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Research Article

Antiulcerogenic effect of Methanolic Root Extract of *Berberis lycium* Linn by Pylorus ligation and Ethanol induced Ulceration

Namdeo Priyanka [1]*, Pandole Madhuri [2], Singh Priti [3]

[1] *Lakshmi Narain College of Pharmacy, Bhopal 462022, M.P. INDIA*

[2] *Saraswati Institute of Pharmceutical Sciences, Gandhinagar 382355, Gujarat INDIA*

[3] *Department of Pharmaceutics, Mittal Institute of Pharmacy, Bhopal 462031, M.P. INDIA*

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*Address for Correspondence:

Priyanka Namdeo, Associate Professor, Lakshmi Narain College of Pharmacy, Bhopal 462022, M.P. INDIA

ORCID ID: https://orcid.org/0000-0002-2939-5726

Abstract

Background: Gastric ulcer is one of the most prevalent gastrointestinal disorders, which affects approximately 5-10% of people during their life. In recent years, abundant work has been carried out on herbal medicine to clarify their potential efficacy in gastric ulcer prevention or management. The present study was carried out to evaluate the antiulcer activity of the methanolic root extract of *Berberis lycium* in albino rats.

Method: The methanolic root extract of *Berberis lycium* was prepared by hot extraction method. Anti-ulcer activity was evaluated and method employed was pylorus ligation and ethanol induced in albino rats. Preliminary methanolic extract of *Berberis lycium* was subjected to the acute oral toxicity study according to the OECD guideline no. 425. Animals were divided into four groups of six animals each. The animals of Group I served as normal control (vehicle) which received distilled water. Group II and III received 250 mg/kg and 500 mg/kg of methanolic root extract, respectively. In pylorus ligation induced ulcer model, various parameters were studied viz. gastric volume, pH, total acidity, free acidity, and ulcer index. Ulcer index and percentage inhibition of ulceration was determined for ethanol induced ulcer model. Group IV received Ranitidine at 50 mg/kg was used as the standard drug. Pretreatment of methanol root extract of *Berberis lycium* showed significant ($P<0.05$) decrease in the gastric volume, total acidity and free acidity. However, pH of the gastric juice was significantly increased only at higher dose 500 mg/kg. It showed also significant ($P<0.05$) decrease in number of ulcers and ulcer score index in pylorus ligation and ethanol induced ulceration models.

Results: The methanol root extract of *Berberis lycium* showed a significant reduction in the total acidity, free acidity and acid volume. The efficacy of plant extract at high dose was comparable with the standard drug Ranitidine.

Conclusion: Our study results support the ethnomedical use of root of *Berberis lycium*.

Keywords: Antiulcer activity, *Berberis lycium*, Pylorus ligation, Ranitidine, Ulcer Index.

INTRODUCTION

The Plant kingdom remains as a virtual gold mine of drugs yet to be discovered there are several hundreds of medicinal plants that's have a history of curative properties against various diseases and ailments. Peptic ulcer disease is one of the most common gastrointestinal disorders, which causes a high rate of morbidity particularly in the population of non-industrialized countries.[1] Peptic ulcer occurs due to an imbalance between the aggressive (acid, pepsin and Helicobacter pylori) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors.[2] Ayurvedic knowledge supported by modern science was necessary to isolate characterize and standardize the active constituents from herbal sources for antiulcer activity. Since, all parts of investigated plant were being used for various human ailments and very less scientific work has been performed. Number of drugs including proton pump inhibitors, prostaglandins analogs, histamine receptor antagonists and cytoprotective agents are available for the treatment of peptic ulcer. But most of these drugs produce several adverse reactions including toxicities and even may alter biochemical mechanisms of the body upon chronic usage.[3] Hence, herbal medicines are generally used in such cases when drugs are to be used for chronic periods. Several natural drugs have been reported to possess anti-ulcerogenic activity by virtue of their predominant effect on mucosal defensive factors.[4]

