Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

Evaluation of Anti-asthmatic Activity of Aqueous Extract of Bark of *Prosopis cineraria* (L.) Druce against Milk-induced Leukocytosis and Eosinophilia

Anirudh Khatri*[1], Anita Rathore[2], Vikas Fenin[1], Chandra P. Jain[1]

[1]*Department of Pharmaceutical Sciences, Mohanlal Sukhadia University, Udaipur, Rajasthan, India*
[2]*Department of Veterinary Pathology, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Navania, Vallabhnagar, Udaipur, Rajasthan, India*

ARTICLE INFO

Article history:
Received: 23 December, 2021
Revised: 19 August, 2022
Accepted: 26 August, 2022
Published: 30 September, 2022

Keywords:
Eosinophilia, Leukocytosis, Oxidative stress, *Prosopis cineraria.*

DOI:
10.25004/IJPSDR.2022.140505

ABSTRACT

The present investigation was conducted to evaluate the anti-asthmatic activity of aqueous extract of the bark of *Prosopis cineraria* (L.) Druce to validate its traditional and ethnopharmacological use. Aqueous extract of the bark of *P. cineraria* (L.) Druce at doses of 100 and 200 mg/kg b.wt *i.p* was evaluated for the assessment of its anti-asthmatic activity using milk induced leukocytosis and eosinophilia, oxidative stress analysis in the liver and kidney, along with histopathological changes in the trachea of mice. The results of the present investigation showed that aqueous extract of bark of *P. cineraria* at (100 and 200 mg/kg b.wt, *i.p*) significantly decreased milk induced leukocytosis and eosinophilia, was effective towards restoration of enhanced levels of lipid peroxidation and reduced levels of superoxide dismutase (SOD) and reduced glutathione (GSH) in the liver and kidney, and showed a reduction in the thickness of the tracheal wall and cartilage along with inflammatory cells in mice in a dose-dependent manner when compared with the control group. It can be concluded that aqueous extract of the bark of *P. cineraria* (L.) Druce possesses anti-asthmatic activity.

Introduction

Asthma is a chronic inflammatory disease of the airways that arises due to a very complex interaction between the immune system and resident cells of the lung that results in bronchial hyper-responsiveness, narrowing of the airways, and increased mucus production. It affects around 300 million people in the world, which may increase further by 100 million by 2025. Herbal alternatives have regained their popularity with their safety and efficacy in the management of asthma by providing symptomatic relief along with inhibition of disease development.[1-3] *Prosopis cineraria* (L.) Druce is known locally as Khejri, Janti and Sami (India), Jand (Pakistan), and Ghaf (Arabic), and has been traditionally used by the rural community for the treatment of various ailments such as helminthiasis, asthma, leucoderma, piles, leprosy, dysentery, bronchitis, tremors of the muscles, and wandering of the mind. Prosopis has been found to contain 5-hydroxytryptamine, flavones prosogerin- C, prosogerin-D, prosogerin-E, l-arabinose, quercetin, luteolin, tannin, apigenin, isorhamnetin-3-diglucoside, and tryptamine. Patulitrin, a glucoside isolated from its flowers. Fatty acids such as palmitic acid, stearic acid, oleic acid, linoleic acid, and fixed oils are found in seeds. [3-9]

***Corresponding Author:** Mr. Anirudh Khatri

Address: Department of Pharmaceutical Sciences, Mohanlal Sukhadia University, Udaipur, Rajasthan, India

Email ✉**:** khatri.anirudh@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The state tree of Rajasthan, *P. cineraria* (L.) Druce, has been traditionally used by rural communities for the treatment of asthma. To justify its traditionally claimed use, the current study was conducted to evaluate scientifically the anti-asthmatic activity of an aqueous extract of the bark of *P. cineraria* (L.) Druce in mice.

Materials And Methods

Plant Material

The bark of *Prosopis cineraria* was collected from the desert areas of Jodhpur (Rajasthan) and authenticated by Vinod Maina, Scientist-D and Head of Office, Botanical Survey of India, Jodhpur (Rajasthan). The voucher specimen (AK-1) was deposited in the herbarium for further use.

