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Evaluation of anti-inflammatory and analgesic activity of methanolic extract of *Berberis lycium* Royle

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Abstract

Objectives: The aim of study was to investigate the anti-inflammatory and analgesic effects of methanolic extract of the shade dried fruits of *Berberis lycium* by oral administration at doses of 250 and 500 mg/kg of body weight in healthy Wistar albino rats.

Methods: The extract was studied for its anti-inflammatory activity in carrageenan-induced and histamine induced hind paw edema in rats and the linear paw circumference was measured at 0, 30, 60, 90 and 120 min. after the administration of phlogistic agent. The methanolic extract was also evaluated for analgesic activity using Eddy's hot plate and Haffner's tail-clip method in Wistar albino rats. The reaction time was taken as the parameter for the evaluation of analgesic activity at time intervals 0, 30, 60, 90 and 120 min. respectively.

Key findings: The methanolic extract of *Berberis lycium*, significantly ($p < 0.05$) reduced carrageenan-induced and histamine induced paw edema in rats. The methanolic extract has also shown analgesic activity as evidenced by increase in the reaction time by Eddy's hot plate and Haffner's tail-clip method in rats.

Conclusion: The anti-inflammatory and analgesic effects of methanolic extract may be due to inhibition of the prostaglandin synthesis.

Keywords: Anti-inflammatory, analgesic activity, methanolic extract of *Berberis lycium* Royle

Introduction

Inflammation or phlogosis is defined as a pathophysiological response of living mammalian tissue to injury from any agent and leads to the local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism to eliminate or limit the spread of injurious agent, but the complex events and mediators involved in the inflammatory reaction can be induced, maintain or aggravate many diseases ^[1]. Sherrington defined pain as "the *physical* (pertaining to mind) *adjunct* (joined to) of an imperative protective reflex", i.e. pain is a sensation which draws attention of the individual as a whole. Pain is also defined as an unpleasant and emotional experience associated with or without actual tissue damage. Pain is an important sensory symptom. Though it is an unpleasant sensation, it has protective benefits by giving warning signal about the existence of a problem or threat.

Due to extensive use of analgesic and anti inflammatory agents, the toxicity and untoward effects do occurs many times especially when therapy of pain, inflammation and fevers involves use of higher dose for longer period. The side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical use ^[2]. Therefore, there arises a need for the development of newer and more powerful anti-inflammatory and analgesic drugs with lesser side effects.

Berberis lycium is an evergreen shrub belongs to family Berberidaceae. *Berberis lycium* was described in 1837 by John Forbes Royle. The family Berberidaceae was first established by (Jussieu A.L.) as 'Berberides' ^[3]. *Berberis lycium* is native to India, Nepal, Pakistan and globally distributed in various parts of world. In India, It occurs in sub-tropical and temperate regions from Kashmir to Uttaranchal on the outer northern-western Himalayas between altitude ranges of 850 - 3500 metres. The various parts of the plant like root, bark, stem, leaves and fruits are used by the people as a medicine or food. This plant has also gained wide acceptance for its medicinal value in ayurvedic drugs.

The plant is known to prevent liver disorders, abdominal disorders, skin diseases, cough, ophthalmic etc.

The various chemical constituent of *Berberis lycium* are berberine, berbamine, chinabine, karakoramine, palmatine, balauchistanamine, gilgitine, jhelumine, punjabine, sindamine, chinabine, acetic acid, maleic acid, ascorbic acid [4]. Phytochemical screening of water extract of *Berberis lycium* by showed the presence of Cardiac glycosides, Saponins, Hydrolysable Tannins, and Alkaloids [5]. The fruits contain malic, tartaric, citric acids and tannins [6]. Fruits also contain moisture, vitamin A, fiber content, cellulose, hemicelluloses, β carotein, anthocyanins, phytic acid and phytate phosphorus [7]. The phytochemical constituents like berberine and palmatine have already shown anticancer activity. Berberine showed positive anti-inflammatory invivo and invitro study in Wistar rats [8]. It has also showed reduced prostaglandin E2 (PG E2) production. Aqueous and methanol root extracts of *Berberis lycium* royle of the plant were studied for the wound healing activity using excision, incision and dead wound space models of wound repair [9]. Berberine has been stated to have potential therapeutic implication in the treatment of RA due to its anti-proliferative effect against rheumatoid arthritis fibroblast like synoviocytes, [10]. Hence based on these observations and evidences it might be expected that *Berberis lycium* may show the anti-inflammatory and analgesic activity.