Berberis lycium is an important medicinal plant belonging to family Berberidaceae and also known as Indian Barberry. It is a rapidly growing herb mainly distributed in India. It is very common in different markets of India as an adulterant and substitute to 'Daruharidra' *B. aristata*. The *Berberis* species are part of British and Indian pharmacology. B. lycium Royle is found at high altitudes in Pakistan. *B. lycium*

is an erect, small, rigid shrub about 1.0–2.5 m tall, with a thick woody shoot covered with thin brittle bark. The root are used for treating a variety of ailments such as eye and ear diseases, rheumatism, jaundice, diabetes, fever, stomach disorders, skin disease, malarial fever [5,6] and management of infected wounds.[7] Major alkaloid of the plant is berberine, which is known for its activity against cholera and acute diarrhea.[8]

Previous studies shows that *Berberis lycium* plant has a wide range of biological activities including anti-hyperglycemic,[9,10] antihyperlipidemic,[11,12] anti-cancer and anti-tumor,[13,14] wound healing,[15] bone healing,[16] antimicrobial,[17,18] anticoccidial,[19] anti-oxidant,[20,21] immunity enhancing, hepatoprotective,[22,23] anti-urolithic and anthelmintic.[24] As mentioned in the earlier reported literature *B. lycium* is found to be an important herb which shows numerous biological activities and used widely as folk medicine in India. Recent screening with plants has revealed many compounds like alkaloids, flavonoids, steroids, tannins, saponins, anthraquinones, carbohydrates, protein and Terpenoids.[25-28] Scientific literatures are continuously reporting herbal drugs having anti-ulcer potential. There is need to evaluate the potential of ayurvedic remedies as adjuvants to counteract side effects of modern therapy.[29]

The present investigation was carried out to exposure the chemical and therapeutically potential by evaluating the phytochemical and antiulcerogenic effect of the methanolic root extract of *Berberis lycium* is presented in the article.

MATERIALS AND METHODS

Plant Material:

The Root of *Berberis lycium* Linn. were collected at in the month of June, 2018 from local area of Rajouri district of Jammu, India. It was identified and authenticated by Dr. Pramod Patil, Professor & Department of Botany of Govt. M.L.B. Girls Autonomous College, Bhopal (M.P.). A voucher was deposited at the Department of Botany for further reference. All the chemicals used were of analytical grade and purchased from S. D. Fine/Merck (India).

Preparation of extract:

Collected roots were dried under shade and kept free from foreign matter like soil, dust, insect, fungal and other extrinsic contamination. Dried plant material was grounded to coarse powder. The powdered roots (500 g) were extracted with methanol (95%) using the continuous hot extraction method for 72 hours.[30] The extracts were filtered, concentrated and dried using a rotary evaporator. The yield was calculated as the weight of the extract/weight of dissolved plant powder × 100. The percentage yield of the ethanolic extract of the root was found to be 2.25%. The dried extract was stored at 4^0C an airtight container in refrigerator for further experimental studies.

Phytochemical Screening:

Methanolic extract of *Berberis lycium* was performed by the standard methods for the presence of carbohydrates, glycosides, saponins, flavonoids, protein and amino acids, tannins, phenolic compounds, Fixed oils, fats, phytosterols and alkaloids.[31]

Experimental Animals:

Healthy Sprague-Dawly (S.D.) albino rats weighing between 150-250g, were used to determine the antiulcer activity of the ethanolic extract. The animals were procured from Mittal Institute of Pharmacy, Bhopal (1555/PO/a/11/CPCSEA). The animals were kept in polypropylene cages (6 in each cage). Animal house was maintained under standard hygienic conditions, at 25 ± 2^0C, humidity (60 ±10%) with 12 hrs light and 12 hrs dark cycles with food and water ad libitum. The animals were maintained in accordance with CPCSEA guidelines. All the procedures described were reviewed and approved by Institutional Animal Ethical Committee.

