Journal of

Pharmacology and Toxicology

ISSN 1816-496X

Academic
Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 2 (4): 391-395, 2007
ISSN 1816-496X

Pharmacological Evaluation of Different Extract of *Asclepias daemia* Leaves

[1]C. Karthikeyan, [1]S. Siva kumar, [1]P. Chandrasekar,
[1]A. Heber, [1]S.J.H. Robert and [2]N.S.H.N. Moorthy
[1]Pearl Peace Medical Mission College of Pharmacy, Tirunelveli – 10, Tamil Nadu, India
[2]School of Pharmaceutical Sciences, Rajiv Gandhi Proudyogiki Vishwavidyalaya,
Airport bypass road, Gandhi Nagar, Bhopal – 462036 (MP) India

Abstract: Antisecretory, gastric transit time and wound healing activity of various extract of the shade-dried powder of *Asclepias daemia* leaves was studied in albino rat and mice respectively. Concentration of gastric hydrogen ions was determined for antisecretory activity by titration with 0.01 N sodium hydroxide using Tofer's reagent as an indicator. The gastric transit activity was determined by using charcoal meal. The results showed that chloroform and aqueous extract produced significant action on antisecretory, gastric transit time and wound healing activities, while petroleum ether extract have no remarkable activity. Aqueous extract showed highly significant ($p<0.01$) antisecretory and gastric transit activities.

Key words: *Asclepias daemia,* antisecretory, wound healing, extract

INTRODUCTION

Asclepiadaceae (Kritikar and Basu, 1999) is a large genus of shrubs, perennial herbs often twining distributed throughout the hotter parts of India. Of the various species known, extensive works have been carried out on only some of them like, *Asclepias curassiva* and *Asclepias daemia*, which are of much medicinal importance. They have been used as stimulating poultice and anthelmintic. *Asclepias daemia* species of Asclepiadaceae, have hairy stems with milky juice, leaves 4-6 cm long, sometimes more ovate or round hairy on lower surface, deeply cordate at base, flowers pale or white small in short cluster. Some pharmacological effects have been reported on the juice of leaves, flavanoids in the leaves are used as an expectorant and also used in the treatment of asthma and rheumatic swelling. Earlier study on leaves extract from our laboratory showed that the leaves extract have anti-inflammatory activity (Karthikeyan *et al*., 2005) and its action on smooth muscles indicate that it may have significant action on GI tract and blood vessels.

Existing literature attributes *Asclepias daemia* leaves with a number of useful properties such as expectorant, anti-asthmatic, anti-rheumatic swelling and anti-inflammatory actions. A review of literature did not reveal any information on the GIT action of this leaves but other species of Asclepias possess GIT action (anthelmentic and poultice). Realizing the potential use of this plant in smooth muscles, the present study is therefore an attempt to assess the efficacy of different extract of this indigenous plant for antisecretory, gastric transit time and wound healing activity.

MATERIALS AND METHODS

The leaves of the plant were collected from Tirunelveli, Tamil Nadu, India and was identified and certified by Mrs. Indrani Bose M.Sc., M.Phil. Dept. of Botany, Rani Anna Govt. College (W),

Corresponding Author: N.S.H.N. Moorthy, School of Pharmaceutical Sciences, RGPV, Bhopal – 462036 (MP) India

Tirunelveli. The study was performed in Department of Pharmacology, Pearl peace Medical Mission College of Pharmacy, Tirunelveli, India on July, 2000. Albino rats of either sex weighing about 120-200 g for antisecretory, male mice for GI transit activity and non-anaesthetized mice for wound healing property were used for the study (animals for these studies were obtained from Govt. Medical College, Tirunelveli).

Concentration of gastric hydrogen ions in free and total acid was determined by titration with 0.01 N sodium hydroxide using Tofer's reagent as an indicator. The gastric transit activity was determined by using charcoal meal and control vehicle or atropine (8 and 16 mg kg^{-1}) was used as control.

About 500 g of dried powder was extracted with petroleum ether (60-80°C) by continuous hot percolation using Soxhlet apparatus (Evans, 2002; Kokate, 1994). The total extract was concentrated by vacuum distillation. The marc left after the petroleum ether was taken and subsequently extracted with chloroform and water.

Extraction

About 500 g of dried powder was extracted with petroleum ether at 60 to 80°C by continuous hot percolation using Soxhlet apparatus. The extraction was continued for 24 h. The petroleum ether was filtered and concentrated using vacuum distillation. A yellowish waxy residue was obtained (3.6%). The marc left after the petroleum ether was taken and subsequently extracted with chloroform extract up to 24 h. The chloroform extract was then filtered and concentrated. A black colour residue was obtained (3.9%). The marc left after the chloroform extract was macerated for 24 h with water to obtain the aqueous extract. Preservative was added to the aqueous extract to prevent fungal growth. This aqueous extract was concentrated to get brown colour residue (14.76%).