Collection of Plant

The ripened fruits of plant *Berberis lycium* were collected directly from the plants in the month of August 2017 from the plants growing naturally in Jammu and Kashmir (Dist. Baramulla). The fruit of the plant was identified by Botanist Prof. Gh. Mohideen. The fruits were washed with tape water and then dried under the shade at room temperature.

Preparation of The Fruit Extract

The fruits of the plant were air dried at room temperature under shade for between 10-12 days and then crushed into coarse powder with a pestle and mortar, the seeds were separated from fruit mass. Maceration was used for the process of extraction using methanol as solvent. About 250 g of the powdered fruits were dissolved in 600 ml of 80% methanol in a 1000 ml conical flask and exhaustively extracted for a period of three days (72 hours) with accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material followed by a second filtration through whatman's no. 1 filter paper, the marc was pressed between filter paper to squeeze out the absorbed solvent. The resulting solution was concentrated on rotary evaporator under reduced pressure at 5 to 6 rpm and at 60 °C temperature. A semisolid gummy concentrate of dark greenish black color was obtained after evaporation of solvent that was designated as crude extract or methanolic extract of *Berberis lycium* (MEBL). The crude methanolic extract was finally dried by freeze drier and preserved. The samples were stored at 10°C till further use.

Acute Toxicity Study

LD₅₀ (lethal dose) determinations were conducted using Lorke's methods (1983) for oral routes in rats [11]. This method was carried out in two phases. In the initial phase, 2 groups each containing two rats were treated with methanolic leaf extract of the plant at doses of 500 and 1000 mg/kg body weight orally and were observed for signs of toxicity and death for 24 hours. In the second phase, 3 groups each

containing one rat were administered with three more specific doses of the extract 1600 mg, 2000 mg, and 3000 mg/kg body weight based on the result of phase one (initial phase). The LD₅₀ value was determined by calculating the geometric mean of the animal survived (0/1 and 1/1). *i.e* the highest non lethal dose (that did not cause death) and the lowest lethal dose for which the rat died.

$$LD_{50} = \sqrt{(\text{Highest non lethal dose}) \times (\text{Lowest lethal dose})}$$

Experimental Animals

Wistar albino rats of both sexes weighing between 100-250 g were obtained from Geetanjali Medical College & Hospital, Udaipur and kept at the Laboratory Animal centre of the college. The animals maintained under standard environmental conditions had free access to standard diet and water *ad libitum*. Rats were housed in groups of six per cage. All the animals were maintained under standard conditions; that is room temperature 26 ± 1 °C, relative humidity 45-55% and 12:12 hours light-dark cycle. The cages were maintained clean, and all experiments were conducted between 9 am and 4 pm.

Ethical Approval

The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) and all the experiments were conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Grouping and Dose Selection

Rats were randomly divided into four groups with each group consisting of six rats. Group I served as a control group were administered with 2 ml/kg (*p.o*) of distilled water. Groups II and III were given 250 and 500 mg/kg of the extract, respectively. Group IV received standard drug 10 mg/kg of Indomethacin for anti-inflammatory models and 10 mg/kg of diclofenac for analgesic models. Administration of all agents was performed *via* oral route using gavage.

