Possible Protective Effects of Omega 3, Diazepam and their Combination Against Yohimbine-Induced Clonic Seizure in Mice: Comparative Study

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Abstract

Yohimbine is actually confirmed in the United States to be utilized for erectile dysfunction; and recently such drug has become commonly used in body-building communities for its presumed lipolytic and sympathomimetic effects. But ingestion of such drug can bring about epileptic effects. Many antiepileptic drugs can be utilized to counteract myoclonic seizure; furthermore, diazepam can be used to oppose such type of seizure; in addition, surrogate therapeutic options such as omega 3 may also be utilized.

In this study, twenty-four (24) mice of both sexes weighing 20-25g were randomly-allocated into 4 groups (6 animals each group) as follows: Group I- Yohimbine-induced clonic seizure [mice orally-administered DMSO (10%)], and after 30 min, animals Sc. injected with 45mg/kg yohimbine). Group II- Diazepam-treated as standard drug: It is Sc. injected at a dose of 2mg/kg, and after 70 minutes, yohimbine at a dose of 45mg/kg is Sc. injected. Group III- Omega 3 (40mg/kg) is orally-administered, and after 30 min 45mg/kg yohimbine is Sc. injected. Group IV- Combination of omega 3 (40mg/kg) is orally-administered then and after ten minutes, diazepam was IP injected 2mg/kg then after 20 minute, 45mg/kg yohimbine is Sc. injected.

The result of this study showed that omega 3 has non-optimal antiepileptic effect; where, it is un-able to reduce the onset and frequency of epilepsy tone in mice during several time periods (30-120min); and data weren’t significantly different when compared to diazepam. But omega 3 reduced onset of epilepsy in combination with diazepam when compared with diazepam alone. As well as omega 3 caused small percent changes frequency of epilepsy tone through 120 min when compared with other groups.

Results of the current research suggested omega 3 fatty acid when is combined with diazepam produces significant role for reducing onset of epilepsy against yohimbine-induced seizure model.

Keyword: Omega, Diazepam, Yohimbine, Epilepsy.
Introduction

Yohimbine is a sympatholytic drug used to treat impotence in male patients (1). Yohimbine possessed physiological and behavioral effects after systemic administration that includes CNS actions which arise from its highly lipophilic and crosses the blood–brain barrier (BBB) (2). Moreover, it also has peripheral effect by increasing the release of norepinephrine (NE). The dual effect of yohimbine can be produced by its antagonistic effect on the central alpha-2-receptors and can act as sympathomimetic agent (3). The sympathomimetic, aphrodisiac, and hallucinogenic properties of yohimbine has succumb resurgence as a street drug; where, it is also used by the body builder community as a nutritional supplement for its presumed lipolytic and sympathomimetic effects; but, such drug was reported to produce a severe acute neurotoxicity in a case of a body builder (4, 5).

Epilepsy is one popular CNS disorder that can cause considerable morbidity and mortality (6), and it is a disabling popular chronic illness of neurological disorder (7). Furthermore, it has variety of etiologic backgrounds and it is diverse group of disorders that accompanying with seizure appearance (8) which include rapid head and eye movement that extend or transient depletion of consciousness to spasms and uncontrolled muscle contractions (9). Many investigators demonstrated that less than 70% of patients stricken with epilepsy achieve seizure control with the available antiepileptic drugs. However, for the remaining 30%, who suffer from intractable epilepsy, treatment options are limited to different multi-drug therapies or surgery if applicable (10). Thus, alternative treatments for patients with refractory epilepsy are therefore needed. Moreover, various types of antiepileptic drugs have several complications and crucial undesirable effects such as agranulocytosis and hepatotoxicity demand new drugs with more advisable margins of safety and more acceptable (11).

Omega 3 fatty acids are n-3 polyunsaturated fatty acids (n-3 PUFA) that are available in marine oils and sea diet, which are fundamental for neurological function and progression (12). The n-3 PUFA are principle structural components of neuronal membranes, and are concerned in cell signaling and modification neurotransmission (13). Research in animals and human has explained a beneficial effect of high n-3 PUFA intake in several neurological disorders, including attention deficit disorder and Alzheimer’s disease (AD) (14). But, there were conflicting results concerning the anti-epileptic effect of omega 3; where, researchers reported that supplementation with omega 3 fatty acid can suppress the epileptic seizures while others mentioned that such fatty acids can elevate the seizure (15).

Thus, this study is designed to evaluate the effect of omega 3 against yohimbine-induced clonic seizure in comparison with 2mg/kg diazepam as a reference drug.

