Potential Mechanisms Involved in the Anticonvulsant Effect of Walnut Extract on Pentylenetetrazole-Induced Seizure

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Key Words
Walnut kernel · Seizure · Rat · Anticonvulsant effect · Pentylenetetrazole

Abstract
Objective: It was the aim of this study to determine the potential effect of walnut kernel extract (WKE) on experimentally induced seizures in rats and to evaluate the role of benzodiazepines and ethosuximide (ESM) within these pathways. Materials and Methods: Male Wistar rats were selected and divided into eight groups. Seizures were evoked by intravenous infusion of pentylenetetrazole (PTZ; 2 mg/ml/min). In combination with PTZ, animals were treated with vehicle or WKE (100 mg/kg i.p.), with or without cotreatment with either flumazenil (FMZ; 5 mg/kg i.p.), ESM (150 mg/kg i.p.) or diazepam (DPZ; 0.5 mg/kg i.p.). Results: WKE administration significantly increased the PTZ dose needed to induce the first myoclonic jerk (13.09 ± 1.29 vs. 49.71 ± 12.03 mg/kg; p < 0.001), decreased the severity of seizure grades and reduced the mortality rate to 0%. FMZ did not significantly reduce the anticonvulsant effect of WKE. The combination of DPZ and WKE showed a synergic anticonvulsant effect, whereas ESM had no significant influence (p > 0.05) on the WKE effects. Conclusion: These findings indicated that WKE was effective at reducing seizure severity, at increasing the dose to the first myoclonic jerk and highly efficacious at preventing mortality, because 100% of animals were protected. It seems that this positive effect could apply through signaling pathways other than benzodiazepine-mediated \(\gamma\)-aminobutyric acid receptors and may at least in part be similar to ESM.

Introduction

Epilepsy is one of the most common central nervous system disorders, and uncontrolled seizures increase the comorbidities and the chance of mortality [1]. Antiepileptic drugs only provide symptomatic treatment as they suppress seizures but do not have the ability to cure the disease [2]. There is continual research focusing on new therapeutic approaches to prevent and treat epileptic seizures.

The walnut tree (\textit{Juglans regia} L.) is cultivated throughout Eastern Asia, Southern Europe, Northern Africa, and the United States of America [3]. The Walnut kernel (WK) accounts for 40–60\% of the nut weight. It has high levels of oil (52–70\%) in which polyunsatu-
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PTZ + DMSO + saline group (p < 0.05). Coadministration of WKE with FMZ did not change the threshold dose. However, injection of WKE + DPZ amplified the anticonvulsant effect of WKE and increased the threshold dose (p < 0.001 compared to the PTZ, WKE + PTZ, WKE + FMZ + PTZ, and PTZ + DZP groups). ESM administration also enhanced the effect of WKE but there was no significant difference between the threshold doses of ESM alone or in combination with WKE (fig. 1).

Administration of WKE significantly increased the PTZ dose for GCS induction (p < 0.001 vs. the PTZ group and the PTZ + DMSO + saline group). Moreover, there was no significant difference between the doses of PTZ for GCS in the presence of WKE alone or combined with FMZ or ESM or DZP. In addition, the PTZ dose of GCS in the PTZ + ESM and PTZ + DZP groups in the presence or absence of WKE did not show a significant difference (fig. 2).

The severity of convulsions was significantly reduced by WKE (p < 0.05 vs. the PTZ group and the PTZ + DMSO + saline group). This effect of WKE was not affected by FMZ. Furthermore, jerky movements were not seen in the WKE + DZP group at all (table 1).

In this study, PTZ and PTZ + DMSO-induced convulsions were associated with a high mortality rate (100% of animals). However, pretreatment with WKE alone or along with FMZ, DPZ and ESM decreased the mortality rate to 0%. In addition, the mortality rate in the ESM + PTZ group and the DPZ + PTZ group was 12.5 and 25%, respectively. All groups showed significant differences whenever compared with the PTZ and PTZ + DMSO groups (p < 0.001; table 1).

![Graph showing required doses of PTZ for the induction of threshold convulsions in the different animal groups. Data are presented as the mean ± SEM (n = 8–11). * p < 0.05 versus the PTZ and DMSO + saline + PTZ groups. ** p < 0.01 versus the PTZ, DMSO + saline + PTZ and DPZ + PTZ groups. * p < 0.001 versus the PTZ, DMSO + saline + PTZ and DPZ + PTZ groups. * p < 0.01 versus the saline + walnut + PTZ group.]

![Graph showing required doses of PTZ for the induction of GCS in the different animal groups. Data are presented as the mean ± SEM (n = 8–11). * p < 0.001 versus the PTZ and DMSO + saline + PTZ groups.]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Convulsion severity</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTZ (n = 9)</td>
<td>5 (5–5)</td>
<td>9 (100%)</td>
</tr>
<tr>
<td>DMSO-saline-PTZ (n = 9)</td>
<td>5 (5–5)</td>
<td>9 (100%)</td>
</tr>
<tr>
<td>Saline + WKE + PTZ (n = 11)</td>
<td>4 (0–5)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>FMZ + WKE + PTZ (n = 8)</td>
<td>3.5 (1–5)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>ESM + WKE + PTZ (n = 11)</td>
<td>0 (0–5)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>DPZ + WKE + PTZ (n = 8)</td>
<td>0 (0–0)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>ESM + PTZ (n = 8)</td>
<td>0 (0–5)</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td>DPZ + PTZ (n = 8)</td>
<td>4 (1–5)</td>
<td>2 (25%)</td>
</tr>
</tbody>
</table>