Extraction

Dried and coarsely ground bark of *Prosopis cineraria* (480 g) was macerated with distilled water (3 days) to obtain an aqueous extract. Solvent was evaporated in a rotary evaporator under reduced pressure to produce an aqueous extract at 1.85% w/w.

Animals

Swiss albino mice of either sex, weighing (20–30 g) were housed under standard laboratory conditions. The animals had free access to food and water. The animal ethical committee approval was taken for all the protocols of the study (Registration No. 05/IAEC/CCPER/ CPCSEA/2018).

Milk-induced Leukocytosis and Eosinophilia

Mice were divided into four groups, with six in each group. Blood samples were collected from the retro-orbital plexus under light anesthesia. Group-I served as control and received a 1% Tween-80 solution. Group-II served as standard and received dexamethasone 50 mg/kg b.wt *i.p*; Group-III received aqueous extract of Prosopis@ 100 mg/kg b.wt *i.p* and Group-IV administered aqueous extract of prosopis@ 200 mg/kg b.wt *i.p*. Boiled and cooled milk (4 mL/kg, s.c.) injected into all the groups 30 minutes after treatments. Total leukocyte and eosinophil counts done in each group before drug administration and 24 hours after milk injection. The difference in total leukocyte and eosinophil counts before and 24 hours after treatment was calculated.[3]

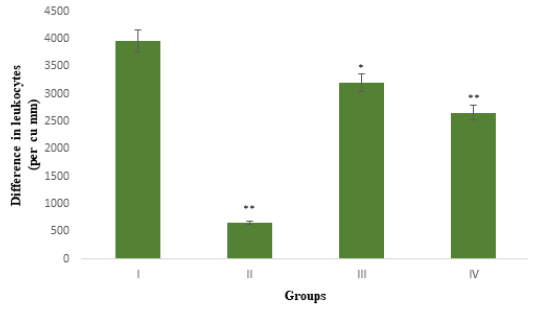

Fig. 1: Effect of aqueous extract of bark of *Prosopis cineraria* on milk induced leukocytosis in mice. ** p < 0.01, * p < 0.05 when compared with control.

Table 1: Effect of aqueous extract of *P. cineraria* bark on lipid peroxidase (in terms of MDA) levels against milk induced leukocytosis and eosinophilia in mice

Group	*Treatment*	*LPO (nmole/g)*	
		Liver	*Kidney*
I	Control (Vehicle)	60.59 ± 1.12	52.92 ± 1.17
II	Standard (Dexamethasone) @ 50 mg/kg b.wt	26.85 ± 0.88**	19.56 ± 0.55**
III	Aqueous extract of Prosopis @ 100 mg/kg b.wt	45.79 ± 0.78**	39.10 ± 1.15**
IV	Aqueous extract of Prosopis @ 200 mg/kg b.wt	42.82 ± 0.95**	30.62 ± 0.69**

Values are expressed as mean ± SEM, n = 6 in each group, ** $p < 0.01$ as compared to control group.

Oxidative Stress Analysis

About 500 mg of tissue (liver and kidney) was weighed and taken in 5 mL of ice-cold PBS (pH 7.4). The homogenates (10%) prepared with the IKA homogenizer under ice-cold conditions were centrifuged for 10 minutes at 3000 rpm. The supernatant was stored at -20°C until assayed for different oxidative stress-related biochemical parameters.[10-12] A double beam UV-vis spectrophotometer was used for recording the absorbance of the test sample.

Histopathology

The tissues of the trachea of mice were collected in 10% neutral buffered formalin from mice of each group at the end of the treatment period. For histopathology, the fixed tissues were processed mechanically for paraffin embedding by acetone and benzene technique (Lillie, 1965).[13] The sections of 4–6-micron thickness were cut and stained with the routine hematoxylin and eosin staining method.

Statistical Analysis

The results are reported as mean ± SEM and analyzed statistically using one-way ANOVA followed by the Dunnets test, using graph pad software. $p < 0.05$ was considered as significant.

