Analgesic, Diuretic and CNS Activities of *Anethum sowa Roxb.* Stem and Leaves.

Sanjaya Kumar Y.R*

Assistant Director (Pharmacology)
National Ayurveda Research Institute for Panchakarma
(Central Council for Research in Ayurvedic Sciences), Ministry of AYUSH,
Cheruthuruthy, Thrissur, Kerala- 679 531
Mobile No.: 09446090579
E mail: drsanjayayr@yahoo.com

Sudesh Gaidhani N

Assistant Director (Pharmacology)
Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH.
New Delhi 110 058.

Deep V.C

Research Officer (Ayurveda)
National Ayurveda Research Institute for Panchakarma
(Central Council for Research in Ayurvedic Sciences), Ministry of AYUSH,
Cheruthuruthy, Thrissur, Kerala- 679 531

Swamy G.K.

Director
National Ayurveda Research Institute for Panchakarma
(Central Council for Research in Ayurvedic Sciences), Ministry of AYUSH,
Cheruthuruthy, Thrissur, Kerala- 679 531

Abstract - The decoction of Anethum sowa Linn. leaves and stem was evaluated for analgesic, diuretic and CNS activities in rats and mice. The test drug showed significant analgesic activity against acetic acid induced writhing in mice. It also reduced threshold tail flick response in rats and paw licking and jumping response in mice. It produced marked diuresis as compared to that of control group. The groups of mice which received the decoction test drug in different doses exhibited significant increase in exploratory behaviour and spontaneous motor activities.

Key words: Anethum sowa, Rats, Mice, Analgesic, Diuretic, CNS

Introduction

Anethum sowa Roxb. (Umbelliferae) is a sparse looking plant with feathery leaves .The plant is native to Europe, Mediterranean region and is also found in India and Pakistan. It is sometimes regarded as a variety of Anethum graveolens Linn. (1) The medicinal property of the plant is mainly due to the essential oil (Dill) in it and is beneficial in indigestion, stomach-ache, flatulence, etc. In the present study an attempt has been made to study the some of the pharmacological activities of the decoction of leaves and stem of the plant.

Materials and Methods

Test drug

Fresh leaves and stem of Anethum sowa collected from local markets of Tumkur and Bangalore districts in Karnataka were authenticated and dried. Decoction of the test drug was prepared by boiling 1 part of the material in 16 parts of water and reducing the final volume to 4 parts.

Dose

Decoction of the test drug was administered at dose of 0.5 ml (Lower dose) equivalent to 125 mg and 1 ml (Higher dose) equivalent to 250 mg per 100 g body weight for efficacy studies. Control group received distilled water.

Animals

Adult Wistar albino rats (150 to 200 grams) and Swiss albino mice (25 – 30 grams) of either sex procured from Small Animal Breeding Station (CPCSEA approved), Veterinary College, Mannuthy, Thrissur, Kerala were used for the study. The animals were housed at ideal temperature and humidity with 12 hour light dark cycle. Animals were fed with standard laboratory diet and *ad libitum* water.

Ethical Clearance

The present trial was conducted with the approval of Institutional Animal Ethics Committee (IAEC) meeting held at National Ayurveda Research Institute for Panchakarma, Cheruthuruthy, Thrissur, Kerala

Experimental design

Acute Toxicity studies

Acute toxicity study was carried out in female Wistar rats as per OECD guideline 423 (2). The test drug was administered at dose of 2 ml equivalent to 500 mg per 100 gram body weight in 3 female wistar rats once orally and the animals were observed for a period of 14 for mortality and signs of toxicity. The test was repeated at the same dose levels with another set of 3 female rats.

Analgesic activity

Acetic acid induced writhing

Test drug was administered in different doses and 45 minutes later 3% acetic acid (v/v – 0.1 ml per 10 g. b.w. I.P.) was used to induce writhing in mice (3). No. of stretching episodes (writhings) exhibited by the animal were counted for a duration of 30 minutes post acetic acid injection.

Hot plate method

Analgesic effect of the test drug was studied in mice using hot plate (4). Basal reaction time in terms of hind paw licking and jump response was noted by keeping the animals individually on hot plate maintained at a constant temperature of 55^0C. A cut off period of 15 seconds was observed to avoid damage to the paws. The animals were retested at 60, 120 and 180 minutes post test drug administration. A difference in reaction time was noted and compared with that of control.

Radiant Heat method

Effect of decoction on threshold tail flick response in rats was noted using analgesiometer (5). Tail flick response was measured before and at 60, 120, 180 and 240 minutes after drug administration.

