

ANXIOLYTIC AND ANTIEPILEPTIC EFFECTS OF ASAFOETIDA OLEO-GUM RESIN

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ABSTRACT

Swiss albino mice were grouped and treated with either distilled water or diazepam (3 mg/kg p.o.) as standard or Asafoetida (286 or 667 mg/kg p.o.) 1 h before to evaluate anxiolytic activity by using Elevated Plus Maze (EPM) test, Light & dark test, Locomotor activity test and Rota rod test whereas Maximal electroshock (MES- 42 mA, 0.2 s) induced convulsion test and pentylenetetrazole (PTZ- 80 mg/kg i.p.) induced convulsion test were used for the assessment of the antiepileptic activity where phenytoin (25 mg/kg p.o.) or diazepam (5 mg/kg p.o.) was administered as reference standard respectively. Both the doses of Asafoetida did not show statistically significant anxiolytic effect in EPM Test and Light & Dark Test but showed significant ($P < 0.001$) CNS depression/ sedation by reducing locomotor activity in

mice. Asafoetida did not show effect on motor co-ordination. 286 mg/kg dose of Asafoetida was more effective as anxiolytic. The same dose also showed statistically significant sedative activity (91%) even after 2 h of treatment. Both the doses of Asafoetida showed antiepileptic effect against PTZ induced Seizures but the dose 286 mg/kg of Asafoetida caused 67% mortality in MES model which was not observed in control as well as 667 mg/kg dose of Asafoetida treated group. The effect of Asafoetida is probably by acting through GABA_A receptor. Vanillin is probably one of the phytoconstituent responsible for anxiolytic and antiepileptic effects of Asafoetida, in addition to other phytoconstituents.

KEY WORDS: Anxiolytic, Elevated plus maze, Light and dark, PTZ, GABA.

INTRODUCTION

Anxiety disorders span the full range of human existence from childhood to old age. Anxiety disorders in youth appear to be a risk factor for the subsequent development of major depression in late adolescence and young adulthood. But even in later life, new onset of anxiety disorders can occur, often in the context of medical illness or other sources of life stress.^[1]

About 50 million people worldwide have epilepsy, with almost 90% of these people being in developing countries.^[2] Epilepsy is usually controlled, but not cured, with medication, although surgery may be considered in difficult cases. However, over 30% of people with epilepsy do not have seizure control even with the best available medications.^[3]

Benzodiazepines are most widely prescribed for the treatment of anxiety and epilepsy. Regular use of benzodiazepines causes deterioration of cognitive functioning, addiction, physical dependence, and tolerance. So to avoid these adverse effects, researchers of today are exploring natural resources to discover safer and cost effective drugs based on their use in traditional systems of medicine.^[4]

Asafoetida oleo-gum resin is obtained as an exudation by incising the rhizomes and roots of different *Ferula* species belongs to Family: Umbelliferae ^[5] which are distributed from the Mediterranean region to Central Asia ^[6] and contains about 40-64% resin (ester of asareninotannols, ferulic acid, pinene, vanillin and free ferulic acid), 25% endogeneous gum and 4-20 % volatile oil. ^[7]

Various activities of different *Ferula* species like antispasmodic and hypotensive activity,^[8] Molluscicidal activity, ^[9] antioxidant activity,^[10] antinociceptive and anti-inflammatory activity,^[11] antifungal activity,^[12] antibacterial,^[13] Influenza A (H1N1) Antiviral ^[14] and cytotoxic activity ^[15] have been reported. The root acetone extract of *Ferula gummosa* is reported for its anticonvulsant property.^[16] Asafoetida is regularly consumed by the Indian population in food preparations so we decided to conduct our studies on regular available Asafoetida in India. In Ayurveda, asafoetida is considered to be one of the best spices for balancing the vata dosha.^[17] Asafoetida oleo-gum-resin is used traditionally for digestion, asthma, bronchitis, hysteria ^[18,19] and as contraceptive/abortifacient ^[20] and also has been claimed to be antiepileptic in classical Unani as well as ethnobotanical literature. ^[18] So the present study was carried out to evaluate the anxiolytic and antiepileptic effect of Asafoetida.

MATERIALS AND METHODS

Collection of Drugs and chemicals

Asafoetida oleo-gum resin was obtained as gift sample from Yucca Enterprises, Mumbai. A voucher specimen of drug has been deposited in the department of pharmacognosy, Acharya & B.M. Reddy College of Pharmacy, Bangalore, Karnataka. Diazepam (Calmpose, Ranbaxy lab. Ltd., India), Phenytoin (M-toin, Medopharm, India) and Pentylenetetrazole (Sigma-Aldrich, St. Louis, USA) were used for the study.

