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Evaluation of diuretic and laxative activity of aqueous extract of Argemone mexicana leaves in rats

Evaluación de la actividad diurética y laxante del extracto acuoso de hojas de *Argemone mexicana* en ratas

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RESUMEN

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Objetivos: Argemone mexicana ha sido ampliamente estudiada por sus diversos beneficios farmacológicos y se ha utilizado en la medicina tradicional para tratar síntomas de estreñimiento. El presente estudio evaluó el extracto por su potencial diurético y laxante.

Método: El extracto acuoso de *Argemone mexicana* se preparó utilizando el método de percolación y se sometió a análisis fitoquímico. La evaluación de la actividad diurética y laxante se llevó a cabo utilizando unas jaulas metabólicas y un fotómetro de llama según el método estándar descrito con anterioridad. Se administró frusemida (20 mg / kg) y picosulato de sodio (5 mg / kg) como control positivo de la actividad diurética y actividad laxante respectivamente.

Resultado: El extracto mostró una actividad diurética significativa a una dosis de 250 mg / kg en comparación con la frusemida estándar. Incluso, este extracto también es efectivo para aumentar la concentración de electrolitos. Mientras que el extracto a 250 mg/kg mostró un aumento significativo en la producción fecal, y también aumentó significativamente el peso de las heces en ambas dosis.

Conclusión: El hallazgo significativo anterior apoya el uso tradicional de *Argemone mexicana* por sus potencialidades diurética y laxante.

Palabras clave: *Argemone mexicana*, Diurético, Laxante, Frusemida, Picosulfato de Sodio, Loperamida.

ABSTRACT

Objectives: *Argemone mexicana* have been widely studied for its several pharmacological benefits and has been used in traditional medicine to treat constipation like symptoms. The present study carried out to evaluate the extract for its diuretic and laxative potential.

Method: The aqueous extract of *Argemone mexicana* prepared using percolation method and subjected to phytochemical analysis. Evaluation of diuretic and laxative activity was carried out using metabolic cage apparatus and flame photometer as per the standard method reported earlier. Frusemide (20 mg/kg) and sodium picosulate (5 mg/kg) were served as positive control for diuretic activity and laxative activity respectively.

Result: The extract showed significant diuretic activity at 250 mg/kg dose when compared to standard frusemide. Even this extract also effective in increasing electrolyte concentration. Whereas the extract at 250 mg/kg showed significantly increasing in fecal output, and also significantly increased the weight of feces at 100 mg/kg and 200 mg/kg dose.

Conclusion: The previous significant finding supports the traditional use of *Argemone mexicana* for its diuretic and laxative potential

Keywords: *Argemone mexicana*, Diuretic, Laxative, Frusemide, Sodium Picosulfate, Loperamide.

INTRODUCTION

Argemone mexicana Linn. (Family: Papaveraceae) is commonly known as 'Mexican prickly poppy' and 'Satyanashi'. It is a widely distributed plant throughout the subtropical and tropical regions of the world. It is an erect, prickly annual herb, up to 1.2 meters in height, naturalized throughout India up to an altitude of 1,500 meters1. An analgesic, antispasmodic, possibly hallucinogenic and sedative weed-plant that is known to lend itself as a traditional healing agent in the treatment of malaria, warts, cold sore, skin disease, skin disease, itches. The plant extract reported for various activities like a sedative and anxiolytic activity[2]. *Argemone mexicana* of reported the presence of alkaloids protopine, allocryptopine sanguinarine and chelerthrine[3] and no trace of morphine or codeine. *Argemone mexicana* has been studied for its antimicrobial activity[4]. Anticancer activity[5] in-vitro antioxidant activity[6] antidiabetic activity[7] anti-inflammatory and analgesic activity[8]. However, there is no report in the literature validating its medicinal use in constipation or other gastrointestinal disorders. In this investigation, we studied the leaf extract of *Argemone mexicana* using the in vivo and in-vitro assays, to provide a scientific base for its medicinal use in indigestion and constipation.

MATERIAL AND METHOD

Preparation of crude extract

The leaves of *Argemone mexicana* were locally collected from the campus of MES College of Pharmacy, Sonai in March 2014. The plant was authenticated by A. Benniamin, Scientist and HOD, Botanical Survey of India, Pune and the specimen has been preserved at BSI, Pune, with voucher no. BSI/WRC/Tech./PSB01.