Diuretic activity

Diuretic activity of the test drug was studied in Wistar rats (6). Animals were hydrated with normal saline (2.5 ml per 100 g. b.w. per oral). Volume of urine excreted was measured at an hourly interval up to 5 hours after administration of test drug at different dose levels was noted and compared with standard drug group that received frusemide.

Effect on Spontaneous Motor activity

Effect of test drug on the locomotor activity was studied in mice (7). Basal activity was noted in each animal using Actophotometer for 10 minutes. Test drug was administered in different doses and 45 minutes later each animal was retested for activity scores for a period of 5 minutes. The difference in activity prior and after drug treatment was noted and compared with that of control.

Evaluation for exploratory activity

The test drug was evaluated for its effect on exploratory activity in mice using a tunnel board (8). Drug treated mice were placed on left hand corner of the board one at a time and observed for 5 minutes. Total number of tunnels entered during the first 5 minutes of observation period was noted.

Results and Discussion

No clinical signs of toxicity and death were observed in rats and mice which received the test drug decoction up to a dose of 2 ml per 100 g. body weight

Decoction of the Anethum sowa Roxb. leaves and stem produced significant reduction in the number of acetic acid induced writhing in mice Significant reduction was observed in number of writhings at 0.5 ml ($P<0.05$) and 1 ml ($P<0.01$) per 100 g. body weight. (Table I) in mice. Significant increase duration of licking/jumping response was observed in mice at both the dose levels (Table II). It also significantly ($P<0.05$) increased the threshold tail flick response in rats at the dose of 1 ml. per 100 g. body weight. (Table III).

The analgesia was more pronounced in Acetic acid induced model than the tail flick and hot plate method indicating its predominant involvement in peripheral pain mechanism than that involving higher center. The leaves and aerial parts of the plant were found to contain flavinoids (Teuber H and Henmann K, 1978) and analgesic property might be attributed to their presence (9). Flavinoids have antiulcer and gastroprotective properties too, which justify the traditional use of dill in colic mixtures especially in infants.

The test drug produced marked increase in the volume of urine voided at high dose level as compared to that of control in rats depicting its diuretic activity (Table IV)., but is less potent and of late onset when compared to standard drug, frusemide. Diuretic activity of the test drug thus evident might be due to apiol content. The decoction also significantly ($P<0.05$) increased the spontaneous motor activity and exploratory behaviour in mice at higher dose level which indicate the possible CNS stimulant activity (Table V and VI) .These psychoactive properties might be attributed to Myristicin / Methoxy safrole present in the plant.

Conclusion

Findings of the study suggested that decoction of stem and leaves of the Anethum sowa Roxb. has significant analgesic and diuretic activites along with CNS stimulant effects. The analgesia was more pronounced in Acetic acid induced model than the tail flick and hot plate method indicating its predominant involvement in peripheral pain mechanism.

Acknowledgement

Authors are thankful to Professor Vaidya Dhiman K.S., Director General , CCRAS, New Delhi for providing necessary facilities and encouragement.

References

[1] Wealth of India.Vol.1 Publications and Information Directorate,CSIR, New Delhi.1968: 78.
[2] OECD guidelines for testing of chemicals section 4 Health effects OECD france.2001.
[3] Witkin L.N. Huebner C Pharmacology of 2- aminoindane hydrochloride. A potent non-narcotic analgesic. Journal of Pharmacology and Experimental therapeutics. 1961. 133: 400.
[4] Kulkarni S.K.Hand book of Experimental Pharmacology, 3rd Ed. Vallabh Prakashan, Delhi 1999: 125-127.
[5] Gujral M.C and Khanna B.K Comparative evaluation of some narcotic analgesics. Jour. Sci. Indu. Research, 1956. 168: 11-13.
[6] Lipschitz W.L, Hadidian Z and Kerpscar.A.Bioassay of Diuretics. J. Pharmacol Expt. Ther. 1943 :79: 97.
[7] Dews P.B. The measurement of the influence of drugs on voluntary activity in mice. British Jour. of Pharmacology .1953, 8: 46-48
[8] Shilito E.E A method for investigating the effect of drugs on the exploratory behaviour of mice. Brit .J. Pharmacology. 1970, 40 : 113-123.
[9] Teuber H and Henmann K. Flavonol glycosides of leaves and fruits of dill (A.graveolens Linn.) II Phenolics of Spices.Z.Bebensm Unters Forsch 1978. 167: 101-104.

