Mintage Journal of Pharmaceutical & Medical Sciences
RESEARCH ARTICLE

EFFECTS OF METHANOLIC EXTRACT OF CELERY SEEDS ON EPILEPSY IN MICE

ZENA HASAN SAHIB

Lecturer in Pharmacology department, College of Medicine, University of Babylon, Babylon, Iraq.Email: drzinahasan@yahoo.com

Received -13.05.2015; Reviewed and accepted -31.05.2015

ABSTRACT

Background: Epilepsy is a neuropsychological disorder, characterized by recurrent spontaneous seizures. all current drugs used have synthetic origin that causes severe side effects. One major disadvantage of drugs acting via the benzodiazepine site of GABA receptors is their tolerance, dependence, and abuse tendency. Hence, search for antiepileptic compounds with same effects and lower side effects. Celery seed has been found to help regulate nervous system by producing a calming effect. **Objectives:** activity of methanolic extract of celery *(apium graveolens)* seed (AGM) on seizure were studied using lidocaine to induce seizure in mice. **Methods:** the efficacy and interaction of the extract (200mg/kg) was compared with standard anticonvulsant drug the diazepam (1mg/kg). **Results:** The extract extremely significant delay the onset of ataxia, and latency of seizures ($p<0.001$) and decreased the duration of convulsion ($p<0.001$) and provide a non significant protection in 4 out of 7 mice with no change in the onset of drowsiness. The diazepam group also showed delay in the onset of ataxia ($p<0.05$), and latency of seizures ($p<0.001$) and decreased the duration of convulsion ($p<0.001$) and provide protection in 5 out of 7 mice ($p<0.01$), while the onset of drowsiness were extremely significant decreased ($p<0.001$). The combination of extract and diazepam showed extremely significant delay in the onset of ataxia, the onset of drowsiness were extremely significant decreased ($p<0.001$), with full protection 7/7 of mice that experience the induced seizures with lidocaine in compare to control group. **Conclusion:** these findings suggest that the methanolic extract of apium graveolens seed has anticonvulsant properties, and potentiate the diazepam effects.

Keywords: anticonvulsant, diazepam, celery seeds.

INTRODUCTION

Epilepsy come in the third most common neurological disorders in the word after stroke and Alzheimer's disease [1], and affects about 40 million people worldwide. [2].

The term epilepsy is used to describe a group of disorders distinguish by recurrent spontaneous seizures that results from complex processes involving several neurotransmitter systems like the glutamatergic, cholinergic, and gabaergic systems [3].

There are many causes of seizures including neurologic diseases like infection, neoplasm and head injury. Heredity has proved to be a predominant factor in some subgroups. [4]. The oxidative stress in the brain may play an important role in the pathophysiology of seizures. [5, 6]

Seizure can be induced by free radical through the inactivation of glutamine synthase [7], or inhibition of glutamate decarboxylase decreasing the GABA inhibitory neurotransmitter. [8]

The mechanisms by which antiseizure drugs work are by either prolong inactivation state of voltage-dependent Na+channels in a use-dependent fashion or act by increase the effectiveness of inhibitory GABA transmission via the GABA A receptor, or by Inhibition of Ca++ currents through T-type Ca++channels, also by inhibition of excitatory glutamate transmission via ionotropic receptors. [9]

Lidocaine is a local anesthetic and has antiarrhythmic properties, it act as a local anesthetic by blocking the voltage-gated sodium channels, that stabilize the neuronal membrane by inhibiting the ionic fluxes necessaries for the initiation and conduction of impulses [10]

Lidocaine induced convulsion has a clear focal onset in experimental models, whereas convulsion occur in patients given intravenous lidocaine are almost generalized and without any signs of focality. [11, 12]

Lidocaine-induced convulsion involves different mechanisms:

1. Increase intracellular calcium concentrations in the brain, which frequently result in convulsion in laboratory animals and man. [13]
2. High dose of lidocaine binds to the GABA recognition site and to another site in the GABA-ionophore receptor complex, and that depress GABA effects.[14].
3. Lidocaine-induced seizures are mediated by excitatory glutamate transmission through both N-methyl-D-aspartate (NMDA) and non-NMDA receptor systems. Also lidocaine enhanced one type of glutamate transporter, EAAT3 activity in certain concentrations and the role of protein kinase C (PKC) and phosphatidylinositol 3-kinase (PI3K) which mediates the lidocaine effects [15].
4. Lastly lidocaine affects the redox environment and the antioxidant enzymatic system resulting in oxidative stress in the amygdala and hippocampus of adult rats, that causes an decreased concentration of GSH and increased lipid peroxidation. [16]

Despite the fact that there is a number of classic and modern anticonvulsant drugs for the treatment of epilepsy patients worldwide, there is a refractory seizures in more than 20% of the cases [3]. Moreover, all current drugs causes severe side effects as it have synthetic origin [17].