Determination of Anti-Inflammatory Activity Carrageenan induced paw edema

The anti-inflammatory activity of the test compounds were evaluated in Wistar rats employing the method suggested by Diwan *et al.*, 1989. Anti inflammatory activity of aqueous methanolic extracts in the dose of 250 and 500 mg/kg was evaluated against carrageenan induced paw edema model in rats. Twenty four animals were divided into four groups consisting of six animals each. Group I served as control group and received distilled water (2 ml/kg *p.o.*), Groups II and III received aqueous methanolic extract of *Berberis lycium* at 250 and 500 mg/kg dose through oral route, respectively. Group IV received standard drug Indomethacin in a dose of 10 mg/kg *p.o*. One hour after the treatment, edema was induced by injection of carrageenan (0.1 ml, 1%, w/v in saline) into the subplantar tissue of the right hind paw. The paw volume was then measured at 0, 30, 60, 90 and 120 min of the administration of phlogistic agent, using the Plethysmometer. The following formula was used to calculate percentage of inhibition.

$$\text{Percentage of inhibition} = 100 (1 - V_t / V_c)$$

Where, V_t and V_c represent edema volume of treated and control animals, respectively [12, 13].

Histamine induced paw edema

Wistar rats weighing between 150 - 250 g were used for the study and fasted overnight prior and during the experiment but have free access to water. Anti inflammatory activity of aqueous methanolic extracts in the dose of 250 and 500 mg/kg was evaluated against carrageenan induced paw edema model in rats. The rats were divided into 4 groups of 6 animals each. Group I served as control group and received distilled water (2 ml/kg *p.o.*), Groups II and III received aqueous methanolic extract of *Berberis lycium* at 250 and 500 mg/kg dose through oral route, respectively. Group IV received standard drug Indomethacin in a dose of 10 mg/kg *p.o.* One hour after the treatment, edema was induced by injection of histamine (0.1 ml, 1%, w/v in saline) into the subplantar tissue of the right hind paw. The paw volume was then measured at 0, 30, 60, 90 and 120 min of the administration of phlogistic agent, using the Plethysmometer. The following formula was used to calculate percentage of inhibition.

$$\text{Percentage of inhibition} = 100 (1 - V_t / V_c)$$

Where, V_t and V_c represent edema volume of treated and control animals, respectively [14, 15].

Determination of Analgesic Activity

Eddy's Hot plate method (thermal method)

Evaluation of analgesic activity of the plant extract was carried out using hot plate method [16]. Following administration according to respective grouping, the rats were placed on a hot plate maintained at $55 \pm 1^\circ\text{C}$. The latency of nociceptive response such as licking, flicking of a hind limb or jumping was measured [17]. The reaction time in control and treated animals was recorded at 0, 30, 60, 90 and 120 min after drug administration. The cut-off time is 15 sec. as to avoid lesions to the animals' paws [18].

Haffner's Tail clip method (mechanical method)

Twenty four Wistar albino rats were used in the experiment. The selected animals were divided into four groups each of six animals. A metal artery clip was applied to the root of mouse's tail to induce pain [19]. A sensitivity test was carried out and animals that did not attempt to dislodge the clip within 15 seconds were discarded. Analgesic activity was evaluated 0, 30, 60, 90 and 120 min after oral administration of the extracts and controls. A metal artery clip is placed at the root of tail (1 cm from the body) and a positive analgesic response was indicated if there was no attempt to dislodge the clip within 5 sec. in any of the five consecutive trials. The reaction time between application of the clip and response is noted by a stop watch. The mean value was evaluated [20].

Statistical Analysis

The results are expressed as the means \pm standard error of mean (SEM). Parametric data were compared to control group and were assessed by the method of one-way ANOVA followed by Dunnett's Multiple Comparisons Test using Graph pad Prism-7. Values $p < 0.05$ was considered as statistically significant.