Preparation of dosage forms

Asafoetida was suspended in distilled water. Diazepam and Phenytoin were suspended in 0.5% gum acacia in distilled water. Pentylenetetrazole was dissolved in distilled water. The dosage forms of drugs and chemicals were prepared freshly on the test days.

Identification test ^[21]

Based on the chemical identification tests Asafoetida oleo-gum resin drug was identified.

Experimental Animals

Healthy swiss albino mice (20-25 g) were procured from Indian Institute of Science, Bangalore. Animals were housed in polypropylene cages and maintained under standard conditions (12 h light/dark cycle, 22 ± 2 °C and 55 ± 5 % relative humidity). They were fed with standard diet and water ad libitum. The animals were maintained in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experimental Animals) guidelines for the care and use of laboratory animals. The study protocol was approved by Institutional Animal Ethics Committee (IAEC) of Acharya & B.M. Reddy College of Pharmacy, Bangalore (Ref. number ABMR: 59: EST: 2009-10)

Pharmacological Activities

I. Acute toxicity studies ^[22]

Powdered Asafoetida oleo-gum resin was suspended in distilled water. The animals were fasted for 3 h before the experiment but water was provided. Acute oral toxicity test was conducted according to OECD guideline 425 (Up and down procedure). Limit test was conducted by using 2000 mg/kg dose of Asafoetida. 2000 mg/kg dose was administered orally to one mice and observed for initial 30 min to 4 h and periodically during the first 24 h. Additional 4 mice were also administered with the same dose of Asafoetida with a interval of 48 hours. All the mice were observed further up to 14 days.

II. Anxiolytic Activity

Elevated Plus Maze Test ^[23]

The apparatus comprises of two open arms (35 cm × 5 cm) and two closed arms (30 cm × 5 cm × 15 cm) that extend from a common central platform (5 cm × 5 cm). The entire maze is elevated to height of 50 cm above the floor level. Swiss albino mice were divided into four groups, each group comprising of six animals. After one hour of oral administration of the distilled water or standard drug Diazepam (3mg/kg) or Asafoetida (286 & 667 mg/kg), each mouse was placed in the centre of the maze facing towards one of the open arm. For a five minutes period, the following parameters were noted; time spent in the open arm, and time spent in the closed arm.

Light and dark test ^[23, 24]

Light dark box consisted of a rectangular open top wooden box of 46 x 27x 30cm (l x b x h), which is divided into 2 compartments- Dark chamber which was painted black and top was covered with plywood and Light chamber which was painted white and a 40-W lamp was placed above the light chamber to illuminate. Both compartments were connected through a small open door way (7.5 x 5 cm) situated on the floor level at the center of the partition. Animals were divided into four groups, each group comprising of six animals. Distilled water or Diazepam (3mg/kg) or Asafoetida (286 & 667 mg/kg) were administered orally to different groups. After one hour of oral administration, each mouse was placed individually in the illuminated part of the Light dark box. The following observations were recorded during the test session of 5 min; time spent in the light chamber and time spent in the dark chamber.

Locomotor activity test

Swiss albino mice were divided into four groups, each group comprising of six animals. Each animal was placed individually in the Actophotometer and the basal activity score was recorded for 10 minutes for all the animals. Distilled water or Diazepam (3 mg/kg) or Asafoetida (286 & 667 mg/kg) was administered orally to mice. After 30 min, 60 min, 90 min and 120 min of oral administration each mouse was retested for activity for 10 min. by placing individually in actophotometer. The difference in the activity was recorded considering before treatment values and after distilled water or diazepam or Asafoetida treatment values. Finally percentage change in locomotor activity was calculated.

Rota rod test ^[25]

The animals which were able to remain for 5 min on the rotating rod at a speed of 15 rpm selected for study and divided into four groups containing six animals in each group. After one hour of oral administration with distilled water or Diazepam (3mg/kg) or Asafoetida (286 & 667 mg/kg), all animals were subsequently assessed for their performance on the rota rod. The fall of time from the rod was noted for each animal.

III. Aniepileptic Activity**Pentylenetetrazole induced convulsions test** ^[23]

Swiss albino mice were divided into four groups, each group comprising of six animals. After 1 h of distilled water or standard drug Diazepam (5mg/kg p.o.) or Asafoetida (286 & 667 mg/kg) treatment, PTZ (pentylenetetrazole 80 mg/kg) ²⁶ was administered by intraperitoneal route to all the animals. Each animal was placed in to individual plastic cage and was observed initially for 30 min and later up to 24 h. The following parameters were recorded during test session of initial 30 min ; Onset of convulsions, Onset of tonic convulsions, Duration of tonic convulsions, Onset of clonic convulsions, Duration of clonic Convulsions, Status of animal after 24 h, Mortality Percentage.