Determination of LD_{50} (Gosh, 1984; Loomis, 1968)

Mice were kept for overnight fasting and they were divided into groups of 10 mice each. The drugs under study were administered by oral route on different doses groups. The animals were then observed for signs of toxicity and mortality for two hours and then at the end of 24 h number of animals dead was counted in each group. Percent death was then calculated and was transferred to profits using probit table. Probits were then plotted (Y-axis) against the log dose of the drug and the LD50 was determined by the dose that corresponds to probit 5.

Evaluation of Antisecretory Activity (Hossein *et al.*, 2002; Sertie *et al.*, 2000)

Albino rats of either sex weighing between 120 and 200 g were starved for 48 h with water *ad libitum*. To prevent coprophogy animals were housed in individual cages having specially designed grid floor. The pyrolic ligation was done under ether anaestheias, care was taken not to include blood vessels within the ligature. The *Asclepias daemia* extracts was administered subcutaneously immediately after pyloric ligation. Four hour later, the stomachs were removed under ether anaesthesia after oesophagal ligation. The gastric contents were collected and volume and pH were determined. Free and total acidity were determined by titrating 0.1 mL of gastric juice against 0.01 N sodium hydroxide using Topfer's reagent as an indicator. The activity was expressed in milliequivalent of gastric juice. The total acid output was also calculated as $\mu eq\ h^{-1}$.

Gastrointestinal Transit Activity (Yamauchi *et al.*, 1987)

The charcoal meal transit test was conducted on groups of 5 male mice. Each animal was administered PO with the extracts, control vehicle or atropine (8 and 16 mg kg^{-1}) and 45 min later all the animals received orally a 5% deactivated charcoal suspension in 10% Arabic gum solution (0.2 mL/20 g). Twenty minutes after the charcoal meal, the animals were sacrificed by cervical

dislocation and the intestine from the pylorus to the rectal end was excised and aligned parallel to a ruler. The distance traversed by the charcoal meal was measured and expressed as a percentage of the total length of the intestine. Atropine was used as a reference drug since it inhibits intestinal motility by its antimuscarinic effects on the gastrointestinal tract.

Wound healing Property (Duke, 1910; Mielke, 1978)

Bleeding time was investigated in non-anaesthetized mice (8 mice/group). About 0.5 mm of the mouse-tail was cut off and the blood was carefully sucked using filter paper. The number of bleeding time the compounds tested were administered PO 60 min before the mouse-tail was cut off.

Statistical Analysis (Armillage and Berry, 1985)

The results were expressed as means±S.E. and test with analysis of variance. The level of significant was accepted for $p<0.05$.

RESULTS AND DISCUSSION

These results indicate that the extracts of *A. daemia* leaves have effective GIT and wound healing activities.

The LD_{50} of the various extracts of *Asclepias daemia* were found to be 2404 mg kg^{-1} for petroleum ether extract, 2499 mg kg^{-1} for chloroform extract and 2409 mg kg^{-1} for aqueous extract. In respect of LD_{50} values, the petroleum ether extract was more toxic than chloroform extract. As high doses of chloroform extract have toxic compared with aqueous extract (Table 1).

All the three extracts of *Asclepias daemia* in doses 200 mg kg^{-1} subcutaneously (SC) reduced the volume of gastric juice and total acid output. The decrease in volume of gastric juice with the pet-eth-extract given SC was statistically in significant ($p>0.05$). Whereas all the other two extracts, Chloroform extract and aqueous extract produced significant reduction in all parameters. Aqueous extract showed total acid output of 13.84±3.60 meq h^{-1} compared with petroleum ether extract (39.45±13.34). The pH of gastric juice was increased by all the compounds. The extract provoked a marked decrease in total gastric acid together with an increase in pH value. However, both extracts showed similar potency in reduction of total gastric acid together with an increase in pH value (Table 2).

The extracts have the property to diminish hydrochloric acid induced gastric lesions in rats. This may be related to an antacid effect or cytoprotective properties in gastric mucus. It is possible that the inhibitory effects of extracts are due, at least partly, the presence of terpenes in leave. Terpens were associated to antiulcerogenic activity in other plants.

Table 1: Determination of LD50 of *Asclepias daemia*

				Probit		
Group	Dose (mg kg^{-1})	Log dose	Dead total	Pet ether	Chloroform	Aqueous
1	2000	3.3010	0/10	3.04	3.04	3.04
2	2250	3.3521	2/10	4.16	4.16	4.75
3	2500	3.3979	6/10	5.25	5.25	5.25
4	2750	3.4393	7/10	5.52	5.52	6.28
5	3000	3.4771	10/10	6.96	6.96	6.96

Table 2 : Gastric antisecretory activity of various extracts of *Asclepias daemia*

