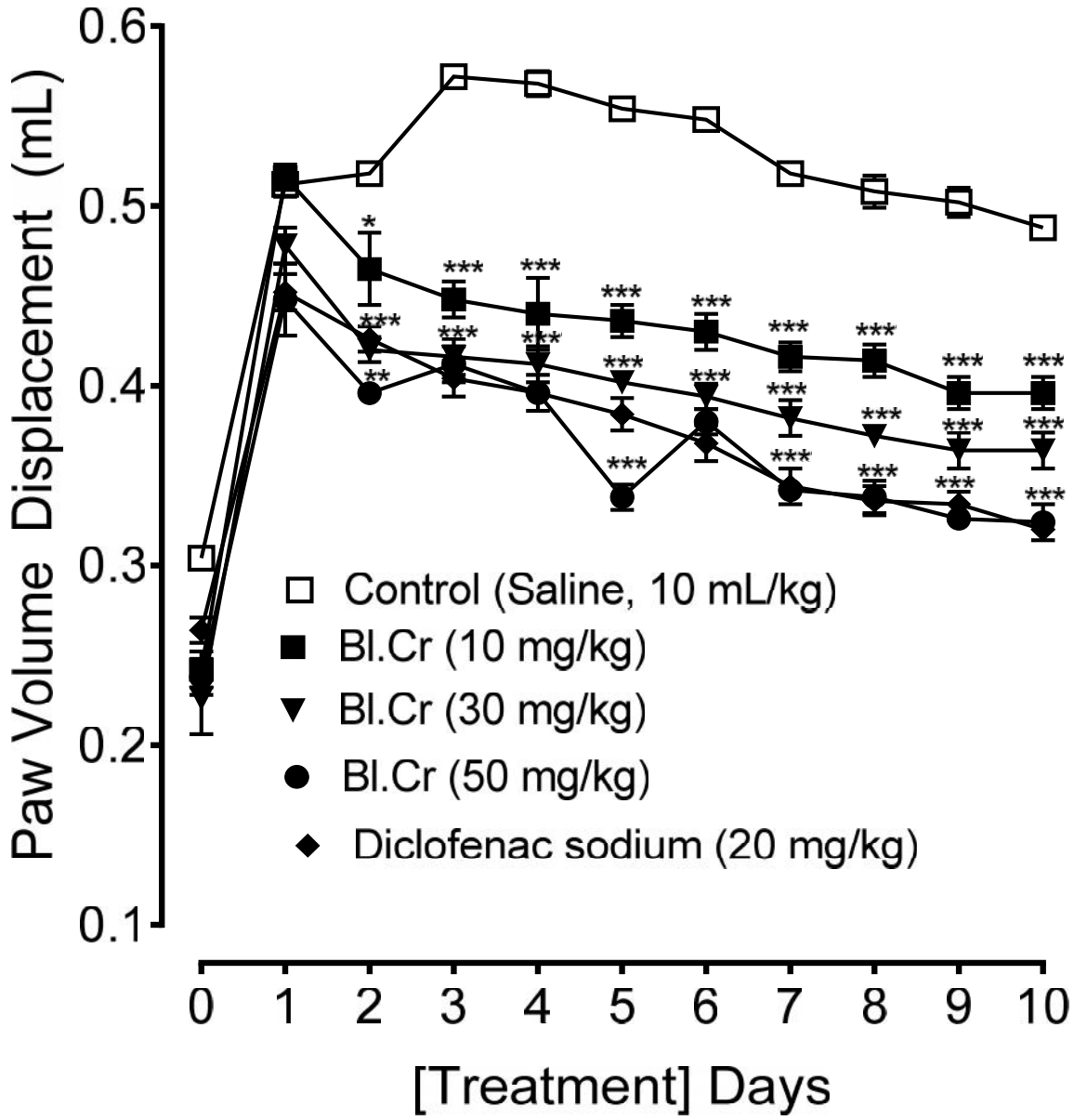

Figure 2. Effect of *Berberis lycium* crude extract (Bl.Cr) and diclofenac sodium on formalin-induced arthritis in hind paw of mice. Values shown are mean ± SEM, n=5. *P < 0.05, ** P < 0.01, ***P < 0.001 vs. saline group, one-way analysis of variance with post-hoc Tukey test.

