Anticonvulsant activity of Apium graveolens in male mice

Entisar J. Al Mukhtar*

* = Lecturer in Pharmacology department. College of Medicine. University of Babylon. Babylon - Iraq. Email: ejhas28@yahoo.com

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Summary:
Epilepsy is a heterogeneous symptom complex—a chronic disorder characterized by recurrent seizures. Seizures are finite episodes of brain dysfunction resulting from abnormal discharge of cerebral neurons. The aim of this study was to investigate the anticonvulsant effect of Apium graveolens. Convulsions were induced by lidocaine in four groups of male albino mice. Group one and two received two different oral doses of Apium graveolens (A. graveolens) daily for 10 days, at day eleven convulsions were induced by lidocaine, group three and four were injected with distilled water (D.W) and diazepam (DIZ) respectively 30 minutes before lidocaine injection. Onset and duration of convulsions were recorded and compared as a main effect to lidocaine, the onset of ataxia and drowsiness were also recorded. No significant differences were found (p>0.05) in the onset of convulsion between D.W and other treated groups. A. graveolens at 15 g/kg not significantly delayed the onset of convulsion in comparison to other groups. Regarding the duration of convulsion it was high significantly increased (p<0.01) in D.W. group in comparison to other groups, The duration of convulsion was non significantly (p>0.05) reduced in 15 g/kg A. graveolens compared to DIZ, while it was high significantly decreased (p<0.01) in A. graveolens 15 g/kg compared to 7.5 g/kg.

Ataxia did not occurred in both 7.5 g/kg A. graveolens and D.W groups. The 15 g/kg A. graveolens significantly (p<0.05) delayed the onset of ataxia in comparison to DIZ. The onset of drowsiness was significantly (p<0.05) delayed in 15 g/kg A. graveolens compared to D.W, while it was non significantly (p>0.05) delayed in 15 g/kg A. graveolens compared to 7.5 g/kg A. graveolens and DIZ groups. In conclusion Apium graveolens has anticonvulsant activity. At the dose 15g/kg the anticonvulsant effect of A. graveolens was higher than that of DIZ, while at the dose 7.5g/kg of A. graveolens it was less than that of DIZ.
Introduction:
With over 10 billion neurons and an estimated $10^{14}$ synaptic connections, the human brain boasts unparalleled electrical complexity, proper functioning of the brain requires distinct isolation of electrical signals, and thus demands a far higher level of regulation. Abnormal function of ion channels and neural networks can result in rapid, synchronous, and uncontrolled spread of electrical activity, which is the basis of a seizure. The seizure disorder belongs to a heterogeneous group comprising a variety of clinical presentations and many different causes. They represent clinical manifestations of abnormal electrical activity in the brain and should be distinguished from epilepsy.\(^1\) Epilepsy is a neurological disorder characterized by repeated seizures over time and is caused by abnormal electrical activity in a population of neurons in the central nervous system.\(^2\) The causes of seizures are many and include the full range of neurologic diseases from infection to neoplasm and head injury. In some subgroups, heredity has proved to be a predominant factor.\(^3\) Seizures vary broadly in both symptoms and severity depending on the area of the brain affected. Some seizures may cause minor staring spells in an individual, whereas others can cause brief blackouts, drooling or frothing from the mouth, teeth clenching, and uncontrollable muscle spasms. The symptoms can last anywhere from seconds to hours.\(^4\) Epilepsy is the third most common neurological disorder after stroke and Alzheimer’s disease, \(^5\) it affects about 40 million people worldwide.\(^6\)

The frequency of epilepsy, coupled with inaccessibility of some antiepileptic, makes it a major public health problem in developing countries.\(^7\) Current available anticonvulsant drugs are able to control epileptic seizures efficiently in about 50% of the patients and lead to improvement in another 25% whereas the remainder do not benefit significantly.\(^5\) The therapy is symptomatic in that available drugs inhibit seizures, but neither effective prophylaxis nor cure is available. Compliance with medication is a major problem because of the need for long-term therapy together with unwanted effects of many drugs used clinically which often render treatment difficult;\(^9\) so that a demand for new types of anticonvulsants exists. One of the approaches to search for new antiepileptic drugs is investigation of naturally-occurring compounds, which belong to new structural classes.

