Nat. Volatiles & Essent. Oils, 2021; 8(5): 11479-11485

NVEO
Natural Volatiles & Essential Oils

Effect of methanolic extract of bark of *Prosopis cineraria* (L.) Druce on milk induced leukocytosis and eosinophilia in the management of asthma

Anirudh Khatri*[1], Anita Rathore[2] and Chandra Prakash Jain[1]

[1]*Department of Pharmaceutical Sciences, Mohanlal Sukhadia University, Udaipur- 313001, Rajasthan, India.*

[2]*Department of Veterinary Pathology, College of Veterinary and Animal Science, RAJUVAS, Navania, Vallabhnagar, Udaipur-313601, Rajasthan, India.*

Abstract

Objective: To evaluate the effect of methanolic extract of bark of *Prosopis cineraria* (L.) Druce for the management of asthma. **Methods:** In the present study, methanolic extract of bark of *Prosopis 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 liver and kidney along with histopathological changes in trachea of mice. **Results:** The results of the present investigation showed that methanolic extract of bark of *Prosopis cineraria* at (100 and 200 mg/kg b.wt, *i.p*) significantly decreased milk induced leukocytosis and eosinophilia, effective towards restoration of enhanced level of lipid peroxidation and reduced level of GSH and SOD in 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 control group **Conclusion:** It can be concluded that methanolic extract of bark of *Prosopis cineraria* (L.) Druce may be used in the management of asthma.

1. Introduction

Asthma is one of the chronic diseases which affects around 300 million people in the world. In 1989 the global initiative for asthma program was commence to raise awareness of this disease on the increasing worldwide patients a further 100 million by 2025.[1-2] *Prosopis cineraria* (L.) Druce is known locally as Khejri, Janti & Sami (India), Jand (Pakistan), and Ghaf (Arabic) has been traditionally used by rural community for 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 acid such as palmitic acid, stearic acid, oleic acid and linoleic acid and fixed oils are found in seeds.[3-8] The present study was conducted to evaluate anti-asthmatic activity of bark of *Prosopis cineraria* (L.) Druce in mice to validate its traditionally claimed use in the management of asthma.

2. Materials and methods

2.1 Plant material

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

2.2 Extraction

Dried and coarsely powder of *Prosopis cineraria* bark (480 g) was defatted with petroleum ether and the marc remaining was extracted successively with 95% methanol in in Soxhlet apparatus. Solvent

was evaporated in rotary evaporator under reduced pressure to produce methanolic extract at 1.92% w/w.

2.3 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).

2.4 Milk induced leukocytosis and eosinophilia

Mice were divided into four groups with six in each group. Blood samples were collected from retro-orbital plexus under light anesthesia. Group-I served as control and received 1% Tween-80 solution. group-II served as standard and received dexamethasone 50 mg/kg b.wt *i.p*, group-III received methanolic extract of Prosopis@ 100 mg/kg b.wt *i.p* and group-IV administered methanolic extract of Prosopis@ 200 mg/kg b.wt *i.p*. Boiled and cooled milk (4ml/kg, s.c.) injected to all the groups 30 minutes after treatments. Total leukocyte and eosinophil count done in each group before drug administration and 24 hr after milk injection. Difference in Total leukocyte and eosinophil count before and 24 hr after treatment was calculated.[2]

2.5 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 IKA Homogenizer under ice-cold condition were centrifuged for 10 min at 3000 rpm. The supernatant was stored at -20°C until assayed for different oxidative stress-related biochemical parameters.[9-11] A double beam UV-VIS spectrophotometer was used for recording the absorbance of the test sample.

2.6 Histopathology

The tissues of 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)[12]. The sections of 4–6-micron thickness was cut and stained with routine Hematoxylin and Eosin staining method.