Major disadvantage of drugs acting via the BZD site of GABA receptors is their tolerance, dependence, and abuse liability [18,19,20]. It has lead scientists to investigate plants, which are commonly employed in traditional and alternate system of medicine for sleep disorders and related diseases [21].

The old medicines provide many options for these problems with wide source of medicinal plants, which are free of undesirable effects and have popularity in most of the developing countries [22]. And since the plants rich in GABA-active flavones are not common food plants, instead it fall within the classes of medicinal plants [23]. Also many medicinal plants contain large amounts of antioxidants such as vitamin C, vitamin E, carotenes, and phenolic components.[24]. Vitamin C, vitamin E and related analogues can scavenge singlet oxygen and ROS. [25, 26]

Hence, search for antiepileptic compounds with selective activity and lower toxicity should encourage to develop newer agents for epilepsy treatment.

Apium graveolens is a member of the Apiaceae family and is known as celery.[27]. It was shown that *A. graveolens* extracts have different beneficial biological effects as it reported by Jiao *et al.* 2003 regarding its antibiotic activity. The isolated compounds from the seeds exhibited antioxidant and inhibitory effects of cyclooxygenase and topoisomerase enzymes (type I and II) [28]. The methanol extract of celery showed a significant

hepatoprotictive activity compared to the paracetamol and thioacetamidein treated rats [29,30]. Celery was also found to have anticarcinogenic and antiproliferation activities [31]. *A. graveolens* was reported to exhibit anti-inflammatory activity in experimental animals [32]. The seeds also have gastro protective effect probably mediated through non-prostaglandin E2 production [33]. Celery which is also known as marsh water parsley, and its seeds extract contain powerful healing factor, a compound known as 3n butylphthalide or (3nB). 3nB was discovered as the active component of celery in response to investigations by researchers seeking to explain some of the medicinal effects of celery including lowering of BP & cholesterol and reducing formation of arterial plaques in experimental studies. [34]

Extracts of root and leaves of *A. graveolens* show a potential activity as scavenger of free OH and DPPH radicals as well as inhibiting of the liposomal peroxidation. Therefore, they can act as antioxidants [35]. *A. graveolens* has carminative, diuretic and uricosuric activities, it also exhibit spasmolytic and sedative properties, which opens new possibitilities of using *A. graveolens* in modern phytotherapy. [36]. In addition to that it has been found that it helps to regulate nervous system by producing a calming effect and stimulates sees drives and produce sedative effect.[37]

Hence present study was done to assess anticonvulsant activities of methanolic extract of *Apium graveolens* seeds.

Aim of the study

To assess the effects of methanolic extract of *Apium Graveolens* seeds and its interaction with diazepam on seizures in mice.

MATERIAL AND METHODS

Preparation of Extract

Celery seeds were obtained from Al-Hilla local market, The seeds were washed carefully, then air dried in shade at room temperature, then grinded to fine powder. The plant extract was prepared by mixing 40 gm of leaves powder with 80 ml methanol by refluxing for 36 hrs at 50-60 º by using Soxhlet apparatus. Appearance of colorless solvent in the siphon tube was taken as the termination of extraction. Pellets of the extract were obtained by evaporation of its liquid contents in the incubator. Hence forth the Methanolic extract of *Apium graveolens* will be called as AGM. The required dose for treatment was prepared by dissolving the pellets in distilled water and administered by stomach tube at doses of 200 mg/kg daily for 14 consecutive days. [38]

Animals

Twenty eight Swiss adult mice (weighting 25- 30 g) of either sex 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.

Experimental design

The animals were randomly divided into 4 groups (7 mice in each) after 2 weeks of adaptation, as follows:

Group (1) Non-preventive control group: they were injected with 0.1 ml of distilled water (D.W.) intraperitoneally (I.P.) 30 minutes before induction of seizure with lidocaine I.P. injection.

Group (2) The AGM extract only treated group: the AGM extract in a dose of 200 mg/kg were given by gastric tube as a single daily dose for 14 days. At day fifteen seizer was induced by lidocaine injection.

Group (3) Preventive positive control They were injected with 1 mg/kg i.p. diazepam (ALSAVAL, Syria) 30 minutes before the induction of seizure with lidocaine injection.

Group (4) The combination group: AGM extract in a dose of 200 mg/kg were given by gastric tube as a single daily dose for 14 days then injected with 1 mg/kg i.p. diazepam (ALSAVAL, Syria) 30 minutes before the induction of seizure by lidocaine injection.