Table I. Effect of A. sowa (Leaves and Stem) decoction on acetic acid induced writhing in mice

Group	**No. of mice protected from writhing**	**Stretching episodes during 30 minutes after acetic acid injection (Mean ± SEM)**
Control (Distilled water)	0/6	37.16 ± 3.76
Lower dose group (A.sowa decoction-0.5 ml per 100 g. B.W.)	0/6	29.16 ± 1.22*
Higher dose group (A.sowa decoction-1 ml per 100 g. B.W.)	0/6	27 ± 2.98**
Standard drug Group Acetyl salicylic Acid 100 mg. per kg. B.W.)	0/6	17.5 ± 2.75**

* $P<0.05$ **$P<0.01$

Table II. Effect of A. sowa (Leaves and Stem) decoction on hot plate analgesia in mice

Group	**Reaction time in seconds (Mean ± SEM)**			
	Recording at various time intervals (Min.) after drug administration.			
	Initial	**60**	**120**	**180**
Control (Distilled water)	3.16 ± 0.30	3.33 ± 0.33	2 ± 0.93	3.33 ± 0.71
Lower dose group (A.sowa decoction-0.5 ml per 100 g. B.W.)	3.66 ± 0.33	3.5 ± 0.34	4.5 ± 0.42*	4.33 ± 0.33*
Higher dose group (A.sowa decoction-1 ml per 100 g. B.W.)	3.16 ± 0.30	3.5 ± 0.42	4.33 ± 0.21*	4..16 ± 0.40*
Standard drug Group Ibuprofen (100 mg. per kg. B.W.)	3 ± 0.25	4.33 ± 0.49*	4.33 ± 0.49**	4.5 ± 0.42**

* P<0.05 ** P<0.01

Table III. Effect of A. sowa (Leaves and Stem) decoction on latency of tail flick response in rats

Group	**Reaction time in seconds (Mean ± SEM)**				
	Recording at various time intervals (Min.) after drug admn.				
	Initial	**60**	**120**	**180**	**240**
Control (Distilled water)	3.5±0.22	3.16±0.30	3.16±0.30	3.83±0.30	3.83±0.30
Lower dose group (A.sowa decoction-0.5 ml per 100 g. B.W.)	4±0.25	4.16±0.30	4.33±0.21	4.33±0.21	4.5±0.22
Higher dose group (A.sowa decoction-1 ml per 100 g. B.W.)	3.83±0.16	4.16±0.16	4.83±0.30*	5±0.36*	4.33±0.33
Standard drug group Ibuprofen 100 mg. per kg. B.W.)	4±0.25	5±0.25*	5.33±0.33**	5±0.36*	4.5±0.22

* P<0.05 **P<0.01

Table IV. Effect of A. sowa (Leaves and Stem) decoction on diuresis in rats

Group	**Volume of urine (in ml) voided at different time intervals (in minutes) after drug administration (Mean ± SEM)**				
	60	**120**	**180**	**240**	**300**
Control (Distilled water)	0.2±0.05	0.46±0.04	0.53±0.06	0.4±0.08	0.1±0.04
Lower dose group (A.sowa decoction-0.5 ml per 100 g. B.W.)	0.26±0.04	0.6±0.05	0.73±0.08	0.3±0.04	0.06±0.03
Higher dose group (A.sowa decoction-1 ml per 100 g. B.W.)	0.3±0.04	0.46±0.06	0.86±0.08*	0.85±0.12**	0.13±0.03
Standard drug group (Frusemide-20 mg. per kg. B.W.)	0.86±0.13**	1.36±0.14***	1.16±0.06**	0.4±0.10	0.1±0.04

* P<0.05 ** P<0.01 *** P<0.001

Table V. Effect of A. sowa (Leaves and Stem) decoction on Spontaneous Motor Activity (SMA) in mice

Group	**Photocell counts at various time intervals (Minutes) after drug admn.**			
	Initial	**60**	**120**	**180**
Control (Distilled water)	142.66±12.38	146.16±12.24	150.33±10.23	146.66±13.28
Lower dose group (A.sowa decoction-0.5 ml per 100 g. B.W.)	153.66±7.34	158±7.02	160.83±8.20	176.83±7.25*
Higher dose group (A. sowa decoction-1 ml per 100 g. B.W.)	168.33±9.54	174.5±8.91	184.33±11.41*	188.66±9.82**

* P<0.05 **P<0.01

Table VI. Effect of A. sowa (Leaves and Stem) decoction on exploratory behavior in mice.

Group	**No. of different tunnels entered during first minute (Mean ± SEM)**	**Total number of tunnels entered during 5 minutes (Mean ± SEM)**
Control (Distilled water)	1.00 ± 0.36	6.83 ± 0.60
Lower dose group (A.sowa decoction-0.5 ml per 100 g. B.W.)	1.16 ± 0.30	7.33 ± 0.66
Higher dose group (A.sowa decoction-1 ml per 100 g. B.W.)	0.83 ± 0.30	7.5 ± 0.84*

*P<0.05