Materials and Method

The yohimbine powder was dissolved in dimethylsulfoxide (DMSO) to produce a stock solution of 0.2mg/ml; and the dose of yohimbine (45mg/Kg). Diazepam (Bristol, UK) as solution for injection was diluted with 10% DMSO to produce a stock solution of 0.1mg/ml; and the dose of diazepam was prepared according to the body weight of the animal to produce (2mg/Kg). Omega 3 (TAD, Germany) was diluted in DMSO to produce a stock solution of 0.4mg/ml; where, the dose of omega 3 is (40mg/Kg).

Twenty-four mice weighing 20-25g were obtained from The College of Pharmacy, University of Baghdad. Animals were kept in the animal house of the Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad, at 25 ± 2 °C and light: dark cycle of 12:12 h for 1 week before starting experiments. Animals were provided with standard rodent pellet diet and the food was withdrawn 12h before the experiment, though water was allowed ad libitum. All experiments were performed according to the guidelines of laboratory animals’ care and the ethical guidelines for the investigations on experimental animals.

Groups of animal

Twenty-four mice of both sexes weighing 20-25g were randomly allocated into 4 groups (6 animals each group) as follows:

Gr I- Yohimbine-induced clonic seizure: Mice were orally-administered 10% (DMSO), then after 30 minutes, animals were Sc. injected with 45mg/kg yohimbine (16).

Gr II- Diazepam as reference drug: Mice were IP injected with a dose of 2mg/kg diazepam (17), then after 20 min, animals were Sc. injected with 45mg/kg yohimbine.

Gr III- Omega 3-administered animals: Mice were orally-administered omega 3 at a dose 40mg/kg by gavage tube (18); then after 30 min, yohimbine at a dose of 45mg/kg was Sc. Injected.

Gr IV- Combination therapy: Mice were orally-administered omega 3 at a dose 40mg/kg by gavage tube then after ten min, diazepam was IP injected at a dose of 2mg/kg. Then after 20 min from diazepam injection, yohimbine at a dose of 45mg/kg was injected Sc. (16-18).
**Measured parameters**

In adult mice or rats, the clonic seizure starts as forelimbs rhythmic movements, often accompanied with facial clonus that can be either unilateral or a synchronized bilateral clonus with or without rearing and the tail erection (strab tail) (19).

Mice were observed for in a range of 30-120 min (20) for:

1. The onset clonic seizures.
2. The number of clonic seizures attack at 30-60min, 60-90min, and 90-120min after yohimbine injection.
3. Measurement of the percent (%) changes in number of clonic seizures attack induced by yohimbine HCl at 120min according to the following equation:

\[
\% \text{ change} = \frac{\text{mean of control group-mean of treated group}}{\text{mean of control group}} \times 100
\]

**Statistical Analysis**

The results were expressed as mean ± S.E.M. The significance of differences between the mean values has been calculated using unpaired Student t-test. Comparison among multiple groups was made by using analysis of variance (ANOVA). P-values less than 0.05 (P<0.05) were considered significant for data of this work.

**Result**

**Observation of the onset of epilepsy seizure within 30 min**

Mice of Group I (10% DMSO + yohimbine) exhibit onset of epilepsy at 5±0.258 min; while in Group II (Diazepam + yohimbine) mice, the onset of epilepsy is 24.2 ±0.478 min; and there was a significant different (P<0.05) in the onset of epilepsy in minutes by comparing such time in Group II to that of Group I as shown in table 1 and figure 1.

In Group III (omega 3 fatty acids + yohimbine) mice, the onset of epileptic seizure is 5.8±0.307min; and there were non-significant differences (P>0.05) in the onset of epilepsy in minutes by comparing such time in Group III to that of Group I mice (10% DMSO + yohimbine). (5±0.258 min). Table 1 and figure 1.

In Group IV [combination therapy (omega 3 fatty acids + Diazepam + yohimbine)] mice, the onset of epilepsy is 29.3±0.66min; and there were significant different (P<0.05) in the onset of epilepsy between Group IV and Group I (10% DMSO + yohimbine); (5±0.258 min); similarly, there were significant differences (P<0.05) in the onset of epilepsy between Group IV and Group III (5.8±0.307min) as shown in table 1 and figure 1.

Furthermore, by comparing the onset of epilepsy among Group II (diazepam + yohimbine), with Groups III (Omega 3 fatty acids + yohimbine HCl) there were significant differences (P<0.05) in the onset of epilepsy. Table 1 and figure 2.