Drug (mg kg^{-1})	Gastric juice in Vol/100 g	pH	Total acid output (Meq h^{-1})
Control	3.23±0.28	1.96±0.34	80.57±9.26
Petroleum ether extract	2.57±0.56*	4.32±0.40***	39.45±13.34*
Chloroform extract	2.06±0.19**	3.71±0.37**	29.34±3.60***
Aqueous extract	1.30±0.22***	4.70±0.27***	13.84±4.60***

p value : *$p<0.1$, **$p<0.01$, ***$p<0.001$

Table 3: Effect of *Asclepias daemia* extracts on gastro intestinal transit time

Drug (mg kg^{-1})	Dose (mg kg^{-1}) (P.O)	Intestine traversed by charcoal meal (5) Mean±SEM	Variation (%)
Control	---	59.70±1.78	---
Petroleum ether extract	200	37.39±2.92*	-37.37
Chloroform extract	200	57.75±4.68	-3.27
Aqueous extract	200	59.02±2.38*	-1.16
Atropine	8	37.97±2.91*	-34.27
	16	37.97±2.91*	-36.40

p value : *p<0.01

Table 4: Hemostatic activity of *Asclapias daemia*

Drug (mg kg^{-1})	Dose (mg kg^{-1}) (P.O)	Intestine traversed by Charcoal meal (5) Mean±SEM	Variation (%)
Control	---	4.40±0.34	---
Petroleum ether extract	200	3.33±0.69	-24.32
Chloroform extract	200	2.36±0.46**	-46.36
Aqueous extract	200	2.94±0.47*	-33.18
Asprin	100	6.01±1.30**	36.59

p value : *p<0.05, ** p<0.01

Table 3, showed the effects of *Asclepias daemia* extracts on gastrointestinal transit, the propulsion of the charcoal meal through the gastrointestinal tract was significantly increased by the extracts with the exception of petroleum ether extract (37.39±2.92), these effects were much higher than that induced by atropine. It is well known that the increase in intestinal motility could be a useful action for the treatment of constipation.

The potential of *Asclepias daemia* extracts to affect the bleeding time was tested in mice showing in Table 4, that all the extracts assayed, with the exception of petroleum ether extract (3.33±0.69) possess haemostatic activity as they significantly reduced the bleeding time when compared with control values. This effect may at least due to the presence of tannins, which are endowed with an astringent property.

In conclusion, *Asclepias daemia* markedly inhibits acid secretion and the occurrence of lesions in stomach. The aqueous soluble chemical constituents in the plant possess the activity against acid secretion, gastric transit time and hemostatic activity compared with non-polar solvent extract.

The aqueous and petroleum ether extracts have significant action in GIT and smooth muscles, which can be used for the treatment of constipation, ulcer and wound healing. The present findings provide scientific evidence to some of the ethanomedical properties of *Asclepias daemia* and also have statistically significant results with the reference compounds. So this study will be extended for further investigation and isolation of active ingredients from leaves (*Asclepias daemia)*, which are responsible for various pharmacological activities.

ACKNOWLEDGMENT

Author wishes to thank the Principal, Pearl Peace Medical Mission College of Pharmacy, Tirunelveli for providing necessary facilities for this study.

REFERENCES

Armillage, P. and G. Berry, 1985. Statistical Methods in Medical Research, 2nd Edn., Oxford, Blackwell Scientific Publications, pp: 186-190.

Duke, W.W., 1910. The relation of blood platelets to hemorrhage diseases description of a methods for determining the bleeding time and coagulation time and report of three cases of hemorrhagic disease relived by transfusion. J. Am. Med. Assoc., 15: 1185-1192.

Evans, W.C., Ed., 2002. Trease and Evans Pharmacognosy, 15th Edn., New York, W. B. Saunders, pp: 471-473.

Gosh, C.F., 1984. Statistical Analysis, Fundamentals of Experimental Pharmacology, pp: 187-190.

Hossein, H., R.K. Gholam and A. Maryam, 2002. Effect of *Anethum graveolens* L. seed extracts on experimental gastric irritation models in mice. BMC Pharmacol., 2: 21-31.

Karthikeyan, C., S. Siva Kumar, P. Chandrasekar, A. Heber, S.J.H. Robert and N.S.H.N. Moorthy, 2005. Antiinflammatory Activity of *Asclepias daemia* leaves. Ind. Drugs, 42: 533-534.

Kokate, C.K., Ed., 1994. Practical Pharmacognosy, 3rd Edn., New Delhi, Vallabh Prakashan, pp: 107-108.

Kritikar, K.R. and B.D. Basu, 1999. Indian Medicinal Plants. Blutter, E., J.F. Cains and K.S. Mhaskar (Eds.), Dehradun, Intl. Book Distributors, 2: 1617-1620.

Loomis, T.A., 1968. Essentials of Toxicology, Lea and Febiger. Philladelphia, pp: 67-78.

Mielke, C.H.M., 1978. Mechanisms of Hemostatics and Thrombosis. Mielke, C.H.M. and R. Rodvien (Eds.), Miami, JL, Symposis Specialists Inc., pp: 21-57.

Sertie, J.A.A., J.C.T. Carvalho and S. Panizza, 2000. Antiulcer activity of the crude extract from the leaves of *Casearia sylvestris*. Pharmac. Biol., 38: 12-18.

Yamauchi, H., K. Nishimura, H. Suda, T. Iso, M. Shimizu and Y. Hiramsatu, 1987. General Pharmacological properties of the potent angiotension converting enzyme inhibitor rentiapril. Arzneimittel Forschung/Drug Res., 37: 157-164.