Test Compound Formulations:

The aqueous suspension of methanolic root extract of *Berberis lycium* was prepared in 0.5 % carboxy-methylcellulose (CMC) solution in distilled water prior to oral administration to animals. It was used within seven days and stored at 8^oC while for further freshly prepared solution was used. The vehicle alone served as control.

Chemicals:

All the drugs and chemicals were of analytical grade. Ranitidine were procured from Merck, Bangalore, India. Ethanol and Methanol (S.D. Fine-Chem, Mumbai, India)

Acute Toxicity Studies:

Acute toxicity studies were performed according to organization for economic co-operation and development (OECD) guidelines.[32] Animals were divided in groups (n=5). The animals were fasted for 4 h. with free access to water only. The methanolic root extract of *Berberis lycium* was administered orally in doses of 2500 and 5000 mg/kg to different groups of mice and observed over 14 days for mortality and physical/behavioral changes.

Assessment of Anti-Ulcer Activity:

Pyloric ligation induced gastric ulceration:

Albino rats of either sex were divided into four groups of six animals each. Animals were fasted for 24 h before the study, but had free access to water. Just 2 hrs before starting the experiment, water is also removed. Group I which served as negative control received distilled water. Group II received methanolic root extract of *Berberis lycium* at the dose of 250 mg/kg and Group III received methanol root extract of *Berberis lycium* at the dose of 500 mg/kg. Group IV received ranitidine (50 mg/kg) was used as a standard. After 1 h of drugs treatment, they were anaesthetized with the help of anesthetic ether the abdomen was opened by a small midline incision below the xiphoid process. Pyloric portion of the stomach was slightly lifted out and ligated, avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall was closed by interrupted sutures. Rats were sacrificed by an over dose of anesthetic ether after four hours of pyloric ligation. The abdomen was opened, cardiac end of the stomach was dissected out and the contents were drained into a glass tube.

The volume of the gastric juice was measured and centrifuged at 2000 rpm for 10 min. From the supernatant, aliquots (1 ml of each) were taken for the determination of pH, total and free acidity. The stomach is then open along the greater curvature and is examined for any ulceration. The degree of ulceration is graded from zero to five depending on the size and severity of ulcers.

Macroscopic evaluation of stomach:

The stomachs were opened along the greater curvature, rinsed with saline to remove gastric contents and blood clots and examined by a 10 X magnifier lens to assess the formation of ulcers. The numbers of ulcers were counted.

Ulcer scoring was done by the following scoring system:

0 = Normal colored

0.5 = Red coloration

1 = Spot ulcer

1.5 = Hemorrhagic streak

2 = Deep Ulcers

3 = Perforation

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows:

Ulcer index (UI) was measured by using following formula:

$$UI = UN + US + UP \text{ X } 10^{-1}$$

Where UI = Ulcer Index, UN = Average number of ulcers per animal, US = Average number of severity score, UP = Percentage of animals with ulcers

Percentage inhibition of ulceration was calculated as below:

$$\% \text{ Inhibition of Ulceration} = \frac{\text{Ulcer index Control} - \text{Ulcer index Test}}{\text{Ulcer index Control}} \times 100$$

Biochemical parameters used to investigate gastric juice:

Determination of pH:

An aliquot of 1 ml gastric juice was diluted with 1 ml of distilled water and pH of the solution was measured using pH meter.

Determination of free acidity and total acidity:

The total volume of gastric content was measured. The gastric contents were centrifuged and filtered. One ml of the gastric juice was pipetted out and the solution was titrated against 0.1 N sodium hydroxide using 2 to 3 drops of Topfer's reagent as indicator, to the end point when the solution turned to yellowish orange colour was observed. This indicated the volume of NaOH required neutralizing the free hydrochloric acid present in the gastric juice. Then 2 to 3 drops of phenolphthalein solution was added and titration was continued until a definite red colour appears. The difference between the two readings indicated the volume of NaOH required neutralizing the combined acid present in the gastric juice. The sum of the two titrations was the total acid present in the gastric juice.

