

Assessment of Xanthine Oxidase Inhibitory Activity and Nephroprotective Potential of *Berberis vulgaris* in Experimental Models of Hyperuricemia.

Prakshi Manral^{1*}, Neelam Painuly¹, Vijaya Laxmi², Deepika Biswas³, Md Shabbir Alam⁴, Shadab Ali⁵, Arshi Aquil⁶

^{1*}Affiliation: School of Pharmacy and Research, Dev Bhoomi Uttarakhand University, Dehradun-248007, Mail id- prakshimanral2222@gmail.com, contact no. 7505544305

¹Affiliation: School of Pharmacy and Research, Dev Bhoomi Uttarakhand University, Dehradun-248007, Mail id- neelu.painuly@gmail.com, contact no. 7055860790

²Affiliation: Department of Pharmacy IBMER, Mangalayatan University, Aligarh, U.P. Mail id- vijaya2562002@gmail.com, contact no. 7250808981

³Affiliation: Arya College of Pharmacy, Jaipur, Rajasthan Mail id- deepikabiswas001@gmail.com, contact no. 7807197033

⁴Affiliation: MJRP University, Jaipur, Rajasthan Mail id- shabbibrobra786@gmail.com, contact no. 7877557097

⁵Affiliation: Sundaram Institute of Medical Sciences Gaurichak, Patna, Bihar, 800007 Mail id- shadabaliw@gmail.com, contact no. 8521047542

⁶Affiliation: Bihar University of Health Sciences Mail id- arshiaquil999@gmail.com, contact no. 7667259203

ABSTRACT

Hyperuricemia is a metabolic disease associated with excess uric acid in the blood. It is associated with gout, renal failure, and other metabolic issues. The present work was planned to evaluate the xanthine oxidase inhibitory and nephroprotective potential of *Berberis vulgaris* in in vitro and in vivo experimental models. The activity of inhibition of xanthine oxidase of the *Berberis vulgaris* ethanolic extract obtained by the Soxhlet method was studied. The extract showed a significant inhibitory activity against xanthine oxidase. At 500 µg/mL, the percentage inhibition was found to be 60%, and the IC₅₀ value was found to be 282.21 µg/mL.

The in vivo experiment was performed on Wistar rats. Hyperuricemia-associated kidney damage was induced by potassium oxonate (250 mg/kg, i.p.) in rats. Animals were divided into five groups: normal control, disease control, standard therapy and two extract-treated groups, which received *Berberis vulgaris* at the doses of 250mg/kg and 500mg/kg, respectively. Renal function was measured by serum creatinine and blood urea nitrogen (BUN). The administration of potassium oxonate dramatically elevated the blood creatinine and BUN values. Administration of *Berberis vulgaris* resulted in a significant dose-dependent improvement in renal function biomarkers, including serum creatinine and blood urea nitrogen. The protective effect was greater in the higher dose (500 mg/kg). Statistical differences were substantial ($P < 0.0001$).

The results indicate that *Berberis vulgaris* has an inhibitory effect on xanthine oxidase and a nephroprotective potential against potassium oxonate-induced kidney damage. Further research is necessary to explain the processes involved and to discover the bioactive molecules responsible for such pharmacological effects.

Keywords: *Berberis vulgaris*, xanthine oxidase, nephroprotection, potassium oxonate, Wistar rats, renal dysfunction.

How to cite this article: Prakshi Manral^{1*} Neelam Painuly¹ Vijaya Laxmi² Deepika Biswas³ Md Shabbir Alam⁴ Shadab Ali⁵ Arshi Aquil⁶. Assessment of Xanthine Oxidase Inhibitory Activity and Nephroprotective Potential of *Berberis vulgaris* in Experimental Models of Hyperuricemia.. Int J Drug Deliv Technol. 2026;16(85s):98-105. DOI: 10.25258/ijddt.16.85s.8.

1. INTRODUCTION

Hyperuricemia is a metabolic disease in which blood levels of uric acid are elevated. This may be due to overproduction of uric acid, underexcretion of uric acid, or both. Persistent hyperuricemia is the main risk factor for gout and has been linked with chronic renal illness, hypertension, cardiovascular disorders and metabolic abnormalities ((1-4). The increased incidence of hyperuricemia and its clinical consequences underline the need for efficient treatment techniques to regulate urate homeostasis and to avoid the onset of sickness.