Free radicals and epilepsy:
The neuronal hyper excitability and excessive production of free radicals have been implicated in the pathogenesis of epilepsy. The oxidative stress to which the brain is highly susceptible could play an important role in the pathophysiology of seizures.\(^10,11\) Seizure can be induced by free radical through the inactivation of glutamine synthase\(^12\), or inhibition of glutamate decarboxylase decreasing the GABA inhibitory neurotransmitter.\(^13\) Superoxide radicals can initiate a chronic mitochondrial oxidative stress that is sufficient to increase seizure susceptibility due to aging, environmental stimulation, or excitotoxin administration.\(^14,15\) The reactive oxygen species (ROS) of mitochondrial origin were suggested to be involved in the epileptic cell damage and that free radical scavenging may prevent status epilepticus-induced cell loss.\(^16\)

Antioxidants and herbal therapy:
Many medicinal plants contain large amounts of antioxidants such as vitamin C, vitamin E, carotenes, and phenolic components.\(^17\) Vitamin C, vitamin E and related analogues are nutraceuticals that can scavenge singlet oxygen and ROS.\(^18,16\) The mRNAs of antioxidant enzymes may upregulated by polyphenols present in herbs and thus may counteract the oxidative stress-induced by chemotherapy and radiotherapy. Also the protective activity may be due to the reduction in lipid peroxidation and elevation in non-protein sulphydryl groups.\(^19\)
Apium graveolens (Celery):

*Apium graveolens* (*A. graveolens*) is a herbal member of the *Apiaceae* family (*Umbelliferae*) commonly known as celery. It is an annual or biennial plant native to Mediterranean regions.\(^2\)\(^0\)\(^1\)\(^2\) A. *graveolens* is ingested as part of a normal diet.\(^2\)\(^2\)\(^3\)

Uses of *A. graveolens*:

The root, fresh leaves, and seeds of *A. graveolens* are used as food and spice such as in salads, soups, etc. Both the whole plant and the seeds have been consumed as a medicine.\(^2\)\(^4\) This plant has reported antimicrobial,\(^2\)\(^5\) adulticidal, larvicidal, repellent, and hepatoprotective activity.\(^2\)\(^7\) *A. graveolens* has carminative, diuretic and uricosuric activities, it also exhibit spasmylytic and sedative properties, which opens new possibilities of using *A. graveolens* in modern phytotherapy.\(^2\)\(^8\) *A. graveolens* has been used in the treatment of bronchitis, asthma, liver and spleen diseases and valuable in weight loss diets.\(^2\)\(^9\) In southeastern China the juice squeezed from fresh celery leaves has long been used for the treatment of epilepsy.\(^3\)\(^0\)

Lidocaine induced convulsion:

Lidocaine is a local anesthetic with antiarrhythmic properties, as a local anesthetic it blocks the voltage-gated sodium channels, thereby stabilizes the neuronal membrane by inhibiting the ionic fluxes required for the initiation and conduction of impulses.\(^3\)\(^1\) In experimental models lidocaine induced convulsion has a clear focal onset, whereas convulsion emerging in patients given intravenous lidocaine are almost invariably generalized and without any clear signs of focality.\(^3\)\(^2\)\(^3\)

Lidocaine-induced convulsion occurred due to either increment in intracellular calcium concentrations in the brain and or binding to the GABA recognition site and to another site in the GABA-ionophore receptor complex, thereby depressing GABA effects, and or it may be mediated by excitatory glutamate transmission through both N-methyl-D-aspartate (NMDA) and non-NMDA receptor systems. In addition lidocaine-enhanced one type of glutamate transporter, EAAT3 activity at certain concentrations and the role of protein kinase C (PKC) and phosphatidylinositol 3-kinase (PI3K) that mediate the lidocaine effects.\(^3\)\(^6\) Lidocaine affects the redox environment and the antioxidant enzymatic system causing oxidative stress in the hippocampus and amygdala of adult rats. Lidocaine causes an decreased concentration of GSH whereas the lipid peroxidation increased.\(^3\)\(^7\)

Diazepam:

