Anti-seizure Activity of Allium cepa. L (red onion) Bulb on Maximal Electroshock (MES) Induced Seizure in Mice

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ABSTRACT

Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures and by the neurobiological, cognitive psychological and social consequences of this condition. Allium cepa. L (onion) is claimed in traditional medicine for the treatment of epilepsy. The study is aimed to know the protective effect of methanolic extract of Allium cepa. L obtained by cold maceration method on maximal electroshock induced seizures in mice. Mice were divided into four groups with six mice in each, control group (0.25% sodium CMC p.o), standard drug diazepam (5mg/kg i.p), and Allium cepa. L extract (8mg/20g and 16mg/20g p.o). Mice were treated 30 minutes before inducing seizures and the presence or absence of hind limb tonic extension, % protection and % mortality in the treatment and control groups were observed. Allium cepa. L extract showed significant anti-seizure property shortening the duration taken for hind limb tonic extension and showed better protection (%) when compared with control group.

Keywords: Epilepsy, Allium cepa extract, maximal electroshock, diazepam, hind limb tonic extension.

INTRODUCTION

Epilepsy disease is still not properly out of shadow and observed globally that affects almost all groups of society. Throughout the world, epilepsy affects approximately 70 million people of all generation, ages, race, ethnicity, and socioeconomic. The worldwide prevalence of epilepsy is inconsistent and diversified among countries but roughly estimated with overall prevalence of 10 per 1000 persons¹. Epilepsy involves spontaneous frequent seizures due to electrical disturbance in brain². The contributing factor of seizure in certain type of epilepsy could not clearly be defined as it is not presented together with other neurological disorder³. Epileptic seizure categorized in range of severity such as starting from staring spells to violent convulsions and loss of consciousness⁴. Wide variety of factors that can cause epilepsy which is dependent location on seizure localized. Most epileptic event will present with abnormal electrical brain activity and measured with electroencephalograph⁵. Seizure is when there is a sudden transient alteration of neuronal discharge occurrence due to hyper synchronous and rhythmic firing in brain⁶. A seizure epileptic’s brains often form excitotoxic lesions which destroy neurons by initiating apoptosis. The current management for epilepsy is limited to controlling seizure attack. A specific drug chosen for a patient is based on their past seizure history, and underlying conditions that contributed to epilepsy⁷. Most of Antiepileptic drug have a limited spectrum of activity and produce side and adverse effects that limit its use. At least 25% of epilepsy patients remain refractory to antiepileptic drug. Seizure management achieved only at expense of various severities results from AED adverse effects⁸. The side effects include dependence, anemia, psychotic reaction, blood dyscrasias, agranulocytosis, sedation, tiredness, and hepatotoxicity⁹. Besides that, patient might develop resistance to AED. Alternative therapy including herbal drugs and complementary medicine is becoming increasingly popular to overcome the side effects resulted from modern drug¹⁰. Onion is one of traditional herb used in treatment of epileptic seizures¹¹. This laid the basis for selecting to determine the protective effect of onion on MES induced seizures.

MATERIALS AND METHODS

Plant material

Allium cepa. L bulbs were used. The material was collected and sent for authentication to Department of Biodiversity (Bioscience) of University Putra Malaysia (UPM) to be verified for authentication.

Preparation of extract

Fresh red Allium cepa. L was obtained, outer covering peeled off and fleshy part washed with distilled water. 150 grams was subjected to size reduction by cutting down into smaller parts and blended. The material was soaked in 150 ml of 90% methanol for 24 hours and subjected to cold maceration. The concentrated extract constantly stirred and allowed it to evaporate at room temperature. The preparation was filtered after 24 hours. The filtrate was evaporated at room temperature, the residual pulp from the filtration was soaked again in 150 ml of 90% methanol for 24 hours and filtered once again.

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Table 1: Anti-seizure activity of *Allium cepa*L (onion) bulbs on maximal electroshock induced seizures in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Duration of Hind Limb Tonic Extension (seconds)</th>
<th>Status recovery death / recovery</th>
<th>Percentage protection (%)</th>
<th>Percentage mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control (0.25% Sodium CMC p.o)</td>
<td>42.83 ±0.946</td>
<td>6 / 0</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>B</td>
<td>Diazepam (5mg/kg i.p)</td>
<td>0</td>
<td>0 / 6</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td><em>Allium cepa</em>L extract (8mg/20g p.o)</td>
<td>13.83 ±3.135*</td>
<td>2 / 4</td>
<td>66.67</td>
<td>33.33</td>
</tr>
<tr>
<td>D</td>
<td><em>Allium cepa</em>L extract (16mg/20g p.o)</td>
<td>9.25±2.16*</td>
<td>1 / 5</td>
<td>83.33</td>
<td>16.66</td>
</tr>
</tbody>
</table>

Values are mean±SEM, n=6. One way analysis of variance (ANOVA) followed by Dunnett’s t-test where * represents significant at p<0.05 compared to control.