Acidity was calculated by using formula:

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{\text{Volume of gastric juice used}}$$

Ethanol induced ulcer model:

The ulcer was induced by administering ethanol. All the animals were fasted for 36 hours before administration of ethanol. The albino rats were randomly divided into five groups of six animals each. Group I represented the control group, which received ethanol, Group II and III received methanolic root extract of *Berberis lycium* at the dose of 250 and 500 mg/kg. Group IV received Ranitidine (50 mg/kg) were administered orally as reference standard drug. The gastric ulcers were induced in rats by orally administrating 1 ml of 80% absolute ethanol, after 45 min of methanolic extract and ranitidine treatment. They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were sacrificed after 1 hr of ethanol administration. The stomach was opened and the ulcer index and percentage inhibition of ulcer formation was calculated.

Statistical analysis:

The analytical data was expressed as mean ± S.E.M. Statistical analysis was carried out by using one-way analysis of variance (ANOVA) followed by Dennett's Multiple Comparison test using Graph Pad Prism version 5.0 Software. Differences between the data were considered significant at $P<0.05$.

RESULTS

Preliminary phytochemical screening:

The results of preliminary phytochemical screening of methanolic extract of *Berberis lycium* roots are shown in Table 1.

Table 1: Phytochemical screening of *Berberis lycium* roots.

S.N.	Phytoconstituents	Methanolic extract of *Berberis lyceum*
1.	Alkaloids	Present
2.	Protein and Amino acids	Present
3.	Phytosterols and Glycosides	Present
4.	Flavonoids	Present
5.	Polyphenolic compounds	Present
6.	Saponins	Present
7.	Tannins	Present

Acute Oral Toxicity Study:

Acute oral toxicity was carried out by up-down regulation method. It is found that methanolic root extract of *Berberis lycium* were safe at limit dose 2500 mg/kg and 5000 mg/kg with no mortality in studied subjects. 1/10th of these doses 250 mg/kg and 500 mg/kg were used in the subsequent study respectively.

Pyloric Ligation Induced Gastric Ulceration:

Effect of methanol root extract of *Berberis lycium* on pyloric ligation induced ulceration is shown in Table 2. The pyloric ligation has caused the accumulation of gastric secretions of 8.1±0.25ml with pH 2.9±0.23 in a control group. The total acidity and free acidity of the gastric secretions were found to be 115±0.28 and 92.1±1.8 mEq/l respectively. Pretreatment with the *Berberis lycium* root extract, significantly ($P<0.05$) reduced the volume of gastric secretions 4.9±0.13 and 4.1±0. 10ml at the doses of 250 mg/kg and 500 mg/kg respectively. pH of the gastric fluid was significantly ($P<0.05$) elevated up to 4.73±0.66 only at higher dose of the extract. In addition, total acidity and free acidity were also reduced significantly ($P<0.05$) in a dose dependent manner. Further it is observed that pyloric ligation has caused gastric ulcerations and pretreatment with root extract of *Berberis lycium* has reduced them significantly ($P<0.05$) in a dose dependent manner. In this model, percentage inhibition of ulceration was found to be 46.80 and 72.65 at 250 mg/kg and 500 mg/kg respectively. The gastroprotection offered by the test extract was comparable to that of the standard drug, ranitidine (50 mg/kg), as shown in Table 3 and Fig. 1.

Table 2: Effect of methanolic root extract of *Berberis lycium* on gastric content, pH, total and free acidity in pyloric ligation induced ulceration in rats.