Maximal electroshock induced convulsions test ^[25]

Animals were divided into four groups, each group comprising of six animals. The animals were treated with distilled water or Phenytoin (25 mg/kg, p.o.) or Asafoetida (286 & 667 mg/kg). After 1 h of treatment, an electric shock (42 mA, 0.2 s) using an electroconvulsimeter was applied through the ear clip and duration of various phases of convulsions like Flexion, Extensor, Clonus, Stupor and status of animal were recorded for each animal.

RESULTS**I. Acute toxicity studies**

2000 mg/kg dose of Asafoetida did not show mortality in 4 animals but one animal died after 2 days of drug administration. Hypnosis was observed in the animals for duration of 40-45 min after 30 min of dose administration.

II. Anxiolytic Activity**Elevated Plus Maze Test**

The results of EPM test are presented in Table 1. Asafoetida 286 and 667 mg/kg showed 34% and 21% increase in time spent in open arm as well as 27% and 17% decrease in the time

spent in closed arm respectively whereas diazepam showed 67% increase in time spent in open arm and 54% decrease in the time spent in closed arm as compared to control group. But the effects of Asafoetida as well as diazepam were not statistically significant.

Table 1: Effect of Asafoetida oleo-gum resin in EPM Test

Group No. & Treatment	Time Spent (sec) in Open Arm	Time Spent (sec) in Closed Arm
I (Distilled Water p.o.)	134.0 ±22.31	166.0 ±22.31
II (Diazepam 3mg/kg p.o.)	224.0 ± 25.12	76.00 ±25.12
III (Asafoetida 286 mg/kg p.o.)	179.2 ±26.99	120.8 ±26.99
IV (Asafoetida 667 mg/kg p.o.)	162.2 ±50.14	137.8 ±50.14

Light and dark test

Asafoetida 286 and 667 mg/kg showed 74% and 43% increase in time spent in light chamber as well as 51% and 28% decrease in the time spent in dark chamber respectively which were not statistically significant whereas diazepam showed significant ($P < 0.01$) 120% increase in time spent in light chamber and 79% decrease in time spent in dark chamber by mice as compared to control group and the results are presented in Table 2.

Table 2: Effect of Asafoetida oleo-gum resin in Light and dark Test

Group No. & Treatment	Time (sec) spent in light chamber	Time (sec) spent in dark chamber
I (Distilled Water p.o.)	118.83 ±16.37	181.17 ±16.37
II (Diazepam 3mg/kg p.o.)	262** ±32.90	38.0** ±32.90
III (Asafoetida 286 mg/kg p.o.)	206.67 ± 30.01	93.33 ± 30.01
IV (Asafoetida 667 mg/kg p.o.)	169.83 ±22.50	130.17 ±22.50

n= 6, ** $P < 0.01$ vs. control (one way ANNOVA followed by Dunnet's Test)

Locomotor activity test

Asafoetida 286 mg/kg significantly ($P < 0.001$) decreased locomotor activity 88%, 95%, 94% and 91% at 30min, 60 min, 90 min and 120 min respectively as well as Asafoetida 667mg/kg significantly ($P < 0.001$) decreased locomotor activity 63%, 70%, 77%, 85% at 30min, 60 min, 90 min and 120 min respectively whereas diazepam significantly decreased locomotor activity 37%, 72%, 69% and 33% at 30min, 60 min, 90 min and 120 min interval respectively as compared to basal readings and presented in Table 3.

Table 3: Effect of Asafoetida oleo-gum resin in locomotor activity test

Group No. & Treatment	No. of locomotions				
	Basal Reading	30 min.	60 min.	90 min.	120 min.
I (Distilled Water p.o.)	305.84 ±53.94	292 ±51.05	289 ±48.94	304 ±55.67	290.16 ±51.98
II (Diazepam 3mg/kg p.o.)	512.67 ±46.59	320.83 ** 39.29	141.50 *** ±18.19	156.67 *** ±24.47	344.67 * ±45.66
III (Asafoetida 286 mg/kg p.o.)	580.33 ±91.92	72.33 *** 13.69	29.83 *** ±9.210	32.83 *** ±10.18	50.17 *** ±16.73
IV (Asafoetida 667 mg/kg p.o.)	795.17 ±60.68	293.83*** 26.71	236.17*** ±33.01	182.50*** ±43.61	116.50*** ±38.79

n= 6, *P< .05, **P<0.01, ***P<0.001 vs. control (one way ANNOVA followed by Dunnet's Test)

Rota rod test

Rota rod apparatus was used to evaluate the muscle relaxing effect of Asafoetida and the results are presented in Table 4. Both the doses of Asafoetida did not show effect on muscle co-ordination but diazepam showed significant (P< 0.01) 53% and 57% decrease in the fall off time from rota rod at 30 min and 60 min after the drug administration respectively. It indicates that Asafoetida does not possess muscle relaxant property.