Benzodiazepines drugs modulate the effects of gamma aminobutyric acid (GABA) the major inhibitory neurotransmitter in the central nervous system (CNS) by binding to GABAA receptors at a specific binding sites that are sometimes labeled “benzodiazepine receptors.” The benzodiazepine receptor locations in the CNS parallel those of the GABA neurons. Binding of GABA to its receptor triggers an opening of a chloride channel, which leads to an increase in chloride conductance. The influx of chloride ions causes a small hyperpolarization that moves the postsynaptic potential away from its firing threshold and, thus, inhibits the formation of action potentials. The clinical effects of the various benzodiazepines correlate well with each drug’s binding affinity for the GABA receptor–chloride ion channel complex.\(^3\)\(^8\) Diazepam is highly effective benzodiazepine used for stopping seizure activity especially generalized tonic-clonic, status epilepticus. Diazepam is also used in anxiety or insomnia treatment, adjunct in acute alcohol withdrawal, muscle spasm, preoperative use, and in conjunction with local anaesthesia.\(^3\)\(^9\)
Aim of the study:
To investigate the effect of *A. graveolens* against lidocaine induced convulsion in comparison to control (distilled water) and diazepam treated groups in mice.

Material and methods:

Animals: Twenty eight male Swiss adult mice (weighting 25-30 g) were used in this study. The animals were housed in standard cages in the animal house of Babylon Medical College, under controlled temperature around 25 °C and 12 hours light-dark cycles. They were supplied with a standard diet and tap water ad libitum.

Plant: Fresh leaves and stalks of *A. graveolens* plant were added to the animals food as a single dose daily for 10 days. In this study the plant was added in two doses, 7.5 g/kg and 15 g/kg daily.

Experimental design: After 2 weeks of adaptation, the animals were randomly divided into 4 groups (7 mice in each) as follows:

Group (1): They were injected with 0.1 ml of distilled water (D.W) intraperitoneally (I.P.) 30 minutes before the induction of seizure with lidocaine I.P. injection. This group represents the unpreventive control.

Group (2): They were injected with 1 mg/kg i.p. diazepam (ALSAVAL, Syria) 30 minutes before the induction of seizure with lidocaine injection. This group represents a preventive positive control.

Group (3): The leaves and stalks of *A. graveolens* in a dose of 7.5 g/kg were added to their food as a single dose daily for 10 days. At day eleven seizure was induced by lidocaine injection. Group (4): The leaves and stalks of *A. graveolens* were added to their food in a dose of 15 g/kg as a single dose daily for 10 days. At day eleven seizure was induced by lidocaine injection.

In all groups, convulsion were induced by intraperitoneal injection of lidocaine hydrochloride 2% (B. Braun, Germany) in a dose of 75 g/kg.

After the injection of lidocaine each mouse was monitored carefully and recorded by video camera (SONY/Cyber-shot) for at least 30 minutes in order to record the occurrence, onset and duration of convulsion as a main parameter recording in this study which may be prevented by diazepam or *A. graveolens*.

Abolition of clonic convulsions during 30 min of observation was the criterion of anticonvulsant activity, mice that did not convulse 30 min after injection of the lidocaine were considered protected.

The onset (measured from the time of lidocaine injection) of ataxia and drowsiness were also recorded as a lidocaine effect. Ataxia represent a lack of coordination of muscle movements, whereas drowsiness represent a state of impaired awareness associated with a desire or inclination to sleep. Both can occur as a result to lidocaine effect on cerebellar neurotransmitters GABA and glutamic acid.
Statistical analysis:

SPSS version 17.0 was used for the statistical analysis, Analysis of variance (ANOVA) was used for multiple sample analysis. Results were expressed as mean (seconds) ± SD. P-values less than 0.05 and 0.01 were considered as statistically significant and high significant respectively. 

Results:
The dead mice were excluded from the data of this experiment.

1. Onset of convulsion:
Convulsion did not occurred in one mouse of the 15 g/kg A. graveolens group, while it did not occurred in two mice of the DIZ group, but on the other hand three mice were died in DIZ group while no death had occurred in A. graveolens (7.5 and 15 g/kg) groups. No significant differences in the onset of convulsion (p>0.05) were found between D.W. and other groups. Although the onset of convulsion was delayed by 15 g/kg A. graveolens compared to 7.5 g/kg A. graveolens, D.W and DIZ (168.8 ± 144.8 vs. 85.8 ± 20.8, 91.8 ± 20.9 and 100.5 ± 89.5 seconds respectively) significant differences (p>0.05) were not found. Figure (1) shows the differences in the onset of convulsion.