In humans, uric acid is a result of purine degradation. Xanthine oxidase (XO) is a molybdenum-containing

enzyme that catalyses the oxidation of hypoxanthine to xanthine and xanthine to uric acid. Increased XO activity leads to increased generation of uric acid and creation of reactive oxygen species that favour oxidative stress and tissue damage ((2,3,5). Thus, inhibition of XO became a key therapeutic technique in the treatment of hyperuricemia and gout.

Currently, pharmacological therapies for hyperuricemia consist of xanthine oxidase inhibitors (e.g., allopurinol and febuxostat) and uricosuric drugs that enhance renal urate excretion. Clinically, these treatments are effective but long-term use of

these drugs may be associated with side effects such as hypersensitivity syndrome, hepatotoxicity, gastrointestinal disturbances, and renal difficulties (2–4). These deficiencies have led to the quest for additional therapeutic chemicals, especially those derived from medicinal plants with recognized safety profiles.

Natural products have long been a valuable source of pharmacologically active compounds. Plant-derived substances have been found to have antihyperuricemic potential via inhibition of xanthine oxidase, antioxidant activity and modulation of urate transport pathways ((4,6–8). Medicinal plants and herbal preparations have also been widely explored for the management of physiological and pathological conditions in both human and veterinary medicine ((7)). The discovery of botanical candidates with antihyperuricemic and nephroprotective activities may give additional benefits in the prevention of hyperuricemia-induced kidney injury.

Ayurvedic Perspective of Hyperuricemia

Hyperuricemia has not been mentioned as separate disease entity in traditional Ayurvedic literature but clinical features of hyperuricemia are similar to Vatarakta which is illness caused due to vitiation of Vata and Rakta ((9–11). Viruddha Ahara (improper dietary habits), sedentary lifestyle, excessive intake of meat, alcohol and protein rich foods are responsible for the formation of Ama (metabolic toxins), impaired Agni (digestive fire), vitiation of Kapha and Vata and ultimately obstruction of body channels (Srotorodha) (9–11).

These degenerative processes lead to the buildup of metabolic waste products such as uric acid, which causes discomfort, inflammation, swelling and stiffness of the joints. Although uric acid is not directly discussed in Ayurveda, these presentations are quite similar to the clinical symptoms of gout and hyperuricemia as reported in modern medicine (1,2,9,11).

The Ayurvedic care of Vatarakta mainly aims at Deepana (enhancing the digestive fire), Pachana (digesting Ama), Raktashodhana (purifying blood), Shothahara (anti-inflammatory therapy) and Mutrala (diuretic action) to help in removal of stored toxins (9–11).

Daruharidra (*Berberis aristata*), Guduchi (*Tinospora cordifolia*), Gokshura (*Tribulus terrestris*), Punarnava (*Boerhavia diffusa*), Triphala and Varuna (*Crataeva nurvala*) are some of the medicinal herbs used historically in Ayurveda for management of Vatarakta and urinary diseases ((9–12).

Among these, Daruharidra (*Berberis aristata*) is extensively described in the Ayurvedic Pharmacopoeia of India and Bhavaprakasha Nighantu as a medicinal plant possessing Tikta Rasa, Laghu-Ruksha Guna, Ushna Virya, and Katu Vipaka, and is traditionally indicated in inflammatory disorders, skin diseases and disorders involving vitiated blood (Rakta Vikara) ((9–12).

Daruharidra is rich in isoquinoline alkaloids, particularly berberine, which has demonstrated antioxidant, anti-inflammatory, nephroprotective, antihyperglycaemic and xanthine oxidase inhibitory activities in several experimental studies (5,8,12)).

These pharmacological properties provide scientific support for its traditional use and indicate that *Berberis* species may represent promising botanical candidates for the management of hyperuricemia and its associated renal complications (5,8,12).

Table 1. Experimental and Traditional Evidence Supporting the Therapeutic Applications of *Berberis* Species in Hyperuricemia and Renal Disorders ((5,8–12).