2.7 Statistical Analysis

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

3. Results

3.1 Milk induced leukocytosis and eosinophilia

The maximum increase in difference of leukocytes (3966.66 ± 398.05) and eosinophil (265.17 ± 27.99) count was observed in control group 24 hr after milk injection (4ml/kg, s.c.). Mice pretreated with methanolic bark extract of Prosopis plant at the doses of 100 mg/kg (2325 ± 108.59) and 200 mg/kg b.wt (1425 ± 61.57) showed a significant decrease in milk induced leukocytosis in dose dependent manner as shown in figure 1. While in milk induced eosinophilia methanolic bark extract at the doses of 100 mg/kg (147.33 ± 3.94) and 200 mg/kg b.wt (123.17 ± 8.60) showed statistically inhibition in dose dependent manner as shown in figure 2.

3.2 Oxidative stress analysis

Lipid peroxidation (LPO) was measured in terms of malondialdehyde (MDA) produced in liver and kidney of mice treated with methanolic extract of Prosopis. A statistically significantly ($P\leq0.01$) increased level of LPO was observed in control group. The treated mice showed a significant decrease in LPO as compared to control group in 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 methanolic extract of Prosopis. A statistically

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

3.3 Histopathological study

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

4. Discussion

In the present investigation methanolic 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.[2] In present study it was observed that methanolic extract of bark of Prosopis @ 200mg/kg b.wt showed significant inhibition comparable to dexamethasone @ 50mg/kg b.wt. Phytochemical analysis of the crude extracts revealed the presence of flavonoids among the other chemical constituents within them. Several flavonoids reported to possesses smooth muscle relaxant and bronchodilator activity. The Anti-asthmatic activity 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.[13] In the present study there was increase in value of MDA, indicator of lipid peroxidation in liver and kidney after received subcutaneous injection of milk. Methanolic extract of bark restored the increased LPO values significantly suggesting its anti-oxidant nature. Glutathione is the cells natural anti-oxidant, which destroys free radical formed in the cells. In the present study there was decrease in value of GSH in in liver and kidney of mice. Significant depletion in GSH levels was restored towards normalcy by methanolic extract of bark showed its potential to prevent the oxidative stress induced alteration in intracellular thiol status and GSH levels. Superoxide dismutase (SOD) catalyze the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide and prevents formation of hydroxyl radicals thus play an important role in the cellular anti-oxidant defense mechanism. In the present study there was decrease in value of SOD in liver and kidney of mice. Methanolic extract of bark increased the SOD levels due to its ability to scavenge superoxide anions.[14] These efforts are further confirmed by the analysis of the histological section of the trachea in which sensitization with milk caused pronounced increase in thickness and size of tracheal wall and cartilage along with moderate inflammation across the tracheal wall. The treated mice showed a reduction in the thickness of the tracheal wall and cartilage along with inflammatory cells in mice as compared to control group in dose dependent manner suggests that it might be interfering with the sensitization process.

Table 1: Effect of methanolic extract of *Prosopis 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) @ 50mg/kg b.wt	26.85±0.88**	19.56±0.55**
III	Methanolic extract of Prosopis @ 100mg/kg b.wt	38.59±0.58**	27.76±0.56**
IV	Methanolic extract of Prosopis @ 200mg/kg b.wt	32.68±0.53**	23.54±1.02**

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

Table 2: Effect of methanolic 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) @ 50mg/kg b.wt	3.72±0.02**	1.80±0.03**
III	Methanolic extract of Prosopis @ 100mg/kg b.wt	3.25±0.04**	1.51±0.03**
IV	Methanolic extract of Prosopis @ 200mg/kg b.wt	3.43±0.04**	1.67±0.02**

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

Table 4.20: Effect of methanolic 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) @ 50mg/kg b.wt	164.83±3.09**	133.58±2.04**
III	Methanolic extract of Prosopis @ 100mg/kg b.wt	129.40±2.88**	112.95±2.13**
IV	Methanolic extract of Prosopis @ 200mg/kg b.wt	148.97±3.27**	127.32±4.10**