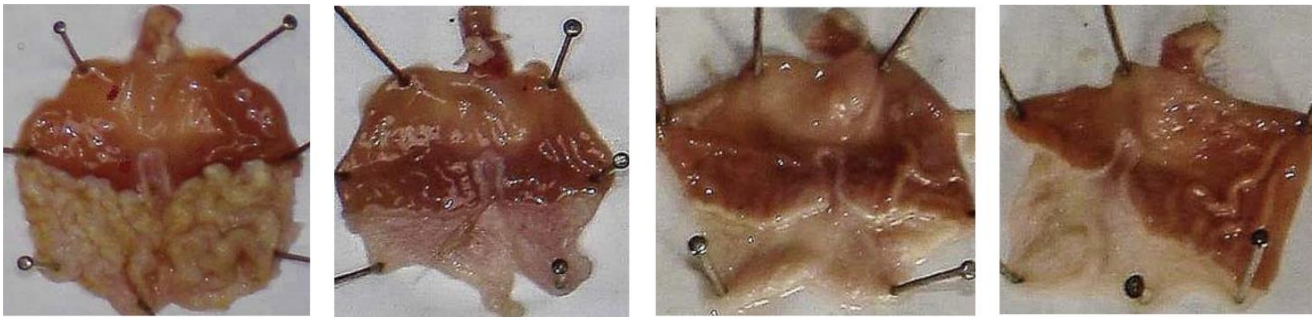
Name of the Group	Gastric Volume (ml)	pH	Total acidity	Free acidity
Group I (Distilled water)	8.1±0.25	2.9±0.23	115±0.28	92.1±1.8
Group II (Extract 250mg/kg)	4.9±0.13*	3.25±0.24	70±0.28*	69±0.25*
Group III (Extract 500mg/kg)	4.1±0.10*	4.73±0.66*	68±0.18*	65±0.22*
Group IV (Ranitidine 50mg/kg)	4.0±0.10*	5.25±0.17*	2±1.21*	33±0.30*

Values are expressed as (Mean ± S.E.M.), n= 6, *p< 0.05 when compared with control group.

Table 3: Effect of methanolic root extract of *Berberis lycium* on gastric ulcer induced by pylorus ligation in rats.

Treatment	Dose (mg/kg)	Ulcer Index	Percentage Ulcer Inhibition
Control (Distilled water)	10	4.0±0.32	-
Methanolic extract	250mg/kg	2.63±0.20*	46.80
Methanolic extract	500mg/kg	3.0±0.17*	72.65
Ranitidine	25mg/kg	1.75±0.59*	82.19

Values are expressed as (Mean ± S.E.M.), n= 6, *p< 0.05 when compared with control group. (Statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's t-test.)



(a) (b) (c) (d)

Figure 1: Photographs showing Pharmacological screening on Pylorus ligation method.

(a) Control group. Showed that the heavy ulcerative condition of the stomach sections was observed, **(b)** Ranitidine 50mg/kg. There is no any sign of inflammation and mild redness of the ulcer index was observed, **(c)** Methanolic root extract of *Berberis lycium* (250 mg/kg). The stomach sections were showed that normal stomach architecture and without any ulceration with mild redness, **(d)** Methanolic root extract of *Berberis lycium* (500 mg/kg). The stomach was observed that no any sign of redness and inflammation.

Ethanol Induced Gastric Ulceration:

Ethanol at dose of 0.5 ml/kg showed superficial, deep ulcers and perforations in the control animals. However, animals treated with methanol root extract of *Berberis lycium* at 250 mg/kg and 500 mg/kg doses showed significant (P<0.05) reduction in the number of ulcer and ulcer index (Table 4). It showed 38.06 and 51.56% ulceration inhibition at the dose of 250 mg/kg and 500 mg/kg respectively whereas ranitidine showed 67.41% ulceration inhibition. Anti-ulcerogenic effect of root extract of *Berberis lycium* in ethanol induced ulcers was comparable to that of ranitidine (50 mg/kg), as shown in Table 4.

Table 4: Effect of methanolic root extract of *Berberis lycium* on ethanol induced ulcers in rats.