The percolation method was used for extraction. The leaves were was collected and shade dried at room temperature. Dried leaves were consistently ground using the mechanical grinder to make delicate powder. The powdered leaves (1 kg) were added in 3.0 l of boiling water and macerated in a percolator for 2 h. The percolation process was continued by gradually adding boiling water till the extraction process was completed, indicated by fade colored menstruum. The percolate was concentrated under reduced pressure, cooled and alcohol was added as the preservative. The extract was collected in an air-tight receptacle and stored at 4^{o}C up for further use[9].

Phytochemical screening

The crude extract of *Argemone mexicana* was subjected to phytochemical analysis qualitatively for the presence of alkaloids, saponins, tannin, resin, glycosides and anthraquinones as according to a standard method.[10-11]

Animals

Healthy Wistar albino rats (n=6) of both sexes (180-220 g) used in this study were maintained at the institutional animal house, kept in standard polypropylene cages with 12 h light-dark cycle at (22±3)^{0}C. The animals were fed a standard rodent chow diet and accessed water *ad libitum*. After proper acclimatization, the animals were used for the study. To carry out the experiment the approval of animal ethical committee was obtained as per the Indian Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines outlined by the Institutional Animal Ethical Committee (IAEC) of MES College of Pharmacy, Sonai and approval number for the study was MESCOP-1211/ac/08/CPCSEA.

Acute oral toxicity study

Wistar albino rats (n=6) (80-220 g) of both sexes were used to determine the toxicity of *Argemone mexicana*, following the acute oral toxic class method of Organization for Economic Co-operation and Development (OECD) according to 423 guidelines.[12] Animals were fasted for 3 h prior to the experiment and doses ranging from 0.1 to 2 g/kg were administered orally. The animals were observed for 48 h following dosing and evaluated for neurological, behavioral and autonomic symptoms, as well as mortality for 14 d following administration of extract.

Evaluation of diuretic activity

The diuretic activity of *Argemone Mexicana* leaves extract was carried out in accordance with slight modification of earlier methods.[13-14] The dose was selected according to Goutam Brahmachari[11] and toxicity study. The animals were randomly divided into four groups of six rats per group. The rats were kept for fasting for 18 h prior to the experiment. The 1st group of animals (control) received saline solution (15 mL/kg, p.o.); in the saline group, 2nd (standard) received frusemide (20 mg/kg, i.p.).[15] The remaining groups that are group third (the test I) and fourth (test II) received the aqueous extract at the doses of 100 and 250 mg/kg (p.o.) respectively, in normal saline. The animals were hydrated with saline solution (15 mL/kg) immediately after dosing and placed in metabolic cages for separate collection of urine and feces (3 per cage; Dolphin, India). Food and water were withheld for 5 h after the animals were placed in their cages, and the cages were maintained at 25.0±0.5 ^{0}C throughout the experiment. The collected volume of urine was measured at the end of 5-h treatment and subjected to analysis.

Urine analysis

The volume, pH, and conductivity of collected urine were estimated using a pH meter and a conductometer. The Na^+ and K^+ concentration in urine were measured using a flame photometer (Mediflame 127, Systronics, Ahmedabad, India). The appropriate filters were used for calibration and the flame intensity of Na+ and K+ determined. The concentration of the Na+ and K+ was calculated from the graphs and expressed in terms of mEq/L.[16] The chloride concentration was determined by titration with silver nitrate solution (N/50) using three drops of 5% potassium chromate solution as an indicator.[17]

Evaluation of laxative activity

Laxative activity was evaluated according to the method of Meite *et al.*[18] with slight modification. Animals fasted for 12 h before the experiment. The animals were placed individually in cages lined with filter paper. Rats were divided into five groups, the first group (negative control) received saline (5 mL/kg, p. o.). The second group (positive control) received sodium picosulfate (5 mg/kg, p.o). The third and fourth groups received 100 and 250 mg/kg p.o. of the *Argemone mexicana* aqueous extract. Immediately after dosing, the animals were kept in individual cages lined with clean filter paper, to collect feces. The fecal production (total number of normal as well as wet) in all groups was monitored for 16 h.[19]

Laxative activity on loperamide-induced constipation

This study was carried out according to the Mikhail O nafiu. *et al.*[20] Animals were divided into five groups of four animals each, they were individually placed in cages lined with clean filter paper, allowed to fast for 18 hours and. The first two groups were treated with the aqueous extract of *Argemone mexicana* (100 and 250 mg/kg, p.o.). Group third of received normal saline (5 mL/kg, p.o) and served as a control. Group fourth received the standard drug sodium picosulfate (5 mg/kg). After 1 h, all group received Loperamide (5 mg/kg, p.o.) by gavage. The feces production (total number) in all groups was monitored for 8 h.