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

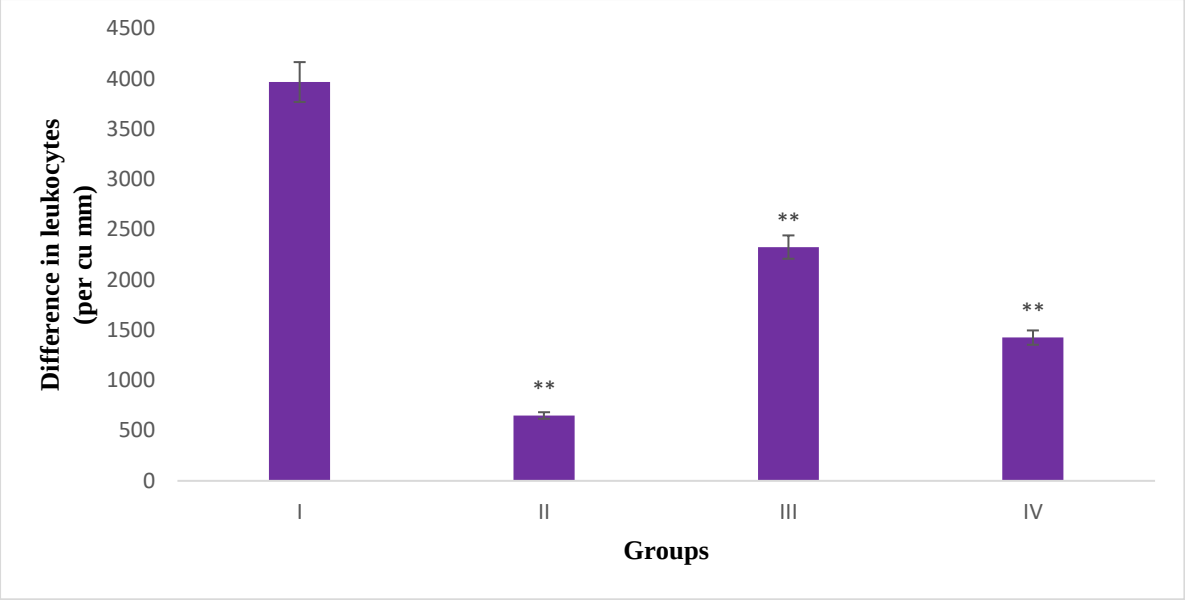


Figure 1. Effect of methanolic extract of bark of *Prosopis cineraria* on milk induced leukocytosis in mice. ** P<0.01 when compared with control.