Results

Carrageenan induced paw edema

The results shown by methanolic extracts of *Berberis lycium* against carrageenan-induced Paw edema are given in Table 1, Fig. A. Methanolic extract at dose of 250 mg/kg showed non-significant reduction of paw oedema (13.36%), but in aqueous methanolic extract administered at a dose of 500 mg/kg, the

paw volume were reduced by (27.92%). This result demonstrated dose time related significant reduction by aqueous methanolic extract. Indomethacin 10 mg/kg, similarly produced significant inhibitory effect of the paw edema (43.50%) as compared to normal control group.

Histamine induced paw edema

In this method, histamine was used to induce paw oedema in rats. The animals were treated with the extracts of the leaves of *Berberis lycium* at the doses of 250 mg/kg, 500 mg/kg and Indomethacin 10 mg/kg respectively and results are tabulated in Table 2, Fig. B. Among the two doses of *Berberis lycium* extract, 500 mg/kg showed maximum reduction in the paw volume (35.14%), while methanolic extract at 250 mg/kg does not show any significant reduction in the paw volume (20.49%) induced by histamine as compared to control group. Indomethacin 10 mg/kg produced significant inhibitory effect of the paw edema (41.89%) as compared to normal control group.

Eddy's hot plate method

In the hot-plate method the extract 500 mg/kg and diclofenac 10 mg/kg prolonged the reaction time significantly ($p < 0.01$) as compared to the control group throughout the observation period as shown in Table 3, Fig. C. The reaction time was taken as the parameter for the evaluation of analgesic activity. The effect produced by 250 mg/kg of the extract does not show any significance. However, with 500 mg/kg significant difference was noted at 0, 30, 60, 90 and 120 min, respectively, in relation to the control group. Comparing different doses of the extract revealed that there is positive relation-ship between reaction time and increase dose of the extract in which, protection against thermal stimuli with 500 mg/kg was significant compared to control.

Haffner's tail clip method

The effect of *Berberis lycium* extract on tail clip test is shown in Table 4, Fig. D. The extract at dose of 250 mg/kg does not show any significance, but methanolic extract at dose 500 mg/kg caused a significant inhibition of pain. However, diclofenac sodium 10 mg/kg was effective than methanolic extracts.

Discussions

Anti-inflammatory

Determination anti-inflammatory activity by using inhibition of carrageenan- induced inflammation, which is one of the most feasible methods to screen anti-inflammatory agents. Carrageenan induces inflammation through the release of prostaglandin, bradykinin and histamine whereas hydroxytryptamine additionally increase the permeability of total blood vessels for various collagens [21]. Edema is due to the exudation of fluids and plasma proteins and the migration of leucocytes, most notably neutrophils and macrophages into the injured area [22]. Carrageenan-induced edema falls in the category of acute inflammation, which involves the synthesis or release of inflammatory mediators at the injured site which further cause pain and fever. The methanolic extract may produce its anti-inflammatory effect by blocking the release of these inflammatory mediators [23]. Carrageenan-induced inflam-matory process is believed to be biphasic. The initial phase seen at first hour is attributed to the release of histamine and serotonin. The second accelerating phase of swelling is due to release of prostaglandin (PGs), bradykinin protease, lysozyme, leukotriene and infiltration of PMNS (polymorphonuclear neutrophils) and macrophages. It has

been reported that the second phase of inflammation is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory drugs, which are generally used to access the edematous effect of natural products [24]. Cyclooxygenase (COX) is a key enzyme in the biosynthesis of prostaglandin from arachidonic acid and has two iso-types. COX-1 is responsible for producing the basal levels of prostaglandin needed for gastrointestinal tract homeostasis, where as COX-2 is an inducible enzyme which is involved in inflammatory events [25].