Also, by comparing the onset of epilepsy among Group II (diazepam + yohimbine), with and Group IV (omega 3 fatty acids +Diazepam + yohimbine HCl), there were also significant differences (P<0.05) in the onset of epilepsy. Table 1 and figure 2.

**Observation of the number of clonic seizures attack at 30-60min in the studied groups**

In table 1 and figure 2, there were significant different (P<0.05) in the number of clonic seizure in Group I (yohimbine-treated) (16.5±0.846) compared to Groups II (Diazepam +yohimbine HCl) (1.33±0.494) and Group IV (omega 3 fatty acids +Diazepam + yohimbine HCl) (0.833±0.477).

Furthermore, there were non-significant (P>0.05) difference in the number of clonic seizure in Group I (16.5±0.846) compared to Group III (15.16±0.477) as shown in table 1 and figure 2.

Moreover, table 1 and figure 2 showed that there were significant differences (P<0.05) in the number of clonic seizure in Group II (Diazepam + yohimbine) (1.33±0.494) compared to that number in Group III (omega 3 fatty acids + yohimbine HCl) (15.16±0.477); but, there were non-significant differences (P>0.05) in the number of clonic seizure in Group II (1.33±0.494) compared to Group IV mice (0.833±0.477).

In addition, there were significant different (P<0.05) in the number of clonic seizure in Group III (Omega 3 fatty acids + yohimbine) (15.16±0.477) compared to that in Group IV (omega 3 fatty acids + Diazepam + yohimbine HCl) mice (0.833±0.477).

**Observation of the number of clonic seizures at 60-90min in the studied groups**

Table 1 and figure 3 showed that, there were significant different (P<0.05) in the number of clonic seizures at 60-90min observed in Group I (yohimbine-treated) (12.3±0.557) compared to that in Groups II (Diazepam +yohimbine HCl) (0±0) and Group IV (omega 3 fatty acids + Diazepam+ yohimbine HCl) (0±0); but, there were non-significant difference (P>0.05) in the number of clonic seizures at 60-90min observed in Group I (12.3±0.557) compared to that in Group III mice (11.66±0.614).

Also table 1 and figure 3 showed that there were significant difference (P<0.05) in the number of clonic seizures at 60-90min in Group II (Diazepam + yohimbine HCl) (0±0) compared to that in Group III (omega 3 fatty acids + yohimbine HCl) (11.66±0.614); but there were non-significant different (P>0.05) in the number of clonic seizures at 60-90min in Group II (0±0) compared to that in Group IV mice (0±0).

Additionally, there were significant different in the number of clonic seizures at 60-90min (P<0.05) in Group III (Omega 3 fatty acids +yohimbine HCl) (11.66±0.614) compared that in Group IV (omega 3 fatty acids +Diazepam + yohimbine HCl) mice (0±0). Table 1 and figure 3.
Observation the number of clonic seizures at 90-120min in the studied groups

Table 1 and figure 4 showed that there were significant different (P<0.05) in the number of clonic seizure at 90-120min in Group I (yohimbine-treated) mice (6.5±0.341) compared to that in Group II (Diazepam + yohimbine HCl) (0±0) and Group IV (omega 3 fatty acids +Diazepam + yohimbine HCl) mice (0±0); but there were non-significant (P>0.05) in the number of clonic seizure at 90-120min in Group I (6.5±0.341) compared to that in Group III (omega 3 fatty acids +yohimbine HCl) mice (5.66±0.494).

Moreover, there were significant difference (P<0.05) in the number of clonic seizure at 90-120min in Group II (Diazepam + yohimbine HCl) (0±0) compared to that in Group III (Omega 3 fatty acids +yohimbine HCl) mice (5.66±0.494); but there were non-significant difference (P>0.05) in the number of clonic seizure at 90-120min in Group II (0±0) compared to that in Group IV mice (0±0).

Table 1 and figure 4 showed that there were significant difference (P<0.05) in the number of clonic seizure at 90-120min in Group III (Omega 3 fatty acids +yohimbine HCl) (5.66±0.494) compared to that in Group IV mice (omega 3 fatty acids +Diazepam + yohimbine HCl) (0±0).

Percent (%) changes in number of clonic seizures within 120min

In table 1 and figure 5 showed that there were significant different (P<0.05) in percent changes in number of clonic seizures attack at 120min between Group I (yohimbine-treated) (0%) compared to that in Group III mice (Omega 3 fatty acids + yohimbine HCl) (13.846%). In contrast, there were non-significant (P>0.05) different in percent changes in number of clonic seizures attack at 120min in Group II (Diazepam + yohimbine HCl) (100%); but there were significant different (P<0.05) in percent changes in number of clonic seizures attack at 120min in Group III (omega 3 fatty acid + yohimbine HCl) each compared to that in Group IV mice (omega 3 fatty acids +Diazepam + yohimbine HCl) (100%) as that shown in table 1 and figure 5.