DISCUSSION

In view of folklore use of *Berberis lycium* in inflammatory conditions such as rheumatism, its extract was evaluated for possible anti-inflammatory action against acute (carrageenan and xylene mediated) and chronic (formalin evoked) models of inflammations in mice, to rationalize aforementioned traditional use of the plant. Carrageenan-induced edema is a well-established *in-vivo* animal model to ascertain the anti-inflammatory effect (Mossa *et al*., 1995; Bukhari *et al*., 2007). Carrageenan induces inflammation through release of histamine, serotonin (early phase), prostaglandins and bradykinin (later phase) (Burch and DeHaas, 1990). These mediators involve in inflammatory response and also induce pain (Rosa *et al*., 1972). It is reported that agents which produce reduction in carrageenan-induced edema, causes inhibition of prostaglandins synthesis via inhibiting the cyclooxygenase (COX) enzyme (Skoutakis *et al*., 1988; Selvam and Jachak, 2004). Bl.Cr in a dose-dependent fashion caused inhibition of carrageenan-induced paw edema, like that exhibited by diclofenac sodium, a standard non-steroidal anti-inflammatory drug (NSAID), which reduces inflammation, swelling and arthritic pain by inhibiting prostaglandins synthesis via inhibition of COX enzyme in the arachadonic acid pathway (Grosser *et al*., 2011). The plant extract was found effective in inhibiting the carrageenan-induced inflammation after 1 hour of administration and effect continuous up to 5th hour. Based on these findings; it can be ascribed that the anti-inflammatory effect of *Berberis lycium* might be possibly occurred via inhibition of prostaglandin synthesi When tested against xylene-induced inflammation, Bl.Cr at dose of 10 mg/kg reduced ear edema induced by xylene while at 30 mg/kg dose, the effect was similar to that of diclofenac sodium. Xylene induces inflammation involving release of various inflammatory mediators, such as kinins, histamine and fibrinolysin, which further causes vasodilation and increase vascular permeability (Li *et al*., 2011). The effectiveness of the plant extract against xylene-induced inflammation may be due to inhibition of phospholipase A_2, which is involved in pathophysiology of inflammation (Sofidiya *et al*., 2014). Bl.Cr was further investigated against formalin-induced chronic inflammation. It is well known that inhibition of edema induced by formalin is simple model to screen anti-arthritic and anti-inflammatory agents, as it resembles human arthritis (Igbe *et al*., 2010). Two sub-aponeurotic injections of formalin were applied to induce inflammation, characterized by increased paw thickness and volume as chronic inflammation responses (Cho *et al*., 2011). These markers were used for testing anti-inflammatory activity. Bl.Cr, like diclofenac sodium was found significantly effective in reducing the formalin-induced cell damage. The results shows that Bl.Cr was found to be effective against acute inflammation (carrageenan-induced paw edema and xylene-mediated ear edema) compared with chronic inflammation (formalin induced paw edema). Free radical generation plays a pivotal role in the pathophysiology of inflammation (Patrono and Rocca, 2009). *Berberis lycium* has been known to possess strong anti-oxidant activity, which is complementing the anti-inflammatory potential of this plant. The presence of flavonoids and phenols may account for the observed anti-inflammatory effect of *Berberis lycium,* as phytochemicals of such classes are known for anti-inflammatory potential (Orhan *et al*., 2007) however, the role of other constituents cannot be ignored. *Berberis lycium* has known to possess berberine, berbamine, palmitine and β-sitosterol as major constituents and these phytochemicals have been validated for anti-inflammatory activities in several studies (Li *et al*., 1999; Liu *et al*., 2010; Loizou *et al*., 2010, Singh *et al*., 2010). The anti-inflammatory effect of *Berberis lycium* may be attributed to its phyto-constituents profile.

Conclusion: The results of the experiment showed that the crude extract of *Berberis lycium* (Bl.Cr) has been more effective against acute inflammation (carrageenan-induced paw edema and xylene-mediated ear edema) than the chronic inflammation (formalin-evoked arthritic inflammation). The results from the present study validates the traditional use of *Berberis lycium* in rheumatism. Further in-depth advance molecular studies are warranted to probe nature of chemicals constituents and to confirm pharmacodynamic basis of the pharmacological action.

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