Seizure severity is expressed as the median, with ranges in parentheses. * p < 0.05 versus the PTZ group. ** p < 0.05 versus the DMSO + saline + PTZ group. * p < 0.05 versus the DMSO + saline + PTZ and ESM + walnut + PTZ groups. * p < 0.05 versus the DMSO + saline + PTZ group. * p < 0.01 versus the DMSO + saline + PTZ, FMZ + walnut + PTZ and DPZ + PTZ groups. * p < 0.001 versus the DMSO + saline + PTZ, saline + walnut + PTZ, FMZ + walnut + PTZ and DPZ + PTZ groups. * p < 0.01 versus the DMSO + saline + PTZ, FMZ + walnut + PTZ and DPZ + PTZ groups. * p < 0.01 versus the DMSO + saline + PTZ and DPZ + walnut + PTZ groups.
Discussion

The results showed that WKE increased the required doses of PTZ for the induction of threshold convulsions and GCS. In addition, WKE significantly reduced the severity of convulsions and completely prevented the deaths caused by seizures. The combined use of WKE and DPZ showed a stronger anticonvulsant effect and increased the dose of PTZ for both threshold convulsions and GCS when compared with WKE or DPZ alone. In addition, the animal group that received a combination of WKE and DPZ did not show jerky movements and all animals survived after seizure induction. Administration of FMZ did not significantly decrease the enhancing effect of WKE on the PTZ threshold dose but did not have a prominent effect on the PTZ GCS dose for seizure induction, seizure severity and mortality rate. On the other hand, similar to DPZ, ESM with or without WKE increased the threshold convulsions and GCS and decreased the convulsion severity and mortality; however, there was no significant difference between the effect of ESM alone or along with walnuts.

The GABAergic system is the most important inhibitory system in the central nervous system, but its function may be disturbed in different conditions [12]; GABA is an inhibitory neurotransmitter that can affect GABA$_A$ and GABA$_B$ receptors [12]. GABA$_A$ are voltage-gated receptors that act with increasing chloride intracellular influx [13].

PTZ is a noncompetitive antagonist of GABA$_A$ receptors that acts through the t-butyl-bicyclo-phosphorothionate site of the receptor and decreases its activity [14]. Another possibility of PTZ action is to change the potassium and calcium channel conductance [15]. DPZ, as a benzodiazepine receptor agonist [16], can increase the conduction of chloride ion through GABA$_A$ receptors and induce the anticonvulsant effect [17]. ESM can decrease the conduction of calcium ion from T-type calcium channels and thereby show its anticonvulsant effect [18]. According to the low impact of FMZ as a benzodiazepine receptor antagonist [16] on WKE effects and the synergistic effect of DPZ and walnuts in this study, it is possible that the major anticonvulsant effects of WKE applies through receptors other than the benzodiazepine pathway. On the other hand, the pattern of interaction between the effects of ESM and WKE on the control of seizures raises the possibility that at least part of the anticonvulsant mechanisms of these agents may be similar.

Other pathways may contribute to the anticonvulsant effects of WKE. There is an increasing number of different studies regarding the role of nitric oxide (NO) in the pathophysiology of disorders such as stroke, trauma and seizure disorders [19, 20] that could help to explain the anticonvulsant effect of WKE. In epilepsy, NO is also considered an essential pathogenic factor and may have a function in the mechanisms underlying seizure induction and progression [21]. Consistent with this, impressive (five-fold) elevations in NO production were found for the duration of the seizures induced by PTZ. The levels of secondary products resulting from lipid peroxidation have also been shown to be significantly increased in the cerebral cortex of rats with PTZ-induced seizures [22]. A recent study confirmed that walnut extracts diminished the production of NO, tumor necrosis factor-$\alpha$ as well as the expression of inducible NO synthase in BV-2 microglial cells activated by lipopolysaccharide [23]. In another study, primary fatty acids were found to be able to suppress NO production in macrophages [24]. In addition, a recent study has revealed that fatty acids decrease NO production in macrophages stimulated by lipopolysaccharide through iNOS protein expression [25]. WKE and its component ellagic acid have also been shown to possess anti-inflammatory activity in human aorta endothelial cells and osteoblastic activity in KS483 cell lines [26]. In addition, it is reported that excessive production of free radicals has been implicated in the pathogenesis of some neurological disorders, including epilepsy, and it has been suggested that antioxidants as adjuncts to antiepileptic drugs may be helpful for better seizure control [27]. Walnuts contain the highest total level of antioxidants, including both free antioxidants and antioxidants bound to fiber [28]. Therefore, part of the anticonvulsant effect of WKE observed in the present study may mediate through mechanisms that modulate NO production, impede the proinflammatory process and also inhibit the redox imbalance.

Conclusion

Our findings show the anticonvulsant effect of WKE and suggest that this effect could be exerted through routes other than the involvement of the benzodiazepine action pathway. Activation of the ESM function pathway, reduction in brain NO production, activation of anti-inflammatory mechanisms and reinforcement of the antioxidant system are possible paths of action for WKE in the control of seizures. However, further studies are needed to elucidate the exact mechanisms in which WKE attenuates the PTZ-induced seizures.
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Disclosure Statement

The authors declare that they have no conflicts of interests.

References