

ANTI-DEPRESSANT ACTIVITY OF METHANOLIC EXTRACT OF *APIUM GRAVEOLENS* SEEDS

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ABSTRACT

In the present study, the anti-depressant effect of methanolic extract of *Apium graveolens* seeds (AGM) was investigated using two behavioural models, the forced swim test (FST) and tail suspension test (TST) in mice. In FST & TST demonstrated a dose dependant, statistically significant reduction in duration of immobility that was comparable to Imipramine (20mg/kg). The effect of 200mg/kg of AGM was better than 20 mg/kg Imipramine. The effect of 100mg/kg of AGM was significant when compared to vehicle treated group. These results showed that the methanolic extract of *Apium graveolens* seeds possessed significant anti-depressant activity in animal models.

Keywords: Anti-depressant, Forced swim test, Tail suspension test, mice, immobility.

INTRODUCTION

Apium graveolens is classified as a member of the Apiaceae family and is known as celery¹. It is a biennial herb that has been used consistently throughout history in medicinal preparations, food flavoring and preparation, and is known in the vernacular as celery. The seeds are typically cultivated in order to form an extract, to make a tea or to mix with salt for use as a flavoring agent; the plant itself (root, foliage and stem) is ingested as part of a normal diet in preparations such as salads, soups, etc. *A. graveolens* has a worldwide distribution of growth, including most of the United States, many countries within the Europe, Asia, Africa and parts of India². Epidemiological evidence supports a relationship between ingestion of *A. graveolens* and a myriad of beneficial health effects, ranging from cardioprotective to anticancer properties³. Celery seed has been found to help regulate nervous system by producing a combing effect. Celery is also known as marsh water parsley. It stimulates sees drives and produce sedative effect⁴.

Despite the numerous scientifically proven pharmacological activities of *Apium graveolens* there was no scientific data on its

potential as an antidepressant agent. As per ethnomedical information the seeds of *Apium graveolens* found to elicit antidepressant activity. Hence present study was done to assess antidepressant activity of methanolic extract of *Apium graveolens* seeds.

MATERIALS AND METHODS

Plant material

The dried seeds of *Apium Graveolens* were used in this study. This was authenticated and confirmed by botanist at Sri. Venkateswara University, Tirupati, Andhra Pradesh, India.

Preparation of Extract

The powdered material was subjected to batch extraction in Soxhlet apparatus. The solvents used were Methanol. The powdered material of *Apium Graveolens* seeds were evenly packed in Soxhlet extractor for extraction with solvent. The temperature was maintained on an electric heating mantle with thermostat control. Appearance of colorless solvent in the siphon tube was taken as the termination of extraction.

The extracts were then concentrated by distilling the solvent. The concentrated extracts were evaporated on a water bath

(40-50 °C) to dryness. Each extract was weighed and percentage yield was calculated. The color and consistency of the extracts were noted. Hence forth the Methanolic extract of *Apium graveolens* will be called as AGM⁵.

Test for Phytochemical Analysis

The conventional chemical tests were carried out for the extract of AGM to identify the presence of various chemical constituents⁶.

IN VIVO STUDIES

EXPERIMENTAL ANIMALS

Adult Swiss albino mice (25-30 g) of either sex were procured from the laboratory animal house, Hindu College of Pharmacy, Guntur, Andhra Pradesh, India and used in the study. The animals were kept under standard environmental conditions of room temperature (22 ± 2°C), relative humidity (50% ± 5%) and 12 h light and dark cycle. The animals were housed in the colony cages (either three rats or six mice per cage) and provided feed (commercial pellets contain a balanced ration obtained from the Sri Venkateswara Enterprises, Bangalore) and water *ad libitum*. All the animals were acclimatized to the laboratory environment 5 days prior to experiment. The animals were fasted overnight just prior to the experiment but allowed free access to drinking water. All the experiments were carried out in accordance with the guidelines of Institutional Animal Ethics Committee. The study was conducted after obtaining ethical committee clearance from the Institutional Animal Ethics Committee.

ANTI-DEPRESSANT ACTIVITY

1. Forced swim test

In this model Swiss Albino mice were divided into 4 groups of six animals each and the test apparatus consists of a transparent rectangular glass jar (25x12x25 cm³) filled to a 15cm depth with water (24 ± 1°C). In the pre-test session, every animal was placed individually into the jar for 15mins, 24hrs prior to the 6mins swimming test, in which the duration of immobility was recorded for the last 5mins. Single administrations (p.o.) of all the test agents were given one hour prior to final swimming test session. 1st group received only saline treatment, the 2nd, 3rd, 4th groups received Imipramine (20mg/kg p.o.) and *Apium graveolens* seed methanolic extracts (100 and 200mg/kg) respectively, The period between when the mouse was immersed and when no further attempts to escape were made (apart from the movements' necessary to keep its head above the water) and were recorded as the immobility time^{7,8}.