The result of this study suggests that methanolic extract of *Berberis lycium* has anti-inflammatory effect in the dose dependent manner as comparable to those of the standard drug, Indomethacin. We observed that methanolic extract at dose 500 mg/kg showed significant inhibition against carrageenan-induced paw edema, but in case of methanolic extract at dose 250 mg/kg failed to possess anti-inflammatory effect (Table 1). The percentage inhibition of the paw volume of the methanolic extract at dose 500 mg/kg were reduced by (27.92%) and produced significant ($p<0.001$) inhibition of paw edema by carrageenan injection in animals and continued during all phases of inflammation. This activity may be assumed probably due to the inhibition of different aspects and chemical mediators of inflammation as established for Indomethacin.

The anti-inflammatory activity of methanolic extract was also evaluated by using histamine induced paw edema in rats (Table 2). Methanolic extract at 250 mg/kg does not show any significant reduction in the paw volume (20.49%) induced by histamine as compared to control group. However, the methanolic extract significantly ($p<0.001$) reduced inflammation at dose 500 mg/kg, the percentage inhibition of the paw volume were reduced by (35.13%) in reference with the control group. It indicates that methanolic extract may inhibit inflammation by blocking the release of 5-HT, which are released by histamine. Histamine is an important mediator of inflammation, a potent vasodilating substance and is also involved in increasing vascular permeability [26]. The present experimental findings tend to suggest that the extract might demonstrate anti-inflammatory actions by inhibiting the synthesis, release or action of inflammatory mediators such as histamine, serotonin, cytokines and prostaglandins [27].

Analgesic Activity

Pain induced by thermal stimulus of the hot plate and is specific for centrally mediated activity which focuses mainly on changes above the spinal cord level [28]. This method was selected for this study because of several advantages including sensitivity to strong analgesics, limited tissue damage, accuracy of results and they are also less time consuming [29]. Analgesic effect mediated through central mechanism indicates the involvement of endogenous opioid peptides and biogenic amines like 5-HT [30, 31]. In the hot plate method, a plate heated at a constant temperature produces two behavioral components that can be measured in terms of their reaction times, namely paw licking and jumping. Both are considered to be supra-spinally integrated responses [32]. As far as analgesic substances are concerned, the paw-licking behavior is affected only by opioids. On the other hand, the jumping reaction time is increased equally by less powerful analgesics such as acetylsalicylic acid or paracetamol [33].

In both methods, the *Berberis lycium* extract does not show significant ($p<0.05$) activity at the doses of 250 mg/kg, however in both hot plate and tail clip methods the extract at dose of (500 mg/kg) and diclofenac prolonged the reaction time significantly ($p<0.01$) as compared the control throughout the observation period but the standard drug (diclofenac) showed the maximum reaction time (Table 3 & 4). This time gap may be due to the time lag between drug entering the central compartment and distribution into the target site or formation of an active metabolites that are endowed with analgesic activity. The results indicated that the extract significantly ($p<0.001$) raised pain threshold as compared to control and the activity was persistent throughout the entire observation period of 120 min. The significant increase in pain threshold produced by *Berberis lycium* in these models suggests involvement of central pain pathways. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems. The analgesic effect produced by the extract may be via central mechanisms involving these receptor systems or via the inhibition of prostaglandins, leukotrienes, and other endogenous substances that are key players in inflammation and pain.

Table 1: Effect of 80% methanolic extract of *Berberis lycium* against carrageenan induced paw edema in rat.

Treatment (mg/kg)	Mean paw volume in ml					% inhibition
	0 min	30 min	60 min	90 min	120 min	
Control (2 ml/kg)	0.81±0.04	1.36±0.16	1.74±0.12	1.82±0.13	1.54±0.04	---
MEBL (250 mg/kg)	0.83±0.11 ^{n.s}	1.46±0.14 ^{n.s}	1.40±0.18 ^{n.s}	0.89±0.16 ^{n.s}	0.79±0.22 ^{n.s}	13.63%
MEBL (500 mg/kg)	0.78±0.13**	0.98±0.12**	0.88±0.16**	0.73±0.15**	0.57±0.15**	27.92%
Indomethacin (10 mg/kg)	0.76±0.09**	0.90±0.11**	0.79±0.15**	0.60±0.16**	0.33±0.05**	43.50%

Values are represented as mean±SEM (n=6 in each group). Data expressed by using one way ANOVA followed by Dunnett's Test. * $p<0.05$, ** $p<0.01$ was considered as significant and n.s = non-significant as compared to control.