In all groups convulsion were induced by intraperitoneal injection of lidocaine hydrochloride 2% (OUBARY PHARMA, Syria) in a dose of 75g/kg. [39]

After the injection of lidocaine each mouse was monitored carefully and recorded by video camera (SONY/Cyber-shot) for at least 30 minute 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 .[39] or AGM extract .

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. [40, 41]. The onset (measured from the time of lidocaine injection) of ataxia and drowsiness were also recorded as a lidocaine effect. [42] 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 of lidocaine effect on cerebellar neurotransmitters (GABA) and glutamic acid. [42, 43]

Statistical analysis

SPSS version 17.0 was used for the statistical analysis; Analysis of variance (ANOVA) was used for multiple sample analysis, while chi-square test was used for convulsion occurrence within groups. Results were expressed as mean (seconds) ± SD. [44]

RESULT

On the onset of ataxia: The AGM only extract and combination preventive groups showed an extremely significant ($p<0.001$), the diazepam showed significant ($p<0.05$) delay in the onset of ataxia in compares to the control group, as shown in figure 1.

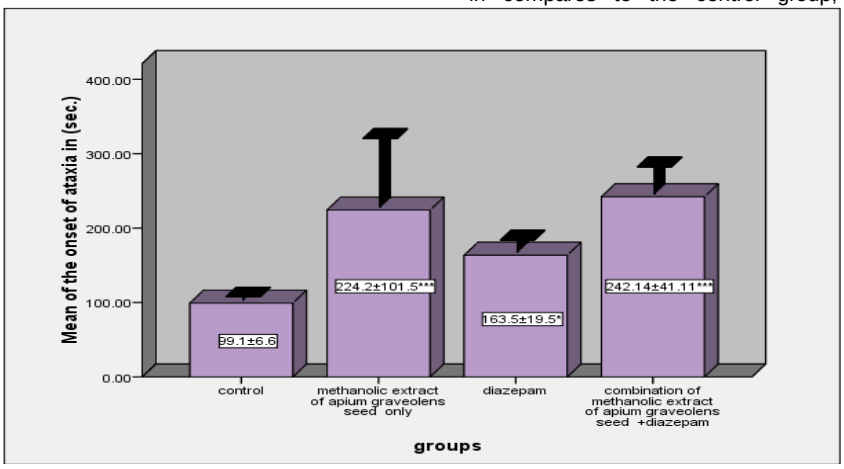

Figure1: The effect of Methanolic extracts of *A. graveolens* seeds (200mg/kg p.o) on the onset of ataxia. (*=p<0.05, *** = p<0.001)

On the onset of drowsiness: both the diazepam and combination groups showed an extremely significant reduction in the onset of drowsiness (p<0.001), while the AGM extract only group showed no significant changes (p>0.05) in compare to the control group, as shown in figure 2.

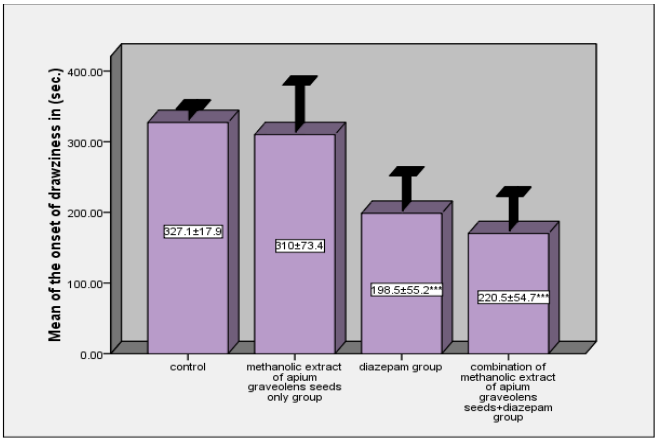

Figure 2: The effect of Methanolic extract of *A. graveolens* seeds (200mg/kg p.o) on the onset of drowsiness. (= p<0.01, *** = p<0.001)**

On convulsion occurrence: the seizures not occurred in 4 animals, that showed a non significant protection (p>0.05), 5 animals that showed a significant protection (p<0.05), and 7 animals that showed an extremely significant protection (p<0.001), of the AGM extract only, the diazepam and combination groups respectively, in compares to the control group in which all the animal were convoluted by lidocaine I.P. injections, as shown in table 1.

Table 1: show the effects of methanolic extract of *A. graveolens* seeds (200mg/kg p.o) on convulsion occurrence.