2. Duration of convulsion:
In comparison to D.W the duration of convulsion was high significantly reduced (p<0.01) with both 15 and 7.5 g/kg doses of A. graveolens (63.6 ± 12.8 vs. 3.2 ± 1.9 and 23.2 ± 4.4 seconds respectively) and with DIZ (63.6 ± 12.8 vs. 8 ± 9.8 seconds). Although, the duration of convulsion was decreased with 15 g/kg A. graveolens compared to DIZ (3.2 ± 1.9 vs. 8 ± 9.8 seconds) significant differences (p>0.05) were not found, while the duration of convulsion was reduced high significantly (p<0.01) with DIZ compared to 7.5 g/kg A. graveolens (8 ± 9.8 vs. 23.2 ± 4.4 seconds). Regarding the two doses of A. graveolens the duration of convulsion was decreased high significantly (p<0.01) with 15 g/kg compared to 7.5 g/kg dose (3.2 ± 1.9 vs. 23.2 ± 4.4 seconds). Figure (2) shows the differences in the duration of convulsion.

3. Onset of ataxia:
Ataxia did not occurred in two mice of the 15 g/kg A. graveolens group, while it did not occurred at all in both 7.5 g/kg A. graveolens and D.W groups. The onset of ataxia is significantly (p<0.05) delayed in 15 g/kg A. graveolens compared to DIZ (146.4 ± 119.4 vs. 66.3± 49.3 seconds). Figure (3) shows the differences in the onset of ataxia.

4. Onset of drowsiness:
Drowsiness did not occurred in two mouse of 15 g/kg A. graveolens group, while it did not occurred in only one mouse of DIZ group. The onset of drowsiness was significantly (p<0.05) delayed with 15 g/kg A. graveolens compared to D.W (70.8 ± 75.3 vs.155.4 ± 23.8 seconds). Although the onset of drowsiness was delayed with 15 g/kg A. graveolens compared to 7.5 g/kg A. graveolens (70.8 ± 75.3 vs. 109 ± 24.3 seconds) and to DIZ (70.8 ± 75.3 vs. 114.5 ± 86.0 seconds) significant differences were not found (p>0.05). Figure (4) shows the differences in the onset of drowsiness.
Discussion:

Effects of Diazepam:

1- On the onset and duration of convulsion:
Compared to D.W, DIZ was found to reduce the duration of convulsion (8 vs. 63.6 seconds) with less effect on the onset of the convulsion (100.5 vs. 91.8 seconds), this result is disagree with what have been found by Uday (2009) (47) who found that DIZ delayed the onset rather than reduced the duration of convulsion.

2- On the onset of ataxia and drowsiness:
Ataxia did not occurred in D.W group and all the animals became convulsed without prior occurrence of ataxia, while it occurred 66.3 seconds after the injection of lidocaine in DIZ group, and this can be attributed to the potency of the brand of lidocaine (Germany lidocaine) used in this study compared to the brand of DIZ (Syrian diazepam) that was available to be used in this study which might be less potent. Regarding the onset of drowsiness it was comparable in both DIZ and D.W groups and this may be attributed to the differences in the sources of the available manufacture of the drugs used in this study (Germany lidocaine and Syrian diazepam).

The insignificant delay in the onset of drowsiness (p>0.05) in D.W. group compared to DIZ group (155.4 vs. 114.5 seconds) disagrees with what have been found by Uday (2009) (47) who found that DIZ had no effect on drowsiness and ataxia when compared to D.W group, this disagreement may be related to the different brand of DIZ (Sult-Jordan) used in his study.

Effects of Apium graveolens:

1- On the onset and duration of convulsion:
Apium graveolens at the dose of 15 g/kg when compared to D.W and DIZ it was found to both delay the onset and reduce the duration of convulsion.

These results goes with Asif et al. (2011) (48) who found that some of A. graveolens constituents have anticonvulsant actions which may be attributed to the antioxidant effect of A. graveolens (49), that may antagonizes the oxidant effect induced by lidocaine as the later can decrease the serum concentration of Glutathione (GSH) and increase serum concentration of malondialdehyde (MDA). (37) This constituent (with antioxidant activity) may be luteolin which is a naturally occurring flavonoid found in foods including A. graveolens, luteolin provided dramatic protection against drug induced free radical damage (50). It have been found that flavonoids and other polyphenols have powerful antioxidant activities, that can scavenge a wide range of ROS (16) and reactive nitrogen species and chelate transition metal ions, often decreasing the pro-oxidant activity of metal ions. (51)