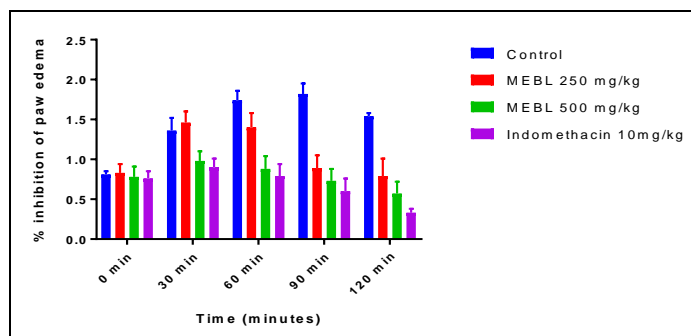
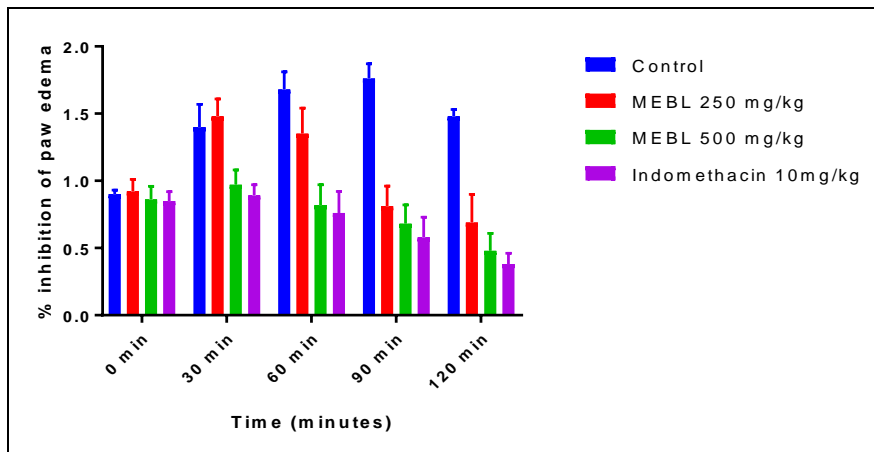


Fig A: Graphical representation of percent inhibition of oedema with 80% methanolic extract of *Berberis lycium* in carrageenan induced paw oedema model. Data represent mean±SEM (n=6).

Table 2: Anti-inflammatory activity of 80% methanolic extract of *Berberis lycium* on Histamine induced paw edema in rats