Results

Milk-induced Leukocytosis and Eosinophilia

The maximum increase in the difference of leukocytes (3966.66 ± 398.05) and eosinophil (265.17 ± 27.99) count was observed in the control group 24 hours after milk injection (4 mL/kg, s.c.). Mice pretreated with aqueous bark extract of Prosopis plant at the doses of 100 mg/kg (3208.33 ± 117.91) and 200 mg/kg b.wt (2658.33 ± 71.20) showed a significant decrease in milk induced leukocytosis in dose dependent manner as shown in Fig. 1. While in milk

Table 2: Effect of aqueous extract of *Prosopis cineraria* bark on reduced glutathione levels against milk induced leukocytosis and eosinophilia in mice

Group	*Treatment*	*GSH (mM/g)*	
		Liver	*Kidney*
I	Control (Vehicle)	2.06 ± 0.04	0.98 ± 0.03
II	Standard (Dexamethasone) @ 50 mg/kg b.wt	3.72 ± 0.02**	1.80 ± 0.03**
III	Aqueous extract of Prosopis @ 100 mg/kg b.wt	2.58 ± 0.04**	1.27 ± 0.04**
IV	Aqueous extract of Prosopis @ 200 mg/kg b.wt	2.95 ± 0.04**	1.40 ± 0.02**

Values are expressed as mean ± SEM, n = 6 in each group, ** $p < 0.01$ as compared to control group.

induced eosinophilia aqueous bark extract at the doses of 100 mg/kg (205 ± 15.72) and 200 mg/kg b.wt (168.83 ± 16.52) showed statistically inhibition in dose dependent manner as shown in Fig. 2.

Oxidative Stress Analysis

Lipid peroxidation (LPO) was measured in terms of malondialdehyde (MDA) produced in the liver and kidney of mice treated with aqueous extract of Prosopis. A statistically significant ($p \leq 0.01$) increased level of LPO was observed in the control group. The treated mice showed a significant decrease in LPO as compared to the control group in a dose-dependent manner, as shown in Table 1. Reduced glutathione (GSH) was measured by estimating free-SH groups, using 5, 5'-dithio-bis-2-nitrobenzoic acid (DTNB) in liver and kidney of mice treated with aqueous extract of Prosopis. A statistically significant ($p \leq 0.01$) decreased level of GSH was observed in the control group. This alteration was significantly restored by plant extract in a dose- dependent manner, as shown in Table 2. Superoxide dismutase (SOD) was measured by generation of superoxide by pyrogallol autooxidation and the inhibition of superoxide dependent reduction of the tetrazolium dye MTT [3-(4-5 dimethyl

Table 3: Effect of aqueous extract of *Prosopis cineraria* bark on superoxide dismutase levels against milk induced leukocytosis and eosinophilia in mice

Group	*Treatment*	*SOD (unit/mg)*	
		Liver	*Kidney*
I	Control (Vehicle)	86.95 ± 2.11	62.10 ± 3.03
II	Standard (Dexamethasone) @ 50 mg/kg b.wt	164.83 ± 3.09**	133.58 ± 2.04**
III	Aqueous extract of Prosopis @ 100 mg/kg b.wt	99.65 ± 1.75*	77.81 ± 2.53**
IV	Aqueous extract of Prosopis @ 200 mg/kg b.wt	112.35 ± 3.68**	98.35 ± 1.57**

Values are expressed as mean ± SEM, n = 6 in each group, ** $p < 0.01$ as compared to control group.

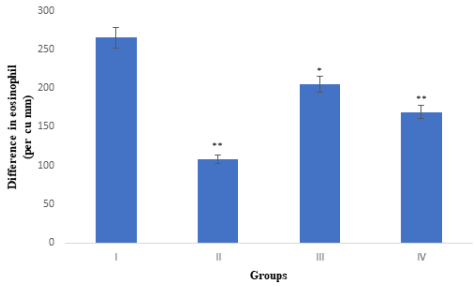

Fig. 2: Effect of aqueous extract of bark of *Prosopis cineraria* on milk induced eosinophilia in mice. ** $p < 0.01$, * $p < 0.05$ when compared with control.

thiazol 2-xl) 2, 5-diphenyl tetrazolium bromide] to its to its formazan in liver and kidney of mice treated with aqueous extract of Prosopis. A statistically significant ($p \leq 0.01$) decreased level of SOD was observed in the control group. This alteration was significantly restored by plant extract in a dose-dependent manner, as shown in Table 3. Aqueous extract of the bark of Prosopis @ 200 mg/kg b.wt showed significant restoration comparable to the standard drug dexamethasone @ 50 mg/kg b.wt.