Table 4: Effect of Asafoetida oleo-gum resin in Rota rod Test

Group No. & Treatment.	Fall off time (sec) after	
	30 min.	60 min.
I (Distilled Water p.o.)	286.5 ±12.15	297.5 ±2.5
II (Diazepam 3mg/kg p.o.)	134.167 ** ±53.28	126.34 ** ±55.82
III (Asafoetida 286 mg/kg p.o.)	296.5 ±2.5	299 ±1.00
IV (Asafoetida 667 mg/kg p.o.)	264.34 ±26.38	276 ±12.97

n= 6, **P<0.01 vs. control (one way ANNOVA followed by Dunnet's Test)

III. Aniepileptic Activity

PTZ induced convulsions test

The results of PTZ induced convulsions test are presented in Table 5. Asafoetida 667 mg/kg dose showed significant (P<0.05) delay in onset of convulsions as well as very significantly (P< 0.001) delayed the onset of tonic convulsions and decreased the duration of tonic phase of convulsions whereas 286 mg/kg dose of Asafoetida showed significant (P< 0.001) reduction in the duration of tonic phase of convulsions only as compared to control group. Convulsions are completely abolished in Diazepam treated group. Asafoetida 286 and 667

mg/kg showed percentage delay in the onset of convulsions (52, 56 %), tonic phase of convulsions (40, 123%), clonic phase of convulsions (27, 0.25%) respectively. While the duration of tonic phase of convulsions (54, 54%), clonic phase of convulsions (29, 87%) was decreased by Asafoetida 286 and 667 mg/kg respectively.

Table 5: Effect Of Asafoetida Oleo-Gum Resin In PTZ Model

Group No. & Treatment	Onset of Convulsion (Sec)	Onset of Tonic Convulsion (Sec)	Duration of Tonic Convulsion (Sec)	Onset of Clonic Convulsion (Sec)	Duration of Clonic Convulsion (Sec)	Mortality after 24 h (%)
I (Distilled Water p.o.)	51.50 ±4.153	65.17 ±6.988	101.0 ±6.245	269.3 ±51.44	40 ±9.041	100
II (Diazepam 5mg/kg p.o.)	-- ***	-- ***	-- ***	-- ***	-- ***	--
III (Asafoetida 286 mg/kg p.o.)	78.50 ±10.82	91.33 ±7.286	46.67 *** ±12.94	341.7 ±77.98	51.83 ±22.19	33
IV (Asafoetida 667 mg/kg p.o.)	80.33 * ±10.45	145.7 *** ±17.64	46.0 *** ±9.399	270.7 ±183	5.0 ±3.173	33

n= 6, *P< .05, ***P<0.001 vs. control (one way ANNOVA followed by Dunnet's Test)

MES induced convulsions test

In MES model, Phenytoin significantly decreased the duration of extensor phase which was not decreased by both the doses of Asafoetida as compared to control group. Results are presented in Table 6. Asafoetida 286 mg/kg dose caused 67% mortality which was not observed in control, phenytoin and Asafoetida 667 mg/kg treated group.

Table 6: Effect of Asafoetida oleo-gum resin in MES Model

Group No. & Treatment	Time spent (sec) in various phases of convulsions				
	Flexion	Extensor	Clonus	Stupor	Mortality (%)
I (Distilled Water p.o.)	1.6 ±0.2108	11.8 ±0.9458	10.8 ±1.167	50.8 ±6.755	0
II (Phenytoin 25 mg/kg p.o.)	2.0 ±0.3651	1.17*** 1.167	10.83 ±2.786	41.33 ±15.03	0
III (Asafoetida 286 mg/kg p.o.)	1.34 ±0.2108	11.17 ±1.759	6.84 ±0.8724	21.17 ±4.593	67
IV (Asafoetida 667 mg/kg p.o.)	1.34 ±0.2108	16.84 ±1.922	11.67 ±1.874	52.67 ±18.58	0

n= 6, ***P<0.001 vs. control (one way ANNOVA followed by Dunnet's Test)