Agent	Active constituent	Experimental model	Major findings
<i>Berberis vulgaris</i> extract	Berberine-rich extract	Potassium oxonate-induced hyperuricemic rats	Reduced serum uric acid and improved renal function
<i>Berberis aristata</i>	Berberine	Traditional Ayurvedic use	Used in Vatarakta and inflammatory disorders
Berberine	Purified alkaloid	Experimental kidney disease models	Antioxidant, anti-inflammatory, renoprote

			ctive effects
--	--	--	------------------

The medicinal value of berberine-containing nutraceuticals and the use of *Berberis* species in Ayurvedic formulations for a long time have also increased the availability of berberine. However, in spite of their ubiquitous usage in traditional medicine, experimental data to support their antihyperuricemic and nephroprotective benefits are still scarce. Thus, a thorough pharmacological investigation of *Berberis vulgaris* is necessary to confirm its traditional claims and to prove its potential as an evidence based treatment choice.

Berberis vulgaris (Family: Berberidaceae) is a medicinal plant widely distributed in Asia and Europe and used traditionally for the treatment of many ailments. The plant contains various active alkaloids, such as berberine, berbamine and palmatine, which have antioxidant, anti-inflammatory, antibacterial and metabolic regulating activities. Berberine, the major phytoconstituent of *Berberis vulgaris* attracted tremendous scientific attention owing to its preventative effects on metabolic and renal diseases ((5,8,12). Preparations from *Berberis vulgaris* may have an antihyperuricemic effect via regulating the activity of xanthine oxidase and the metabolism of urate, and there is accumulating evidence for this (5,8).

Therefore, The present study was designed to evaluate the inhibitory activity of *Berberis vulgaris* extract on xanthine oxidase enzyme and to evaluate the protective effect of *Berberis vulgaris* extract on renal damage induced by potassium oxonate by measuring the serum creatinine and blood urea nitrogen levels in Wistar rats.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

In the current experiment, allopurinol, potassium oxonate and xanthine sodium salt were utilised. Preparation of physiological saline solution: Sodium chloride was used. Serum creatinine and blood urea nitrogen (BUN) were estimated using commercial diagnostic kits as per the manufacturer's instructions. All chemicals and reagents were of analytical grade and used in the investigation.

2.2 Collection, Authentication and Preparation of Plant Extract

Fruits of *Berberis vulgaris* were obtained from Ghansali Village, Tehri Garhwal, Uttarakhand, India and were certified by Prof. Vijay Malik, Department

of Botany, Chaudhary Charan Singh University, Meerut.

The fruits were picked, washed thoroughly, dried in the shade and pulverised to a coarse powder. The powdered material (~100 g) was extracted in Soxhlet using a mixture of ethanol-water as solvent. The extraction procedure was carried out to the ultimate depletion of the plant material. The resulting extract was filtered using Whatman No. 1 filter paper and concentrated under decreased pressure in a rotary evaporator. The dried extract was collected and kept in an airtight container in a cold and dry area for future use (13,14).

2.3 In Vitro Xanthine Oxidase Inhibitory Assay

Xanthine oxidase inhibitory activity of *Berberis vulgaris* extract was evaluated using a microplate assay method. The experiment was carried out in phosphate buffer (pH 7.4). In brief, 20 µL of xanthine oxidase solution (0.003 U/well) and 20 µL of test material were incubated in each well of 96-well microplate at 30°C for 10 min. The reaction was started by adding 20 µL of xanthine substrate solution (0.1 mM) and the reaction mixture was incubated at 30 °C for 30 min. The absorbance was measured at 295 nm, using an ELISA microplate reader. Allopurinol was used as a positive control (6,10).

The percentage inhibition of xanthine oxidase activity was calculated using the following equation:

$$\% \text{ Inhibition} = 100 - \frac{[(\text{Absorbance of control} - \text{Absorbance of test sample})]}{\text{Absorbance of control}} \times 100$$

2.4 Experimental Animals

The in vivo study was performed in healthy adult Wistar rats of either sex weighing 160–180 g. Rats were acclimatised to conventional laboratory settings and kept in polycarbonate cages (six/cage). The temperature was maintained at 22 ± 2°C, the relative humidity at 56 ± 5% and the photoperiod at 12 h light/dark. The animals received a regular pellet meal and drinking water ad libitum throughout the study period ((15,16)).

All experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) according to CPCSEA guidelines (Approval No. CCSEA/IAEC/PH-284).

2.5 Induction of Hyperuricemia

We induced experimental hyperuricemia using potassium oxonate, a recognised uricase inhibitor. In the final session, potassium oxonate was injected intraperitoneally at a dosage of 250 mg/kg body weight to increase the blood uric acid level. In all

experimental groups except the normal control, the hyperuricemia was caused by the injection of potassium oxonate ((8,17,18).

2.6 Experimental Design

A total of thirty Wistar rats were randomly divided into five groups (n = 6):

Group	Treatment Description
Group I	Normal Control
Group II	Disease Control: Potassium oxonate (250 mg/kg, i.p.)
Group III	Standard Treatment Group: Potassium oxonate (250 mg/kg, i.p.) + Allopurinol (10 mg/kg, p.o.)
Group IV	Test Group I: Potassium oxonate (250 mg/kg, i.p.) + <i>Berberis vulgaris</i> extract (250 mg/kg, p.o.)
Group V	Test Group II: Potassium oxonate (250 mg/kg, i.p.) + <i>Berberis vulgaris</i> extract (500 mg/kg, p.o.)

2.7 Drug Administration

Rats were starved for 1 hour before drug delivery. Allopurinol and extract of *Berberis vulgaris* were newly prepared in distilled water before dosage. During the trial period, treatments were given once daily by oral administration. The dosage was calculated based on the body weight of each animal before treatment. All groups except for the normal control group were induced to hyperuricemia ((8,15,17).

2.8 Collection of Blood Samples

Animals were euthanised 24 h after the final treatment under xylazine anaesthesia (10 mg/kg).

Blood samples were collected by cardiac puncture, allowed to clot at room temperature, and centrifuged to obtain serum for biochemical analyses.

2.9 Estimation of Serum Creatinine

Serum creatinine was measured by the Jaffe reaction technique using the Liquid Creatinine Kit (Erba Mannheim; Batch No. 1102508). Serum samples were diluted with distilled water (1:19), and the resulting values were multiplied by the dilution factor of 20 according to the instructions of the manufacturer. Values are reported in mg/dL.

2.10 Estimation of Blood Urea Nitrogen

The blood urea nitrogen levels were determined by the GLDH-Urease Initial Rate Method using a commercial diagnostic kit (Erba Mannheim; Batch No. B092542). Serum samples diluted in distilled water (1:100) were multiplied by a dilution factor of 101 to get the observed values. Results: mg/dl ((8,19)).

2.11 Statistical Analysis

Data were presented as mean $\pm$ SEM. Statistical analysis was done using one-way analysis of variance (ANOVA) and Dunnett's post hoc test. Differences were judged statistically significant at $p < 0.05$. Statistical analysis and graphical depiction of the data were performed using GraphPad Prism (Version 5.0).

3. RESULTS

3.1 In Vitro Xanthine Oxidase Inhibitory Activity of *Berberis vulgaris*

The result of the in vitro study indicated that *Berberis vulgaris* possesses the ability to inhibit xanthine oxidase (XO). The extract showed 60% inhibition at 500 μ g/mL dose, which suggested that the extract might reduce the synthesis of uric acid by inhibiting XO. Moreover, serial dilution tests were performed to evaluate the degree of enzyme inhibition and to estimate the IC 50 value of extract.

The findings showed that *Berberis vulgaris* has significant inhibitory efficacy against xanthine oxidase. The extract showed intriguing potential as a natural source of xanthine oxidase inhibitors, albeit it was not so prominent as allopurinol's inhibitory action.

Table 2. In vitro xanthine oxidase inhibitory activity of *Berberis vulgaris*.