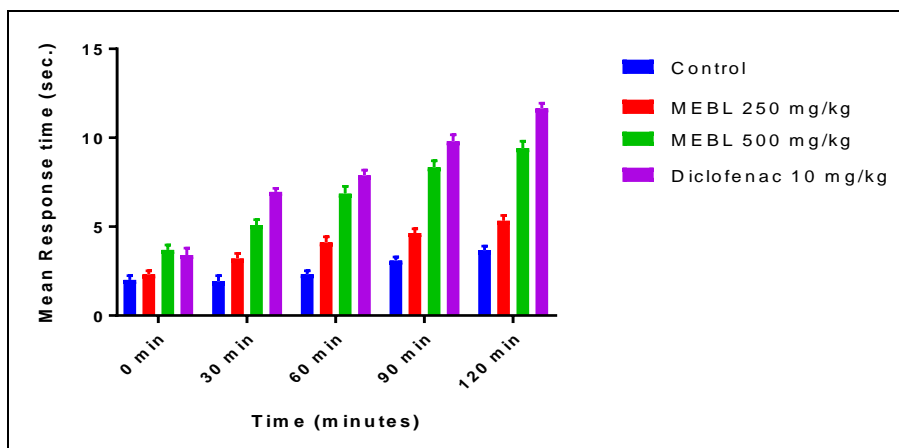
Treatment (mg/kg)	Mean paw volume in ml					% inhibition
	0 min	30 min	60 min	90 min	120 min	
Control (2 ml/kg)	0.90±0.03	1.40±0.17	1.68±0.13	1.76±0.11	1.48±0.05	----
MEBL (250 mg/kg)	0.92±0.09 ^{n.s}	1.48±0.13 ^{n.s}	1.35±0.19 ^{n.s}	0.81±0.15 ^{n.s}	0.69±0.21 ^{n.s}	20.49%
MEBL (500 mg/kg)	0.86±0.10 ^{**}	0.97±0.11 ^{**}	0.82±0.15 ^{**}	0.68±0.14 ^{**}	0.48±0.13 ^{**}	35.14%
Indomethacin (10 mg/kg)	0.85±0.07 ^{**}	0.89±0.08 ^{**}	0.76±0.16 ^{**}	0.58±0.15 ^{**}	0.38±0.08 ^{**}	41.89%

Values are represented as mean±SEM (n=6 in each group). Data expressed by using one way ANOVA followed by Dunnett's Test. * $p<0.05$, ** $p<0.01$ was considered as significant and n.s = non-significant as compared to control.

**Fig B:** Graphical representation of percent inhibition of oedema with 80% methanolic extract of *Berberis lycium* in histamine induced paw oedema model. Data represent mean±SEM (n=6).**Table 3:** Effect of 80% methanolic extract of *Berberis lycium* on hot plate test

Treatment (mg/kg)	Reaction time in seconds at time (minutes)				
	0 min	30 min	60 min	90 min	120 min
Control (2 ml/kg)	2.01±0.23	1.93±0.31	2.23±0.19	3.09±0.20	3.68±0.22
MEBL (250 mg/kg)	2.32±0.20 ^{n.s}	3.20±0.29 ^{n.s}	4.12±0.31 ^{n.s}	4.64±0.24 ^{n.s}	5.33±0.30 ^{n.s}
MEBL (500 mg/kg)	3.69±0.28 [*]	5.09±0.31 [*]	6.86±0.41 [*]	8.34±0.37 [*]	9.42±0.38 ^{**}
Diclofenac sod. (10 mg/kg)	3.41±0.38 ^{**}	6.96±0.20 ^{**}	7.88±0.30 ^{**}	9.81±0.36 ^{**}	11.66±0.28 ^{**}

Values are represented as mean±SEM (n=6 in each group). Data expressed by using one way ANOVA followed by Dunnett's Test. * $p<0.01$, ** $p<0.001$ was considered as significant and n.s = non-significant as compared to control.

**Fig C:** Graphical representation of percent increase in reaction time with 80% methanolic extract of *Berberis lycium* in hot plate. Data represent mean±SEM (n=6).**Table 4:** Effect of 80% methanolic extract of *Berberis lycium* on tail clip test

Treatment (mg/kg)	Reaction time in seconds at time (minutes)				
	0 min	30 min	60 min	90 min	120 min
Control (2 ml/kg)	2.18±0.31	2.07±0.27	2.28±0.19	3.17±0.31	2.45±0.26
MEBL (250 mg/kg)	2.49±0.26 ^{n.s}	3.27±0.21 ^{n.s}	4.53±0.23 ^{n.s}	4.89±0.26 ^{n.s}	5.60±0.32 ^{n.s}
MEBL (500 mg/kg)	3.89±0.31 ^{**}	5.62±0.36 ^{**}	6.67±0.22 ^{**}	8.53±0.31 ^{**}	9.68±0.34 ^{**}
Diclofenac sod. (10 mg/kg)	3.71±0.26 ^{**}	6.37±0.33 ^{**}	8.57±0.34 ^{**}	9.75±0.33 ^{**}	10.17±0.31 ^{**}

Values are represented as mean±SEM (n=6 in each group). Data expressed by using one way ANOVA followed by Dunnett's Test. * $p<0.05$, ** $p<0.01$ was considered as significant and n.s = non-significant as compared to control.