Groups	No. of convoluted animals	No. of non convoluted animals	Total no. of animals
Group (1)	7(100%)	0(0%)	7(25%)
Group (2)	3(42.8%)	4(57.14%)	7(25%)
Group (3)	2(28.5%)*	5(71.4%)*	7(25%)
Group (4)	0(0%)***	7(100%)***	7(25%)
Total	12(24.85%)	16(57.14%)	28(100%)

***= p<0.05, *** = p<0.001**

On the onset and duration of convulsion: a highly significant delay in the onset (p<0.01) with an extremely significant decreased in the duration of convulsion (p<0.001) in the AGM extract only. The diazepam group showed highly significant delay in the onset (p<0.01) and an extremely significant decrease the duration of convulsion (p<0.001) in compare to control group, while the combination of AGM extract and diazepam offered complete protection represented by no convulsion occurred so we could not report the onset and duration of convulsion, as shown in table 2.

Table 2: show the effects of methanolic extract of *A. graveolens* seeds (200mg/kg p.o) on onset and duration of convulsion.

Groups	Onset of convulsion (sec.s)	duration of convulsion (sec.s)
Group (1)	211.0±10.9	173.1±27.7
Group (2)	396.6±107.8**	34.2±43.1***
Group (3)	320.5±16.7**	20.6±17***
Group (4)	-	-

****= p<0.01, *** = p<0.001**

DISCUSSION

It was found that the diazepam group increase the onset and decrease the duration of convulsion with no effect on the onset of ataxia as compare to control group, this results is agree with what have been found by Uday (2009) [45]. While in this study the onset of drowsiness decreased, in which its disagree with him as in his study there was no effect on the drowsiness and this may be certified to the differences in the manufacture of the diazepam. Moreover, the seizures was not occurred in 5 animals. The above diazepam effects come from that its one of the enhancers of GABAergic transmission that represents a large group of traditional and new antiepileptic drugs. [46]

AGM extract only group when compared to control group and diazepam group it was found to delay the onset and reduce the duration of convulsion, they also have protect the treated mice against induced seizure in 4 animals. These results on convulsion goes with Asif *et al*. (2011) [47] who found that some of *A. graveolens* constituents have anticonvulsant actions and that may be come from their antioxidant effects [35]. And also goes with Ehsanullah, *et al;*(1990) who found that the aqueous extract of *A. graveolens* root is useful in Petit-mal epilepsy, and not in Grand-mal epilepsy. [48]

AGM group also delayed the onset of ataxia, with no significant increases in the onset of drowsiness in differ to the diazepam group that indicate advantage of AGM group upon diazepam in antagonize the lidocaine-induced ataxia.

The combination of extract and diazepam showed full protection 7/7mices which indicate a potentiating interaction between diazepam and AGM extract. This additive effect may be resulted from that each drug act on different sites with different effects as the diazepam act on GABA inhibitory receptor so have a sedative effect, while AGM extract has different suggested effects.

The antioxidant effect of *A. graveolens*, [35] 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). [16] Also It have been found that flavonoids and other polyphenols have potent antioxidant activities, that can scavenge a wide range of ROS [25], and reactive nitrogen species and chelate transition metal ions, frequently decreasing the pro-oxidant activity of metal ions. [49] In addition to that other phytoconstituents such as alpha-tocopherol and glucosides are found in *A. graveolens* [25, 50] also have antioxidant effect. [28] Moreover, It has been found that the consumption of roots and leaves juices of *A. graveolens* resulted in a significant elevation in GSH content [51].

Furthermore, its luteolin constituent which is a naturally occurring flavonoid and found in *A. graveolens,* provided dramatic protection against drug induced free radical damage [51], and show inhibition effect on the sarcoplasmic calcium channels and intracellular calcium release [52], which can prevent the increment in the intracellular calcium concentrations in the brain, that were resulted from lidocaine, leading to convulsion in laboratory animals and man. [13] Add to that, luteolin which is one of the lemon palm had been suggested by Wake *et al;* in 2000 to has nicotinic and muscarinic receptor modulating effect in human cerebral cortical cells membrane, [54] that could protect animals from ataxia induced by lidocaine injection in AGM extract treated groups.

Up to our knowledge there is no study to assess the combination of AGM extract and diazepam to compare with it.

CONCLUSIONS

- Findings from this study showed that methanolic extract of *Apium graveolens* possess anticonvulsant activities by itself like that of diazepam.
- The methanolic extract of *Apium graveolens* 200mg/kg potentiates both the anticonvulsant and sedative effects of the diazepam.
- Further Studies are needed to know if consumption of the extract predisposes to interaction with other anticonvulsants on concurrent use.

ACKNOWLEDGMENT

Special thanks to Dr. Intesar, Dr. Selman, and Dr. Hamid for their help and support. Great thanks to the animal house staff and the department of pharmacology/ college of medicine for their help.

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