Group I	: Control (Normal saline 10ml/kg; p.o)
Group II	: Imipramine (20mg/kg; p.o)
Group III	: AGM (100mg/kg; p.o)
Group IV	: AGM (200 mg/kg; p.o)

Statistical analysis

Results were analyzed by one way ANOVA followed by Dunnett's multiple comparison test and the values $P < 0.05$ were considered significant (Table 1).

Table 1: Comparative profile of immobility parameter in mice forced swim test after treatment of AGM

Group	Treatment	Dose (mg/kg), p.o	Duration of Immobility (sec)	
			Pretreatment	Post treatment
1	Control (Normal saline)	10ml/kg	193.0 ± 17.98	176.0±9.977
2	Imipramine	20	184.5 ± 8.958	81.0±22.38**
3	AGM	100	183.2 ± 16.29	123.8±22.02*
4	AGM	200	173.3 ± 9.218	73.0±16.96***

All values are mean ± SEM. (n=6). One-way ANOVA followed by Dunnet's test.* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, when compared to vehicle treated (control) animals.

2. Tail suspension method

Swiss Albino mice were divided into 4 groups of six animals each. Here, the mice were individually suspended 50 cm above the surface of table with an adhesive tape placed 1 cm away from the tip of the tail. Immobility duration was recorded for the last 5 minutes

during 6 minutes. Mice were considered immobile only when they hung passively and were completely motionless. Single administrations (p.o.) of all the test agents i.e. vehicle, Imipramine (20mg/kg p.o.) and *Apium graveolens* seeds extract (100, 200mg/kg) were given one hour prior to test^{7,8}.

Group I	:	Control (Normal saline 10ml/kg; p.o)
Group II	:	Imipramine (20mg/kg; p.o)
Group III	:	AGM (100mg/kg; p.o)
Group IV	:	AGM (200 mg/kg; p.o)

Statistical analysis

Results were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test and the values $P < 0.05$ were considered significant (Table 2).

Table 2: Comparative profile of immobility parameter in mice tail suspension test after treatment of AGM

Group	Treatment	Dose (mg/kg), p.o	Duration of Immobility (sec)	
			Pretreatment	Post treatment
1	Control (Normal saline)	10ml/kg	137.7±14.96	149.8±10.14
2	Imipramine	20	150.2±14.63	68.83±11.46**
3	AGM	100	129.8±11.27	95.83±12.00*
4	AGM	200	133.6±12.43	62.3±9.17***

All values are mean ± SEM. (n=6). One-way ANOVA followed by Dunnett's test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, when compared to vehicle treated (control) animals.

RESULTS AND DISCUSSION

The air dried and finely ground seeds of *Apium Graveolens* was extracted by Soxhlet apparatus with methanol at 40-50^o c for eight hours, when filtered and concentrated under reduced pressure gave the yield of 9.24% w/w. Preliminary phytochemical analysis revealed the presence of carbohydrates, flavonoids, alkaloids, steroids and glycosides in the methanolic extract of seeds of *Apium Graveolens* (AGM).

In *in-vivo* study, the AGM (100, 200 mg/kg) produced significant anti-depressant effect on mice & rats in both FST & TST; its action was found to be similar to imipramine. Both the models of depression are widely used to screen new anti-depressant drugs. These tests are sensitive to all major classes of anti-depressant agents.

In FST, Mice were forced to swim in restricted space from which they cannot escape. This induces a state of behavior despair in animals, which is claimed to reproduce a condition similar to human depression. In TST, immobility reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression⁹.

It was observed that following administration of test formulations of *Apium graveolens* seeds extract demonstrated significant (compared to vehicle treated group) a dose dependant reduction in duration of immobility and produced significant anti-depressant like effects. The behavioral effects of AGM are similar to data obtained by other investigators with classical anti-depressant drugs such as imipramine (or other tricyclic), monoamine oxidase inhibitors and selective serotonin

reuptake inhibitors.

The anti-depressant effects of AGM in FST & TST were more prominent at 200 mg/kg when compared to lower dose of same fraction. The prominent significant antidepressant effects at dose of 200mg/kg could be due to strong and effective concentration of the active constituent.

The swimming and immobility behaviors are sensitive to serotonergic agents, such as the SSRI's agents. Based on these findings it can be suggested that the AGM which is able to reduced the immobility time and increase swimming behavior in the Mice exposed to these paradigms can exert its effect through a mechanism similar to that of the SSRI's via serotonin system¹⁰. More over imipramine belongs to the class of tricyclic anti-depressant drugs which blocks the reuptake of NE & 5-HT into their respective neurons. Hence AGM can also mediate its activity through the same mechanism as that of Imipramine.

CONCLUSION

From the results of our studies, it can be concluded that methanolic extract of *Apium graveolens* seeds possessed antidepressant activity in animal models. The observed effects were nearly equal to the existed familiar standard drug imipramine and antidepressant activity may be due to the presence of multiple phyto constituents such as flavonoids, glycosides, alkaloids and steroids. Hence isolation of active principles from this extract will be more advantageous to produce novel bioactive constituents having antidepressant activity.

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