DISCUSSION

A few classical animal models of anxiety such as the elevated plus maze and light & dark model has been used for measuring anxiety like behavior in mice which are based upon the exposure of subject to unfamiliar aversive place. ^[26] The elevated plus maze test is principally

based on the exposure of animal to spontaneous or natural aversive stimuli i.e. height, unprotected opening and novelty and the natural property of rodents is to preferably stay in dark environment. The animals being exposed to the new environment prefer to stay in closed arm due to fear. Asafoetida 286 mg/kg dose showed more anxiolytic effect than 667 mg/kg dose. Light and dark model was used to evaluate natural aversion of mice for brightly lit places. Anxiolytics reduce the natural aversion to light and increase the time spent in the light chamber and decrease the time spent in dark chamber. Asafoetida 286 mg/kg dose showed more anxiolytic effect than 667 mg/kg dose.

Locomotion is an index of CNS activity and decrease in locomotor activity indicates CNS depression. A significant reduction in locomotion by Asafoetida indicates its sedative property in mice. Asafoetida 286 mg/kg dose showed more CNS depressant effect than 667 mg/kg dose. Both the doses of Asafoetida showed more significant reduction in locomotor activity than diazepam treated group even after 120 min of drug administration.^[27]

Though Asafoetida did not show statistically significant anxiolytic effect but on an average of both the models Asafoetida 286 and 667 mg/kg showed 54% and 32% anxiolytic effect respectively and also it has showed statistically significant sedative effect (91%) even after 2 h of treatment. Hence it may be useful to reduce the fear and anxiety since it has very significant sedative effect. But the sedative effect of Asafoetida is not involved muscle incoordination. Asafoetida 667 mg/kg dose showed more effect in delaying the onset of convulsions, tonic convulsions and decrease in the duration of clonic convulsions as compared to the 286 mg/kg dose. Hence 667 mg/kg dose of Asafoetida shows better antiepileptic effect than lower dose.

Asafoetida 667 mg/kg dose showed significant anticonvulsant effect in PTZ model. But this dose not showed anticonvulsant effect and no mortality in MES model while 286 mg/kg dose caused the death of mice which was not even observed with the control group. Asafoetida 286 mg/kg dose showed 20% more anxiolytic and sedative effect as compared to 667 mg/kg dose. 286 mg/kg dose showed mortality in MES model which was not even observed in control group but 667 mg/kg dose did not cause any mortality in MES Model and showed more antiepileptic effect compared to 286 mg/kg dose in PTZ Model. Reason for the opposite effect of both the doses in different activities cannot be explained with the present study data. Further investigations are required to explore the mechanisms involved in it. Whether the same phytoconstituent/s are showing biphasic effect or the quantity of phytoconstituents

are responsible for it. The effect of most of the anxiolytic agents is due to the enhanced response to GABA, by facilitating the opening of GABA-activated chloride channels. GABA_A receptors were involved in anxiety and their direct activation would have an anxiolytic effect.^[28] Pentylentetrazole produces seizures by blocking GABA_A receptor.^[29] Hence it seems that sedative and antiepileptic effects of Asafoetida are mediated through GABA_A receptor either by facilitating the action of GABA or by increasing the concentration of GABA in the brain. Vanillin is the phytochemical constituent reported to have the calming/sedative and anticonvulsant effect.^[30] Vanillin is one of the phytochemical constituents of Asafoetida. So vanillin is probably responsible for anxiolytic and antiepileptic effects of Asafoetida in addition to other constituents of it. The other phytochemicals of Asafoetida are also responsible for these activities yet to be revealed.

CONCLUSION

Asafoetida oleo-gum resin did not show significant anxiolytic effect but possesses significant sedative effect. 286 mg /kg dose is more sedative than 667 mg/kg. 286 mg /kg dose also showed on average 54% anxiolytic effect and also statistically significant sedative effect even after 2 h of treatment. Hence it may be useful to reduce the fear and anxiety. Both the doses of Asafoetida don't have effect on muscle coordination.

Asafoetida 667 mg/kg dose showed significant anticonvulsant effect in PTZ model but not in MES induced seizures. Hence, it may be useful in myoclonic and petitmal epilepsy but not in grandmal epilepsy. The dose 286 mg/kg of Asafoetida caused 67% mortality in MES model which was not observed in control as well as 667 mg/kg dose of Asafoetida treated group. The mechanism behind this is need to be evaluated. The effects of Asafoetida are probably by acting through GABA_A receptor. Vanillin is probably one of the phytoconstituent responsible for anxiolytic and antiepileptic effects of Asafoetida, in addition to other constituents.

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