Table 1. Onset of clonic seizures during 30min, number of clonic seizure during different period from 30-120min that induced by yohimbine and other treatment groups and percent changes in number of clonic seizures within 120min

<table>
<thead>
<tr>
<th>Treatment group n=6</th>
<th>Type of treatment</th>
<th>onset of clonic seizures (30min) (Mean ± S.E.M)</th>
<th>number of clonic seizures at 30-60min (Mean ± S.E.M)</th>
<th>number of clonic seizures at 60-90min (Mean ± S.E.M)</th>
<th>number of clonic seizures at 90-120min (Mean ± S.E.M)</th>
<th>percent changes in number of clonic seizures within 120min</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>yohimbine HCl</td>
<td>5±0.258# €</td>
<td>16.5±0.846# Є</td>
<td>12.3±0.557# Є</td>
<td>6.5±0.341# €</td>
<td>0%</td>
</tr>
<tr>
<td>II</td>
<td>Diazepam + yohimbine HCl</td>
<td>24.2±0.478* #¥ €</td>
<td>1.33±0.494* #¥</td>
<td>0 ± 0* #¥</td>
<td>0# #¥</td>
<td>100%</td>
</tr>
<tr>
<td>III</td>
<td>Omega 3 fatty acids + yohimbine HCl</td>
<td>5.8±0.307# Є</td>
<td>15.16±0.477# Є</td>
<td>11.66±0.614# Є</td>
<td>5.66±0.494# Є</td>
<td>13.846%</td>
</tr>
<tr>
<td>IV</td>
<td>Omega 3 fatty acids + Diazepam + yohimbine HCl</td>
<td>29.3±0.66* #¥</td>
<td>0.833±0.477* #¥</td>
<td>0 ± 0* #¥</td>
<td>0# #¥</td>
<td>100%</td>
</tr>
</tbody>
</table>

Symbol * refers to difference between Group I and other groups. Symbol; # refers to difference between Group II and other groups; symbol ¥ refers to difference between Group III and other groups; and symbol € refers to difference between Group IV and other groups.
Figure 1. Mean±SE of the onset of clonic seizures(min) that induced by yohimbine HCl (Group I) and other treated groups, Diazepam+ yohimbine HCl (Group II), omega 3 fatty acids +yohimbine HCl (Group III) and omega 3 fatty acids +Diazepam +yohimbine HCl (Group IV). Symbol *refers to difference between group I and other groups. Symbol; # refers to difference between group II and other groups; symbol ¥ refers to difference between group III and other groups; and symbol € refers to difference between group IV and other groups.

Figure 2. Mean±SE of the number of clonic seizures at 30-60min that induced by yohimbine HCl (Group I) and other treated groups (Diazepam+ yohimbine HCl (Group II), Omega 3 fatty acids +yohimbine HCl (Group III) and omega 3 fatty acids +Diazepam +yohimbine HCl (Group IV)). Symbol *refers to difference between group I and other groups. Symbol; # refers to difference between group II and other groups; symbol ¥ refers to difference between group III and other groups; and symbol € refers to difference between group IV and other groups.

Figure 3. Mean±SE of the number of clonic seizures at 60-90min that induced by yohimbine HCl (Group I) and other treated groups (Diazepam+ yohimbine HCl (Group II), Omega 3 fatty acids +yohimbine HCl (Group III) and omega 3 fatty acids +Diazepam +yohimbine HCl (Group IV)). Symbol *refers to difference between group I and other groups. Symbol; # refers to difference between group II and other groups; symbol ¥ refers to difference between group III and other groups; and symbol € refers to difference between group IV and other groups.

Figure 4. Mean±SE of the number of clonic seizures at 90-120min that induced by yohimbine HCl (Group I) and other treated groups (Diazepam+ yohimbine HCl (Group II), omega 3 fatty acids +yohimbine HCl (Group III) and omega 3 fatty acids +Diazepam +yohimbine HCl (Group IV)). Symbol *refers to difference between group I and other groups. Symbol; # refers to difference between group II and other groups; symbol ¥ refers to difference between group III and other groups; and symbol € refers to difference between group IV and other groups.
Thai chronic treatment by omega-3 encourages neuroprotection and positive plastic exchanges in the brain of rats with epilepsy (34), with a reduced in neuronal death in CA1 and CA3 subfields of the hippocampus. This may be assigned to n-3 PUFAs ion channel intonation, and anti-inflammatory activity (36).