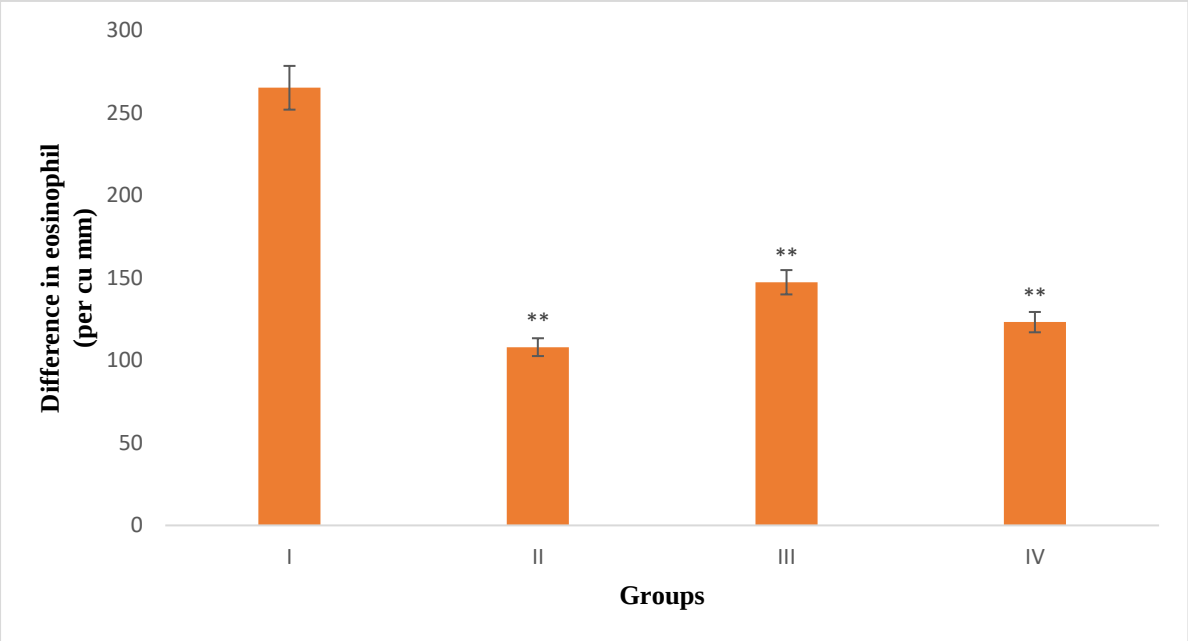
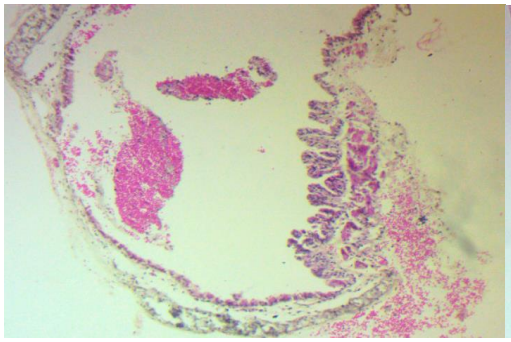
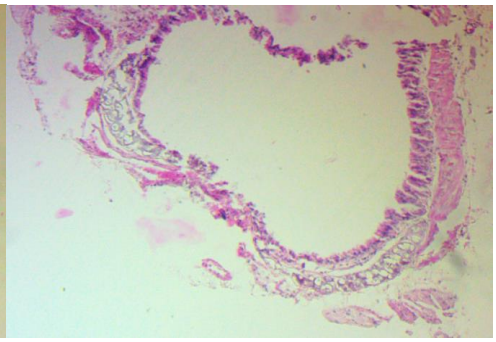


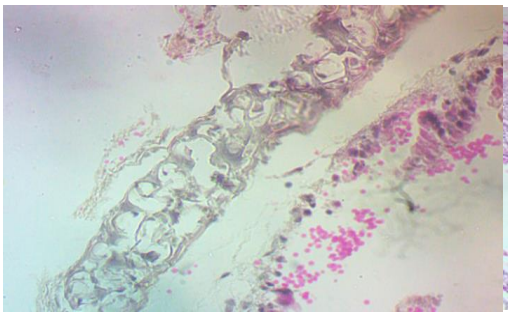
Figure 2. Effect of methanolic extract of bark of *Prosopis cineraria* on milk induced eosinophilia in mice. ** P<0.01 when compared with control.



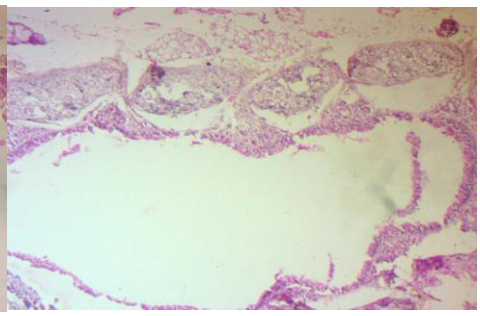
Group I Tracheal section showed mucosa, tracheal cartilage and infiltration of inflammatory cells with moderate transmural oedema H&E, 100 X.



Group II Tracheal section showed mucosa, tracheal cartilage and mild infiltration of inflammatory cells H&E, 100 X.



Group III Tracheal section showed tracheal cartilage with mild infiltration of inflammatory cells H&E, 100 X.



Group IV Tracheal section showed mucosa, tracheal cartilage and mild infiltration of inflammatory cells H&E, 100 X.

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