Statistical analysis (P<0.05)

The results were expressed as the mean ± standard error of the mean (SEM), and data was statistically analyzed by one-way analysis of variance followed by Dunnett's multiple comparison tests. All the results obtained in this study were compared with the vehicle control group.

RESULT

Preliminary phytochemical screening

The leaves of *Argemone mexicana* were extracted using distilled water as a solvent. The percentage yield of extract was found to be 34% (w/w). The preliminary phytochemical screening of crude extract revealed the presence of phytochemical constituents such as alkaloids, reducing sugar, proteins, tannins, flavonoids, glycosides, saponin and terpenoids. However, anthraquinone and resin were absent. The results are shown in Table 1.

Acute oral toxicity studies

It was observed that oral administration of aqueous extract of *Argemone mexicana leaves* to the rat up to 2000 mg/kg dose neither showed any mortality or any visible clinical signs of general weakness in the animals like tremors, diarrhea, convulsion etc.

Diuretic activity

The diuretic activity of Argemone *mexicana* leaves extract evaluated using the standard methods, measuring urinary output, pH, and conductivity. The extract at (100 and 250 mg/kg, p.o.) dose and standard frusemide (20 mg/kg, i.p.) significantly (P<0.05) increased urinary output. (Table 2).

As there was an increase in urinary output in *Argemone mexicana* treated rats at 250 mg/kg, we were interested in investigating the effect of *Argemone mexicana* extract on the concentration of electrolytes in the urine in order to identify the mechanism of its diuretic activity. The *Argemone mexicana* extract produced a significant (P<0.05) increase in excretion of sodium, potassium and chloride ions at both of the treatment doses (100 and 250 mg/kg p.o.; Table 3). Changes in other parameters such as conductivity and pH were not significant when compared with that of the vehicle control group. The saluretic index and Na^+/K^+ ratio were also calculated and are shown in Table 3.

Laxative activity

The extract showed dose dependant increase in fecal output of rats when compared to the control group (Table 4). The effects of *Argemone mexicana* increased significantly fecal output at doses of 100 and 250 mg/kg (p.o.) of rats compared to control group ($p < 0.05$ and $p<-0.01$ respectively). Extract effect at the higher dose of 250 mg/kg (p.o.) was similar to that of the standard drug sodium picosulfate (5 mg/kg, p.o.).

Table. 1 Preliminary phytochemical screening of hydro-alcoholic extract of *Argemonae Mexican* leaves.

Sr. No.	Phytochemical test for	Result
1	Alkaloids	+
2	Anthraquinones	-
3	Flavonoids	+
4	Glycosides	+
5	Proteins	+
6	Resins	-
6	Reducing sugar	+
7	Saponins	+
8	Tannins	+
8	Terpenoids	+

Table. 2 Effects of frusemide and hydro-alcoholic extract of *Argemonae mexicana o*n urinary volume, pH, and conductivity in normal rats

Treatment	Dose	Urine Vol. (ml)	pH	Conductivity
Saline	15 ml/kg	3.46+ 0.14	7.13 + 0.15	15.62 + 0.44
Frusemide	20 mg/kg	8.31 + 0.24*	7.23 + 0.19	18.1 + 1.25
Extract	100 mg/kg	3.71 + 0.33	7.24 + 0.32	17.75 + 0.64
Extract	250 mg/kg	6.51 + 0.14*	7.1 + 0.28	17.9 + 1.27

Values are expressed as the mean + standard error of the mean (n=6). *P<0.05, vs vehicle control (One-way analysis of variance Dunnett's multiple comparison tests).

Table.3 Diuretic activity of hydro-alcoholic extract of *Argemonae Mexicana* in rats

		Concentration of ions (mEq/L)			Saluretic index			Na/K ratio
Treatment	Dose	Na	K	Cl	Na	K	Cl	
Control	15 ml/kg	24.25 + 0.35	8.51 + 0.26	14.34 + 0.59				2.85
Frusemide	20 mg/kg	54.26 + 0.26**	31.14 + 0.34**	34.12 + 0.41**	2.238	3.66	2.381	1.74
Extract	100 mg/kg	26.3 + 0.68*	15.1 + 0.33 **	17.42 + 0.52**	1.104	1.774	1.214	1.71
Extract	250 mg/kg	44.57 + 0.35**	22.9 + 0.47 **	25.3 +0.551*	1.84	2.71	1.764	1.95

Values are expressed as the mean + standard error of mean (n=6). *P<0.05, vs vehicle control (One-way analysis of varience Dunnett's multiple comparision test).