Histopathological Study

Histopathological examination of the tracheal tissue showed a pronounced increase in the thickness and size of the tracheal wall and cartilage along with moderate inflammation across the tracheal wall as evidenced by the presence of oedema and inflammatory cells. In asthmatic patients, there was an increase in eosinophil count, mucus hypersecretion, and airway hyperreactivity were stimulated. Aqueous extract of the bark of Prosopis @ 200 mg/kg b.wt showed a more pronounced reduction in the thickness along with inflammatory cells comparable to dexamethasone @ 50 mg/kg b.wt (Fig. 3).

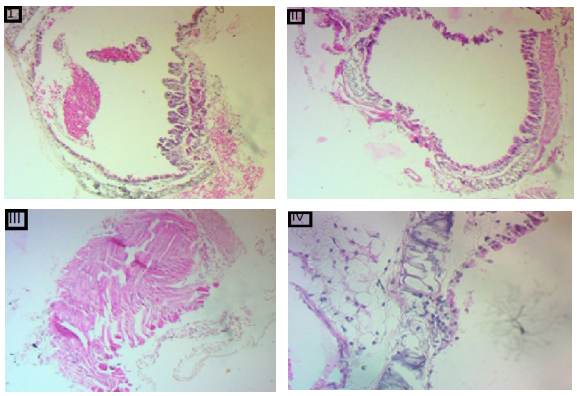

Fig. 3: (I) Tracheal section (Group I) showed mucosa, tracheal cartilage and infiltration of inflammatory cells with moderate transmural oedema H and E, 100 X. (II) Tracheal section (Group II) showed mucosa, tracheal cartilage and mild infiltration of inflammatory cells H and E, 100 X. (III) Tracheal section (Group III) showed mild transmural oedema and infiltration of inflammatory cells H and E, 100 X. (IV) Tracheal section (Group IV) showed tracheal cartilage and mild infiltration of inflammatory cells in sub mucosa H and E, 100 X.

Discussion

In the present investigation, aqueous bark extract of Prosopis plant at the doses of 100 mg/kg b.wt and 200 mg/kg b.wt was evaluated for anti-asthmatic activity by using milk induced leukocytosis and eosinophilia in mice model, as asthma involves various types of inflammatory mediators in pathology. Subcutaneous injection of milk produces a marked increase in leukocytes and eosinophils count after 24 hours by acting as an antigen.[3,14] In the present study, it was observed that aqueous extract of the bark of Prosopis @ 200 mg/kg b.wt showed significant inhibition comparable to dexamethasone @ 50 mg/kg b.wt. Phytochemical analysis of the crude extracts revealed the presence of flavonoids among the other chemical constituents within them. Several flavonoids are reported to possess smooth muscle relaxant and bronchodilator activity. The Anti-asthmatic activity of aqueous extract of bark of Prosopis plant may be due to flavonoids and thereby possess *in-vivo* antiallergic activity. The oxidative damage produced by free radicals is referred to as oxidative stress and has been associated with several degenerative diseases.[15,16]

In the present study, there was an increase in the value of MDA, an indicator of lipid peroxidation in the liver and kidney, after receiving a subcutaneous injection of milk. Aqueous extract of bark restored the increased LPO values significantly, suggesting its anti-oxidant nature.[17] Glutathione is the cells' natural anti-oxidant, which destroys free radicals formed in the cells.[18] In the present study, there was a decrease in the value of GSH in the liver and kidney of mice. Significant depletion in GSH levels was restored towards normalcy by aqueous extract of bark, which showed its potential to prevent the oxidative stress induced alteration in intracellular thiol status and GSH levels. Superoxide dismutase (SOD) catalyses the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide and prevents the formation of hydroxyl radicals, thus playing an important role in the cellular anti-oxidant defence mechanism.[19] In the present study, there was a decrease in the value of SOD in the liver and kidney of mice. Aqueous extract of bark increased the SOD levels due to its ability to scavenge superoxide anions. These efforts are further confirmed by the analysis of the histological section of the trachea in which sensitization with milk caused a pronounced increase in thickness and size of the tracheal wall and cartilage along with moderate inflammation across the trachea. The treated mice showed a reduction in the thickness of the tracheal wall and cartilage along with inflammatory cells in mice as compared to the control group in a dose dependent manner, suggesting that it might be interfering with the sensitization process. Hence, further detailed studies need to be conducted to separate the constituents and individually evaluate these phytoconstituents for their clinical efficacy in the treatment of asthmatic patients.