Tested Sample	% Inhibition	IC ₅₀
Allopurinol (0.5 mmol)	90 ± 0.49	26.1 ± 1.46 µmol
<i>Berberis vulgaris</i> (0.5 mg/mL)	60 ± 0.62	282.21 ± 0.41 µg/mL

3.2 Effect of *Berberis vulgaris* on Serum Creatinine Levels

Effect of *Berberis vulgaris* on serum creatinine level in potassium oxonate-induced hyperuricemic Wistar rats. The blood creatinine level was found to be significantly increased in the disease control group (GP II) as compared to the normal control group (GP I) over the duration of the trial. These results imply that renal impairment follows the administration of potassium oxonate. Serum creatinine level was considerably lower in the allopurinol (GP III) therapy group than in the disease control group. *Berberis vulgaris* extract at doses of 250 mg/kg (GP IV) and 500 mg/kg (GP V) also demonstrated a substantial reduction in blood creatinine level. The protective effect of *Berberis vulgaris* on the kidney was dosage-dependent serum creatinine levels were somewhat better in the 500mg/kg treatment group than in the 250mg/kg group.

Table 3. Effect of *Berberis vulgaris* on serum creatinine levels in potassium oxonate-induced hyperuricemic Wistar rats.

Gro up	Day 1	Day 3	Day 7
GP I	0.70 ± 0.09	0.64 ± 0.09	0.59 ± 0.09
GP II	2.24 ± 0.07	2.15 ± 0.07	2.11 ± 0.07
GP III	1.28 ± 0.11***	1.22 ± 0.11***	1.15 ± 0.11***
GP IV	1.58 ± 0.14***	1.53 ± 0.14***	1.47 ± 0.14***

Gro up	Day 1	Day 3	Day 7
GP V	1.50 ± 0.12***	1.42 ± 0.12***	1.42 ± 0.12***

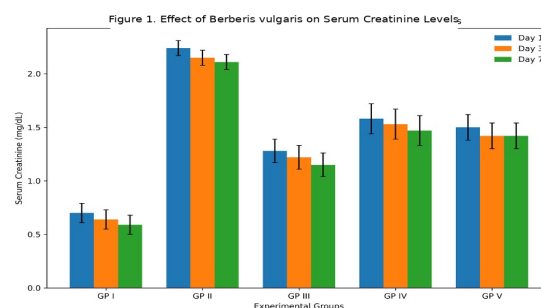


Figure 1. Effect of *Berberis vulgaris* on serum creatinine levels in potassium oxonate-induced hyperuricemic Wistar rats. Data are expressed as mean ± SEM (n = 6). ***p < 0.0001 versus disease control group (GP II).

****Data are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Dunnett's post hoc test. ***p < 0.0001 versus disease control group (GP II).**

3.3 Effect of *Berberis vulgaris* on Blood Urea Nitrogen Levels

It shows the effect of therapy with *Berberis vulgaris* on blood urea nitrogen (BUN) levels in potassium oxonate-induced hyperuricemic Wistar rats. The BUN level of disease control group (GP II) was significantly increased compared to normal control group (GP I), indicating the induction of renal impairment.

However, treatment with allopurinol greatly slowed the rise in BUN levels (GP III). Similarly, treatment with *Berberis vulgaris* extract at 250 mg/kg (GP IV) and 500 mg/kg (GP V) significantly reduced the BUN concentrations as compared to the disease control group. Furthermore, the higher dosages of *Berberis vulgaris* (500 mg/kg) showed more protective effect against potassium oxonate-induced increase in BUN, which indicates dose-dependent effect.

Table 4. Effect of *Berberis vulgaris* on blood urea

nitrogen levels in potassium oxonate-induced hyperuricemic Wistar rats.

Gro up	Day 1	Day 3	Day 7
GP I	21.5 ± 1.1	20.8 ± 1.1	20.2 ± 1.0
GP II	39.0 ± 1.2	37.4 ± 1.3	36.5 ± 1.2
GP III	24.7 ± 0.8***	24.0 ± 0.8***	23.1 ± 0.8***
GP IV	30.1 ± 0.9***	29.0 ± 0.9***	28.0 ± 0.9***
GP V	27.1 ± 0.8***	25.9 ± 0.8***	25.2 ± 0.8***