Treatment	Dose (mg/kg)	Ulcer Index	Percentage Ulcer Inhibition
80% Absolute Ethanol	1ml/kg	4.85±0.32	-
Methanolic extract	250mg/kg	2.46±0.30*	38.05
Methanolic extract	500mg/kg	2.02±0.27*	51.56
Ranitidine	50mg/kg	1.70±0.10*	67.41

Values are expressed as (Mean ± S.E.M.), n= 6, *p< 0.05 when compared with control group. (Statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's t-test.)

DISCUSSION

Peptic ulcer is one of the most frequent diseases of the alimentary tract. Multifactorial elements, including bacterial infection, and genetic-environmental, autoimmune factors, are involved in the development of peptic ulcer. The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanism. To regain the balance, different therapeutic agents including plant extracts may be used. *Berberis lycium* is one such plant, and folklore people used this for the treatment of ulcers. Hence, the present study was oriented to evaluate the phytochemical studies and anti-ulcer activity of methanolic extract of *Berberis lycium* against pylorus ligation and ethanol induced ulcer in rats.

Various standard phytochemical tests were performed for the root extract of *Berberis lycium* and the results were reported in (Table 1). The presence of wide range of phytochemical constituents indicates that plant could serve as lead for the development of novel agents for various pathological disorders. The causes of gastric ulcer pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and/or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid. These factors are associated with the development of upper gastrointestinal damage including lesions, ulcers and life threatening perforation and hemorrhage. Aspirin, phenylbutazone, indomethacin and some non-steroidal anti-inflammatory drugs are also known to cause duodenal and gastric ulceration. Volume of gastric secretion is an important factor in the production of ulcer due to exposure of unprotected lumen of the stomach to the accumulating acid.

Ethanol is also has been reported to cause disturbances in gastric secretion, damage to the mucosa, alterations in the permeability, gastric mucus depletion and free radical production. This is attributed to the release of superoxide anion and hydroperoxyl free radicals during metabolism of ethanol as oxygen derived free radicals has been found to be involved in the mechanism of acute and chronic ulceration in the gastric mucosa. The methanolic root extract of *Berberis lycium* and Ranitidine significantly decreased the total acidity and free acidity and significantly enhance the pH, this suggests that it having an anti secretory effect. Pylorus ligation and ethanol induced ulcer control rats shown perforated ulcer, deep ulceration of granular epithelium and almost reducing the sub-mucosa. The methanolic root extract of *Berberis lycium* at 250 mg/kg dose has shown mucosal erosion, the partial healing of ulcer with few inflammatory cells and the dose 500 mg/kg has shown the healed ulcer, normal mucosa and no inflammatory cells. On the other, the standard drug Ranitidine (50 mg/kg) produces anti-ulcer activity by inhibiting gastric secretion, as shown in Fig. 1.

The antiulcer property of root extract of *Berberis lycium* in pylorus ligation model is evident from its significant reduction in free acidity, total acidity, number of ulcers and ulcer index. A. indicum treated animals significantly inhibited the formation of ulcers in the pylorus ligated rats and also decreased both the concentration and increased the pH, it is suggested that *Berberis lycium* can suppress gastric damage induced by aggressive factors.

Berberis lycium root extracts have been reported to possess antioxidant activity and to contain various types of compounds such as flavonoids, polyphenolic compounds, saponins and tannins. The gastro protective effect exhibited by Ethanolic extract *Berberis lycium* is speculated to be attributed to its antioxidant property, which in turn could be linked to the presence of flavonoids, polyphenolic compounds, saponins and tannins. These compounds most likely inhibit gastric mucosal injury. The results of the present study suggest that the methanol root extract of *Berberis lycium* may be beneficial in the treatment of gastric lesions. Further studies to identify the active moieties responsible for the activity.

CONCLUSION

The preliminary phytochemical screening major constituents present in the compounds are steroids and alkaloids. The phytochemical screening has given the basic foundation in which the class of compounds possesses the antiulcerogenic activity. From the results of pharmacological screening the phytoconstituents showed significant action against pylorus ligated and ethanol induced ulcers in rats. From the evaluation the *Berberis lycium* may be considered as a natural source in modern drug development areas for its versatile medicinal uses.