In addition other phytoconstituents such as alpha-tocopherol (16) and glucosides are found in A. graveolens (52) they also have antioxidant effect. (53) It has been found that the consumption of roots and leaves juices of A. graveolens resulted in a significant elevation in GSH content. (50)

The dose 7.5 g/kg of A. graveolens when compared to D.W was found to reduce the duration of convulsion but its onset was comparable to that of D.W, while when compared to DIZ it resulted in a more rapid onset (85.8 vs. 100.5 seconds) and longer duration of convulsion (23.2 vs. 8 seconds) and this results may be due to low dose of A. graveolens or to the duration of its administration which may be short for such low dose.
Thus DIZ as anticonvulsant was less effective than 15g/kg *A. graveolens* while, it was more effective than 7.5 g/kg *A. graveolens*, this result could be attributed to the more antioxidant activity of *A. graveolens* at the dose 15g/kg.

2- **On the onset of ataxia and drowsiness:**

Ataxia did not occurred in D.W. group, this may be related to the potent effect of lidocaine as convulsion inducer, also it did not occurred in 7.5g/kg *A. graveolens* group which may be attributed to less anticonvulsant activity of *A. graveolens* at 7.5g/kg dose.

The onset of ataxia was significantly delayed (p<0.05) in 15g/kg *A. graveolens* group in comparison to DIZ group (146.4 vs. 66.3 seconds) which indicate superiority of 15g/kg *A. graveolens* upon DIZ to antagonized the lidocaine-induced ataxia.

Regarding the onset of drowsiness it was significantly delayed in D.W group (p<0.05) compared to 15g/kg *A. graveolens* group (155.4 vs. 70.8 seconds) and this can be explained by the good activity of *A. graveolens* at the dose 15g/kg to antagonized the drowsiness as a minor effect of lidocaine, this explanation is more acceptable because drowsiness did not occurred in two mice in 15g/kg *A. graveolens* group, whereas it occurred in all mice of the D.W group.

The onset of drowsiness was insignificantly delayed (p>0.05) in D.W group in comparison to 7.5g/kg *A. graveolens* (155.4 vs. 109 seconds) and this also can be attributed to the activity of *A. graveolens* to antagonized lidocaine-induced drowsiness.

At the dose 7.5g/kg of *A. graveolens* the onset of drowsiness was comparable to that in DIZ group (109 vs. 114.5 seconds), which can be explained by the low activity of *A. graveolens* at such dose.

Although drowsiness did not occurred in one mice in DIZ group, the onset of drowsiness was insignificantly delayed (p>0.05) in DIZ group when compared to 15g/kg *A. graveolens* group (114.5 vs. 70.8 seconds) which can also explained by the good activity of *A. graveolens* at the dose 15g/kg to antagonized lidocaine-induced drowsiness.

Regarding the two doses of *A. graveolens*, the insignificant delay (p>0.05) in the onset of drowsiness in 7.5g/kg *A. graveolens* group compared to 15g/kg *A. graveolens* group (109 vs. 70.8 seconds) can be explained by the occurrence of drowsiness in all mice of 7.5g/kg *A. graveolens* group, whereas it did not occurred in two mice in 15g/kg *A. graveolens* group which indicate the more activity of *A. graveolens* to antagonized lidocaine-induced drowsiness at the dose 15g/kg upon 7.5g/kg dose.

Up to knowledge; this is the first published study dealing with the anticonvulsant effect of *A. graveolens*.

**Conclusions**

1. *Apium graveolens* at both doses (7.5 and 15 g/kg) has anticonvulsant activity compared to D.W
2. The anticonvulsant activity of *Apium graveolens* at the dose 15 g/kg was higher than that of DIZ, while at the dose 7.5 g/kg this activity is less than that of DIZ.

Further studies are needed including clinical ones as *A. graveolens* is a safe, cheap eaten herb and available all over the year, that can be used by epileptic patient as adjuvant or may be an alternative to the anticonvulsant drugs which are associated with many adverse effects.

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Figure (1) shows the differences in the onset of convulsion (seconds).

* * = p<0.01

Figure (2) shows the differences in the duration of convulsion (seconds).
Figure (3) shows the differences in the onset of ataxia (seconds).

![Graph showing differences in the onset of ataxia](image1)

* = p<0.05

Figure (4) shows the differences in the onset of drowsiness (seconds).

![Graph showing differences in the onset of drowsiness](image2)

* = p<0.05
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