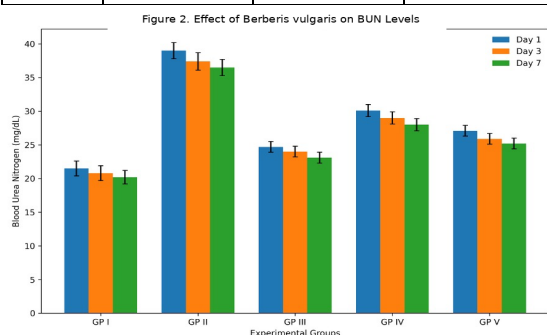


Figure 2. Effect of *Berberis vulgaris* on blood urea nitrogen levels in potassium oxonate-induced hyperuricemic Wistar rats. Data are expressed as mean ± SEM (n = 6). ***p < 0.0001 versus disease control group (GP II).

****Data are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Dunnett's post hoc test. ***p < 0.0001 versus disease control group (GP II).**

4. DISCUSSION

The positive benefits shown in the groups receiving the extract may be due to the phytochemical constituents of *Berberis vulgaris* and its alkaloid compounds, such as berberine. Previous reports of antioxidant and anti-inflammatory effects of berberine may be useful for the maintenance of renal function. In the present study, the reduction in serum

creatinine and blood urea nitrogen levels may be connected with the regulation of oxidative stress and inflammatory pathways implicated in kidney damage associated with hyperuricemia (5,8)

The present findings also confirm the classical Ayurvedic use of Daruharidra in the treatment of Vatarakta, a condition considered similar to gout and hyperuricemia. According to traditional Ayurvedic literature, Daruharidra is an anti-inflammatory and blood-purifying remedy (9–12). The observed inhibition of xanthine oxidase and the improvement of markers of renal function following the ingestion of *Berberis vulgaris* give pharmacological support for the putative therapeutic activities of this plant. The studies suggest that old Ayurvedic wisdom could be able to complement modern approaches in the management of hyperuricemia, although additional mechanistic and clinical study is required.

4.1 Study Limitations

The present study has certain limitations that should be considered while interpreting the findings. Serum uric acid levels were not determined; therefore, a direct assessment of the antihyperuricemic effect of *Berberis vulgaris* could not be established. Moreover, histological examination of renal tissue was not undertaken, which might have helped to link the biochemical improvements with the structural alterations in the kidney. Moreover, the active phytochemicals responsible for the reported pharmacological effects were not identified and measured. It would be interesting to estimate serum uric acid, histological evaluation, oxidative stress indicators, inflammatory mediators and phytochemical characterisation in future studies for a more thorough knowledge of the processes behind the protective benefits of *Berberis vulgaris*.

5. CONCLUSION

The present study showed that *Berberis vulgaris* had considerable xanthine oxidase inhibitory activity and protective effects against potassium oxonate-induced renal impairment in experimental rats. *Berberis vulgaris* considerably improved the renal function by decreasing serum creatinine and blood urea nitrogen levels. The higher dosage (500 mg/kg) was comparatively more effective than the lower dose (250 mg/kg) revealing a dose-dependent nephroprotective response.

However, although allopurinol demonstrated superior therapeutic efficacy, the present findings provide additional experimental evidence supporting the xanthine oxidase inhibitory activity and renoprotective potential of *Berberis vulgaris* in potassium oxonate-induced hyperuricemia. Further

investigations involving serum uric acid estimation, histopathological evaluation, mechanistic studies and phytochemical characterization are warranted to validate these findings and facilitate the development of evidence-based herbal therapeutics.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical Approval

All animal experimental procedures were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and were approved by the Institutional Animal Ethics Committee (IAEC) of TRANSLAM Institute of Pharmaceutical Education and Research (Approval No. CCSEA/IAEC/PH-284).