Table. 4 Laxative activity of aqueous extract of *Argemone mexicana* in rats.

Treatment	Dose		Feces out put (g)
		0-8	8-16 h
Control	5 ml/kg	0.751 +0.48	1.612+ 0.62
Sodium Picosulfate	5mg/kg	5.912 + 0.14**	5.511 + 0.71 **
Extract	100 mg/kg	3.711 + 0.74*	4.131 + 0.31 *
Extract	250 mg/kg	4.871 + 0.97**	4.86 + 0.68 **

Values are expressed as the mean ± standard error of mean (n=6). *P<0.05 compared to control group; **P<0.01 compared to control group (One-way analysis of varience Dunnett's multiple comparision test).

Table. 5 Effect of *Argemone mexicana* aqueous extract on loperamide induced constipation in rats.

Treatment	Dose	Weight of feces (g)
Control	5 ml/kg	0.947 + 0.45
Sodium Picosulfate	5mg/kg	3.851 + 0.63**
Extract	100 mg/kg	2.701 + 0.33*
Extract	250 mg/kg	3.231 + 0.47**

Values are expressed as mean + standard error of mean (n=6). *P<0.05 compared to control group; **P<0.01 compared to control group (One-way analysis of varience Dunnett's multiple comparision test)

Effect of the aqueous extract of Argemone mexicana on loperamide-induced constipation in rats

In loperamide-induced constipation, the aqueous extract of *Argemone mexicana* increased the total number of feces in a dose-dependent manner, and the results were statistically significant ($p < 0.05$) (Table 5). The reduction of loperamide-induced constipation at 250 mg/kg (p.o.) of plant extract treatment was found to be almost comparable with that of treatment by 5 mg/kg of sodium picosulfate.

DISCUSSION

Considering the medicinal use of *Argemone mexicana* in gut disorder like indigestion and constipation. Preliminary phytochemical analysis revealed the presence sugar, proteins, tannins, flavonoids, saponin and terpenoids in the aqueous extract (Table 1). The toxicological study showed that extract was safe at 2 gm/kg dose. Present study supporting the traditional use of an aqueous extract of *Argemone mexicana* as a diuretic. The extract was found significantly increasing the urinary output at 250 mg/kg than the 100 mg/kg extract as compared to the standard. The plasma sodium and potassium are important for homeostasis. Thus, the normal level of sodium and potassium is important for renal control of acid-base balance. Electrolyte concentration revealed that the aqueous extract of *Argemone mexicana* significantly increasing the sodium, potassium, and chloride at both the doses, even these doses increased specific conductivity that indirect measures the ionic content of urine. These findings support the traditional use of the plant for diabetes and hypertension. Thus the diuretic action not be attributed to increasing electrolyte excretion. The preliminary phytochemical analysis of extract revealed the presence of polar compounds such as flavonoids and terpenoids are responsible for its diuretic activity. The current study supports the traditional use of *Argemone mexicana* as a diuretic agent. Further studies are required to isolate the active principal responsible for the diuretic action.

Fecal output depends on the dietary fiber, water-electrolyte balance, the rate of absorption and secretion from the lumen. Many laxatives have common mechanism action that is increasing water electrolyte secretion, decreasing its absorption in the colon. The presence of terpenoids, flavonoids, sterols, phenolic compounds can be responsible for the laxative activity of the plant. Although the phytochemical screening revealed the presence of terpenoids, flavonoids like components.

The laxative activity of *Argemone mexicana* was studied in rats. Oral administration of extract showed the significant and dose-dependent increase in fecal output of rats in regards to the accumulation of water in the intestine.

Our results showed that *Argemone mexicana* extract and sodium picosulfate (standard) exert respectively opposite effects with loperamide on the gastrointestinal function. It is well documented that loperamide abolishes experimental osmotic diarrhea by acting on intestinal motility, and consequently reducing the flow entering the colon.[21-22]

Sodium picosulfate related to the polyphenolic category of stimulant laxatives. After oral administration, it is converted in the intestine to an active form through the action of bacterial enzymes.[23]

Sodium picosulfate increase peristaltic movements and reduces water reabsorption, increases secretion which leading to softening stool. These results suggest that the active principle of extract act through the same way.

CONCLUSION

Current research showed significant diuretic and laxative effect of aqueous extract of *Argemone mexicana* in rats. Thus, this study provides sound mechanism basis for the medicinal use of *Argemone mexicana* in constipation. Further research is required to assess the possible exact mechanism of extract.

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