The current study showed that omega-3 have partial antiepileptic effect but non-significant. It was unable to decrease the number of clonic seizure-induced by yohimbine (Group III) from the onset of induction to 120 min compared to that in yohimbine-treated (Group I) mice; in other word, its effect was not-significant (P>0.05); also, the onset of seizure induction times in Group III mice were shorter than that observed in Group II mice; but, the positive antiepileptic effect of omega3 that include the onset clonic seizures in Group IV mice (combination of omega 3 with diazepam) was significant compared to that in diazepam alone (Group II) mice; but, the effect of omega3 that include the onset time for induction of epilepsy in Group IV mice was significantly prolonged compared to that in Group III (omega 3 fatty acid against yohimbine-treated); moreover the beneficial effect of omega3 in Group IV rats, there were reduction in the number of epilepsy when omega 3 combined with diazepam during various time intervals 30-120 minutes compared to that in diazepam-treated (Group II) mice but, was non-significantly different (P>0.05).

As well as in figure 5, the positive effect of omega 3 (Group III) was shown; where, there were clear difference in the percent of epileptic frequency compared to that of yohimbine HCl (Group I).

The reason for the non-optimal significant activity of omega 3 against yohimbine-induced clonic seizure may be related to short duration of treatment with such fatty acid as researcher mentioned that, prolonged oral administration of alpha-linolenic acid (an omega-3 fatty acid) and linoleic acid in a 1:4 mixture protected rats from having seizures in four different epilepsy models (34). Moreover, investigators reported that 200 mg/kg/day of omega 3 intraperitoneally (I.P) injected for 21 days possessed antiepileptic effect (37).

**Conclusion**

Omega 3 fatty acid produced non-significant effect; and when it is combined with diazepam produced significant role for reducing onset of epilepsy against yohimbine-induced seizure model. But, omega 3 alone has no role for decreasing the onset of epilepsy. Additionally, omega 3 alone caused small changes in percent

**Discussion**

The a2-antagonist yohimbine has a diversity of effects on the noradrenergic, dopaminergic, serotonergic and gamma aminobutyric acid (GABAergic) neurotransmission. Yohimbine-induced seizures, not only intermeditated through impairment of GABAergic transmissions but furthermore, by an endogenous enhancement of the excitatory amino acid transmission (21); and it was and still vastly used in diverse experimental studies in vitro (22, 23, 24) and in vivo in aware animals (25, 26, 27).

In the present study, yohimbine-induced seizures in all groups of the experiment I, II, III, and IV in various time sequences from 30 min after seizure induction by yohimbine to 120 min. Table 1 and figures (1-4). This is parallel with other studies also showed that yohimbine can excite seizure assay (28, 29).

Pharmacological studies revealed that prostaglandin E2 and other pro-inflammatory mediators were upraised in the animal design of epilepsy (30). It has been reported that both of the enzymes, phospholipase A2 (PLA2) and oxidoreductase cyclooxygenase-2 (COX2) can be invigorated by epilepsy; where, the PLA2 enzyme can liberate arachidonic acid (AA) from membrane phospholipids, and the COX2 can transfer AA to pro-inflammatory mediators (31).

Polyunsaturated fatty acids (PUFAs) docosahexaenoic acid (DHA, 22: 6n-3) are major constituents of membrane phospholipids in brain tissue (32).

Diets insufficient in omega 3 PUFA lead to decreased DHA in the brain and enhanced turnover of arachidonic acid to eicosanoids, an effect which is defeat by restoring the omega 3 to diet (33).

Researchers reported that the pro-inflammatory mediators have been decreased by omega-3 fatty acid eicosapentaenoic acid (EPA) through inhibiting PLA2 and COX2 enzymes (34). Others reported that n-3 PUFAs possessed beneficial therapeutic effect in patients suffering from a neurological disorder such as epilepsy, in spontaneous, and in recurrent seizure (35).

**Conclusion**

Omega 3 fatty acid produced non-significant effect; and when it is combined with diazepam produced significant role for reducing onset of epilepsy against yohimbine-induced seizure model. But, omega 3 alone has no role for decreasing the onset of epilepsy. Additionally, omega 3 alone caused small changes in percent
frequency of epilepsy tone within 120min when compared with other groups; also omega 3 in combination with diazepam have role for reduction frequency of epilepsy but was not significant but omega3 alone didn’t have significant role for reduction frequency of epilepsy.

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Competing interests
There are no competing interests to declare.

References