References

1. Clarke R, Lundy FT, McGarvey L. Herbal treatment in asthma and COPD-current evidence. Clinical Phytoscience. 2015; 1(4): 1-7.
2. Mali RG, Dhake AS. A review on herbal antiasthmatics. Oriental Pharmacy and Experimental Medicine. 2011; 11: 77-90.
3. Taur DJ, Patil RY. Anti-asthmatic activity of Ricinus communis L. Roots. Asian Pacific Journal of Tropical Biomedicine. 2011; 1(1):13-16.
4. Kaul RN. Trees or grass lands in the Rajasthan- Old problems and new approaches, Indian Forester.1967; 93: 434-435.
5. Burkart A. A monograph of the genus Prosopis (Leguminosae subfam. Mimosoideae). Part 1 and 2. Catalogue of the recognized species of Prosopis. Journal of the Arnold Arboretum. 1976; 57(3): 219-249, 450-526.
6. Mann HS, Shankarnarayan KA. The role of Prosopis cineraria in an agropastoral system in western Rajasthan. In browse in Africa symposia, H. N. LeHouerou (ed.), Addis Ababa, Ethiopia. 1980; pp. 437-442.
7. Khatri A, Rathore A, Patil UK. Prosopis cineraria (L.) Druce: A boon plant of desert- An overview. International Journal of Biomedical and Advance Research. 2010; 1(5): 141-149.
8. Vyas S, Pandya D, Mankad A. A review on Prosopis cineraria as an important plant of arid regions of India. International Journal of Multidisciplinary Research. 2020; 6(3): 1-6.
9. Singh P, Bangarwa KS, Dhillon RS. Studies on Phenology and Reproductive Biology of Khejri [Prosopis cineraria (L.) Druce]. Indian Journal of Agricultural Research. 2021; 55(1): 110-114.
10. Fernanada BAP, Cibele MCPG, Patricia PA, Ione S. Protective action of hexane crude extract of Pterodon emarginatus fruits against oxidative and nitrosative stress induced by acute exercise in rats. BMC Complementary and alternative medicine. 2005; 5:17-21.
11. Sedalk J, Lindsay RH. Estimation of total protein bound and non-protein bound sulfhydryl groups in tissue with Ellman's reagent. Analytical biochemistry. 1968; 25:192-205.
12. Madesh M, Balasubramanian KA. Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. Indian journal of biochemistry & biophysics. 1998; 35(3):184-188.
13. Lillie RD. Histopathologic technique and practical histochemistry. McGraw Hill Book Co. New York and London.1965;176.
14. Pathan AR, Vadnere G, Sabu M. Evaluation of antiasthmatic effect of Curcuma zedoaria rhizomes by using milk induced leukocytosis in mice. Neuro Pharmac Journal. 2016; 1(1):13-17.
15. Banjarnahor SDS, Artanti N. Antioxidant properties of flavonoids. Medical Journal of Indonesia. 2014; 23(4): 239-244.
16. Rice-Evans, CA, Miller NJ, Paganga G. Structure–antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine. 1996; 20: 933-956.
17. Gaschler MM, Stockwell BR. Lipid peroxidation in cell death. Biochemical and Biophysical Research Communications. 2017; 482(3): 419-425.
18. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. The Journal of Nutrition. 2004; 134: 489-492.
19. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine. 2018; 54(4): 287-293.

HOW TO CITE THIS ARTICLE: Khatri A, Rathore A, Fenin V, Jain CP. Evaluation of Anti-asthmatic Activity of Aqueous Extract of Bark of *Prosopis cineraria* (L.) Druce against Milk-induced Leukocytosis and Eosinophilia. Int. J. Pharm. Sci. Drug Res. 2022;14(5):537-540. **DOI:** 10.25004/IJPSDR.2022.140505