COMPETING INTERESTS STATEMENT

The authors declare no conflicts of interest.

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REFERENCES

1. Falk GW, Cecil essentials of medicine, WB Saunders Company, 5th edition, Edinburgh: 2001; pp. 334-343.

2. Tripathi KD, Essentials of Medical Pharmacology, Jaypee Brothers Medical Publishers (P) Ltd. New Delhi, 1999, pp. 628- 642.

3. Ariyphisi I, Toshiharu A, Sugimura F, Abe M, Matsuo Y, Honda T, "Recurrence during maintenance therapy with histamine H2 receptors antagonist in cases of gastric ulcers" Nikon University J Medical, 1986; 28:69-74.

4. Sairam K, Rao CV, Goel RK, "Effect of Centella asiatica linn on physical and chemical factors induced gastric ulceration and secretion". Indian J Exp. Biol 2001; 39:137-142.

5. Kirtikar KR and Basu BD (1933) Indian. Medicinal. Plants. I, Lalit Mohan Basu and Co., Allahabad, 2422-2423

6. Chopra RN, Chopra TC, Handa KL, Kapoor LD, Chopra's Indigenous Drugs of India, 284. U.N. Dhar and sons Pvt. Ltd.,1958; 503-505.

7. Dutta NK and Panse MV, Usefulness of berberine (an alkaloid from Berberis aristata) in the treatment of cholera. Indian J. Med. Res.,1962; 732-735.

8. Lahiri SC and Dutta NK, Berberine and chloramphenicol in the treatment of cholera and severe diarrhoea. J. Ind. Med. Assoc.1967; 1-11

9. Gulfraz M, Qadir G, Nosheen F, Parveen Z, Antihyperglycemic effects of Berberis lycium Royle in alloxan induced diabetic rats. Diabetologia Croatica. 2007; 36(3):49-54.

10. Mustafaa KG, Ganai BA, Akbar S, Dar MY, Tantry MA, Masood A, The extracts of Berberis lycium and diabetes mellitus in alloxan

monohydrate induced diabetic rats. Journal of Pharmacy Research. 2011; 4(8):2570-2573.

11. Ahmed M, Alamgeer, Sharif T, Zabta M, Akbar A, Effect of Berberis lycium Royle on lipid profile in alloxan induced diabetic rabbits. Ethnobotanical Leaflets. 2009; 13:702-708.

12. Rahimi-Madiseh M, Heidarian E, Rafieian-kopaei M, Biochemical components of Berberis lycium fruit and its effects on lipid profile in diabetic rats. Journal of HerbMed Pharmacology. 2014; 3(1):15-19.

13. Khan M, Giessrigl B, Vonach C, Madlener S, Prinz S, Herbaceck I, Berberine and a Berberis lycium extract inactivate Cdc25A and induce α-tubulin acetylation that correlate with HL-60 cell cycle inhibition and apoptosis. Mutation Research. 2010; 683(1):123-130. https://doi.org/10.1016/j.mrfmmm.2009.11.001

14. Issat T, Jakobisiak M, Golab J, Berberine, a natural cholesterol reducing product, exerts antitumor cytostatic/cytotoxic effects independently from the mevalonate pathway. Oncology Reports. 2006; 16:1273- 1276. https://doi.org/10.3892/or.16.6.1273

15. Asif A, Kakub G, Mehmood S, Khunum R, Gulfraz M, Wound healing activity of root extracts of Berberis lycium Royle in rats. Phytotherapy Research. 2007; 21(6):589-591. https://doi.org/10.1002/ptr.2110

16. Ahmad A, Mehmood S, Gulfraz M, Bone healing properties of Berberis lycium (Royal): A case study. Case Study and Case Report. 2011; 1(1):1-5.

17. Hussain MA, Khan MQ, Habib T, Hussain N, Antimicrobial activity of the crude root extract of Berberis lycium Royle. Advances in Environmental Biology. 2011; 5(4):585-588.