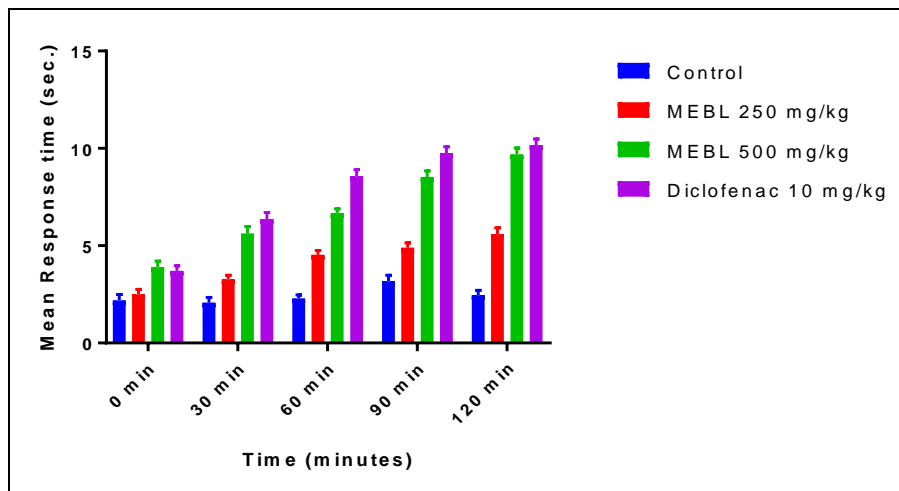


Fig D: Graphical representation of percent increase in reaction time with 80% methanol extract of *Berberis lycium* in tail clip test. Data represent mean \pm SEM (n=6).

Conclusion

It has been reported that a number of flavonoids were reported to possess anti-inflammatory and analgesic activity by reduced availability of prostaglandins^[34]. These activities in the methanol extract may be due to the presence of phenolic compounds and flavonoids in the extract of *Berberis lycium*, which is confirmed by the phytochemical tests. Anthocyanins are one of flavanoid class, and belonging to an important class of plant constituents. In *Berberis lycium*, total twelve anthocyanins were identified in the purified extract of *Berberis berry* by Liquid chromatography- Mass spectrometry and Ultraviolet spectral analysis. Two main anthocyanins, found in *Berberis lycium* are delphinidin-3-glucoside (43.7%) and cyanidin-3-glucoside. Other minor anthocyanins were also characterized and those are glycosides of cyanidin, pelargonidin, peonidin and malvidin^[35].

Flavonoids are known to inhibit the enzyme prostaglandin synthetase, more specifically the endoperoxidase and reported to produce anti-inflammatory effects^[36]. Since, prostaglandins are also involved in the pain perception; inhibition of their synthesis might be the possible reason for the analgesic activity of the methanolic extract. The presence of flavonoid identified might be responsible for the analgesic and anti-inflammatory activities in methanolic extract. Thus, it is concluded that the methanolic extract of *Berberis lycium* produces significant analgesic and anti-inflammatory activities in dose dependent manner.

Berberis lycium is a multi-potential plant with many characteristics. From the morphological, anatomical characters, phytochemical constituents, traditional uses and the pharmacological properties of this herb, it is evident that its use may prove to be useful in the development of some commercial drugs. Thus more work is required to point out the underlying phytochemicals which are responsible for various activities of this plant. The literature also showed that the plant has a leading capacity for the development of new good efficacy drugs in future. These plants are hardy in nature and grown in nature 'premaculture' so does not require chemical or pesticides and are ecofriendly.

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