Figure 1: Duration of Hind Limb Tonic Extension (seconds)

Figure 2: Percentage of protection against maximal electroshock induced seizure

**RESULTS AND DISCUSSION**

Epilepsy is an excitotoxic brain disorder that could lead to increased destruction of neurons. Free radicals are the known cause for epileptic seizures. Free radical is a molecular fragment that contains one or more unpaired electrons and caused oxidative stress by excessive production of reactive oxygen (ROS) species. The brain is prone to damage due to reactive oxidative stress because of its high demand of oxygen requirement and high metabolic rate. Persistent seizures promote the increase of ROS production which result in oxidative damage of the biomolecules. *Allium cepa*L (onion) is one of traditional herbs used in India for treatment of epilepsy. Onion contains organosulfur compounds (cepaenes, Alin and allylpropyl-disulphide and thiosulfimates), sulfur, flavonoid (quercetin and kaempferol), vitamin B (B1, B2, B3, B5, B6, B9), vitamin C, trace elements like calcium, iron, magnesium, manganese, phosphorus, potassium, zinc and selenium.

Maximal electroshock (MES) seizures in mice were produced by using electroconvulsive meter of 50mA current and stimulus 0.2 sec after 30 minutes of drug administration to each group. They receive electrical stimulus of sufficient intensity to induce maximal seizures on their hind limb with tonic extension as the end point of the test.

The following parameters were recorded after seizures induce duration of hind limb tonic extension (HTLE) and percentage of protection. The seizure induced mice were kept under observation for 24 hours for any mortality.
facilitate the chloride channel opening on GABA A receptor. At 5mg/kg dose of diazepam caused complete suppression of MES induced seizure thus no data shown in record. Selenium is a non-trace element that plays a vital role in epilepsy pathogenesis. It is an analogue of selenocysteine, a component of selenoprotein that has some important enzymatic functions. There are three subfamilies of selenoproteins include thioredoxin reductase (TrxR), glutathione peroxidase (GPx), and iodothyronine deiodinases (DIO). GPx and TrxR plays important role in epilepsy. GPx from peroxidase family acts as peroxidase or ROS reduction which provides protection against oxidative damage. TrxR I reduces the hydrogen peroxide and oxidative stress. Selenoprotein T (sel T) acts as calcium regulator in brain. Selenium is required in normal maintenance of cell protecting from any brain disorder such as epilepsy by protecting the cell death in brain. Much of the literature supported the correlation between selenium deficiencies with epilepsy. The enrich selenium Allium cepa.L was tested against maxima electroshock seizures induced mice. It was found that control group (0.25% sodium CMC p.o) does not have any protection against seizure induced by MES which resulted in 100% mortality. A. cepa.L extract (8mg/20g and 16mg/20g, p.o) produced 66.67% and 83.33% protection and standard drug diazepam (5mg/kg i.p) produced 100% protection against seizure induced by MES. Protective effect of A. cepa.L may be due to selenium as the plant is enriched with selenium, allin and allylpropyl-disulphide. Bulbs of Allium cepa.L yield the greater amount of total selenium. Selenium level was found to be 0.024 μg g−1 for onion. Red medium size onion contains selenium in higher amounts than white onions. Certain region in world was well-known to have selenium soil deficiency such as in Africa and Europe and that affects the selenium contain of the crop from the soil. A. cepa.L available in Malaysia’s local market mostly imported from India that comes from selenium enrich soil and produce high profile of selenium crop products.

CONCLUSION
A. cepa.L at 8mg/20g p.o and 16mg/20g is significant at P<0.05 having shorter duration of hind limb tonic extension compared to control group. Diazepam at 5mg/kg i.p had abolished the hind limb tonic extension by preventing the seizure spread and offered 100 % protection. Allium cepa.L is protective against generalized seizure may be due to potential source of selenium, allin and allylpropyl-disulphide. Further studies to be performed in assessing the selenoproteins thioredoxin reductase (TrxR), glutathione peroxidase (GPx) which plays an important role in epilepsy.

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