Data Availability Statement

The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

REFERENCES

1. Prasad Sah OS, Qing YX. Associations between Hyperuricemia and Chronic Kidney Disease: A Review. *Nephrourol Mon.* 2015 May 23;7(3). doi:10.5812/numonthly.7(3)2015.27233
2. Gliozzi M, Malara N, Muscoli S, Mollace V. The treatment of hyperuricemia. *Int J Cardiol.* 2016 Jun;213:23–7. doi:10.1016/j.ijcard.2015.08.087
3. Pacher P, Nivorozhkin A, Szabó C. Therapeutic Effects of Xanthine Oxidase Inhibitors: Renaissance Half a Century after the Discovery of Allopurinol. *Pharmacol Rev.* 2006 Mar;58(1):87–114. doi:10.1124/pr.58.1.6
4. Bai H, Zhang Z, Zhu M, Sun Y, Wang Y, Li B, et al. Research progress of treating hyperuricemia in rats and mice with traditional Chinese medicine. *Front Pharmacol.* 2024 Jul 19;15. doi:10.3389/fphar.2024.1428558
5. Liu YF, Wang HH, Geng YH, Han L, Tu SH, Wang H. Advances of berberine against metabolic syndrome-associated kidney disease: Regarding effect and mechanism. *Front Pharmacol.* 2023 Feb 6;14. doi:10.3389/fphar.2023.1112088
6. TJITRARESMI A, WICAKSONO IA, WICAKSONO IA, HENDRIANI R, SUSILAWATI Y, NOVIANDRI I, et al. SCREENING FOR ANTI-HYPERURICEMIA POTENTIAL OF SOME
7. Varshney B, Malik S, Singh A, Mehta N. Role of Medicinal Plants and Herbs in Veterinary Medicine. In. 2022. p. 32–48. doi:10.4018/978-1-6684-5129-8.ch003
8. Bilal M, Ahmad S, Rehman T, Ghauri AO, Khalid S, Abbasi WM, et al. Anti-Hyperuricemic and Uricosuric Potential of *Berberis vulgaris* in Oxonate-Induced Hyperuricemic Rats. Dose-Response. 2021 Jul 1;19(3):155932582110403. doi:10.1177/15593258211040329
9. Chuneekar KC. Bhavaprakasha Nighantu of Bhavamishra. G.S. Pandey. Varanasi: Chaukhambha Bharati Academy; 2018.
10. Government of India M of A. Government of India, Ministry of AYUSH. The Ayurvedic Pharmacopoeia of India. Part I, Vol. I. New Delhi; 2001. . Part I, Vol. I. New Delhi: Ministry of Health and Family Welfare, Government of India (Department of ISM & H., as applicable to that edition); 2001.
11. P. V. Sharma. Dravyaguna Vijnana. Reprint Edition. Vol. II. Varanasi: Chaukhambha Bharati Academy; 2013.
12. Srivastava S, Rawat AKS. Quality Evaluation of Ayurvedic Crude Drug *Daruharidra* , Its Allied Species, and Commercial Samples from Herbal Drug Markets of India. Evidence-Based Complementary and Alternative Medicine. 2013;2013:1–14. doi:10.1155/2013/472973
13. De Silva GO. Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. *American Journal of Essential Oils and Natural Products.* 2017;5(2):29–32.
14. Pandey A, Tripathi S. Concept of standardisation, extraction and pre-phytochemical screening strategies for herbal drug. *Journal of Pharmacognosy and Phytochemistry.* 2014;2(5):55–61.
15. Diehl K, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, et al. A good practice guide to the administration of substances and removal of blood, including routes and volumes. *Journal of Applied Toxicology.* 2001 Jan;21(1):15–23. doi:10.1002/jat.727
16. National Research Council. Guide for the Care and Use of Laboratory Animals. Washington, DC: National Academies Press; 1986.
17. Hongyan L, Suling W, Weina Z, Yajie Z, Jie R. Antihyperuricemic effect of liquiritigenin in potassium oxonate-induced hyperuricemic rats. *Biomedicine & Pharmacotherapy.* 2016 Dec;84:1930–6. doi:10.1016/j.biopha.2016.11.009
18. Stavric B, Clayman S, Gadd REA, Hébert D. Some in vivo effects in the rat induced by chlorprothixene

- and potassium oxonate. *Pharmacol Res Commun.* 1975 Apr;7(2):117–24. doi:10.1016/S0031-6989(75)80015-4
19. Taylor AJ, Vadgama P. Analytical Reviews in Clinical Biochemistry: The Estimation of Urea. *Annals of Clinical Biochemistry: International Journal of Laboratory Medicine.* 1992 May 1;29(3):245–64. doi:10.1177/000456329202900301