18. Malik AT, Kamili NA, Chishti ZM, Ahad S, Tantry AM, Hussain RP, Breaking the resistance of Escherichia coli: Antimicrobial activity of Berberis lycium Royle. Microbial Pathogenesis. 2017; 102:12-20. https://doi.org/10.1016/j.micpath.2016.11.011

19. Malik TA, Kamili AN, Chishti MZ, Tanveer S, Ahad S, Johri RK, In vivo anticoccidial activity of berberine [18, 5,6-dihydro-9,10-dimethoxybenzo(g)-1,3-benzodioxolo- (5,6-a) quinolizinium]-An isoquinoline alkaloid present in the root bark of Berberis lycium. Phytomedicine. 2014; 21(5):663-669. https://doi.org/10.1016/j.phymed.2013.11.005

20. Mashwani ZUR, Khan MA, Irum S, Ahmad M, Antioxidant potential of root bark of Berberis lycium Royle from Galliyat, Western Himalaya, Pakistan. Pakistan Journal of Botany. 2013; 45:231-234.

21. Sabir S, Tahir K, Rashid N, Naz S, Masood B, Shah AM, Phytochemical and antioxidant studies of Berberis lycium. Pakistan Journal of Pharmaceutical Sciences. 2013; 26(6):1165-1172.

22. Mushtaq M, Naz S, Khan S, Rehman S, Khan RU, In vivo effect of Berberis lyceum and Silybum marianum on production performance and immune status of broiler chickens. Archiv Tierzucht. 2013; 56(91):911-916. https://doi.org/10.7482/0003-9438-56-091

23. Chand N, Durrani FR, Ahmad S, Khan A, Immunomodulatory and hepatoprotective role of feed added Berberis lycium in broiler chicks. Journal of the Science of Food and Agriculture. 2011; 91(10):1737- 1745. https://doi.org/10.1002/jsfa.4399

24. Shah MA, Sherwani SK, Sualeh M, Kanwal S, Khan HN, Kazmi SU, In vitro anthelmintic and antiurolithic assessment of Berberis lycium root bark. Journal of Pharmacognosy and Phytochemistry. 2014; 3(2):205-210.

25. Srivastava P, Srivastava G, Pharmacological and phytochemical screening of Desmodium gangeticum and Moringa oleifera. Research Journal of Chemistry and Environment. 2018; 22(5):6-10. https://doi.org/10.2139/ssrn.3068565

26. Gupta I, Sharma P, Investigation of Phytoconstituents from Leaves, Seeds, Bark and Pods of Cassia fistula Plant. Journal of Chemistry and Chemical Sciences. 2018; 8(5):886-890. https://doi.org/10.29055/jccs/656

27. Auwal SM, Saka S, Mairiga AI, Sanda AK, Shuaibu A, Ibrahim A, Preliminary Phytochemical and Elemental Analysis of Aqueous and Fractionated Pod Extracts of Acacia nilotica (Thorn mimosa). Veterinary Research Forum. 2014; 5(2):95-100.

28. Kumar A, Patra S, Qualitative and Quantitative Analysis of Secondary Phytochemical in Gymnema Sylvestre, Indian journal of scientific research. 2017; 12(2):150- 156.

29. Wagner H, Search for plant derived natural products with immunostimulatory activity (recent advances). Pure and Appl. Chem. 1990; 62:1217-1222. https://doi.org/10.1351/pac199062071217

30. Vasudeva N, Sharma SK, Post coital antifertility activity of Hibiscus rasasinensis roots. Evid Based Complement Alternat Med. 2009; 5(1):91e94 https://doi.org/10.1093/ecam/nem003

31. Tyler, VE, Brady LR, Robbers JE, Pharmacognosy, 1993; pp: 59-64

32. OECD Guidelines for testing of chemical, revised draft guidelines 425:30, Acute Oral Toxicity-Up-and-Down Procedure: 2001.