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The effect of *Ferula assa-foetida* Hydroalcoholic Extract on Pentylenetetrazole-Induced Seizures in Male Rats

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ABSTRACT

Epilepsy is a common neurological disorder with treatment resistance remaining a challenge. *Ferula assa-foetida* (asafoetida) is traditionally used in Persian medicine for neurological conditions, but its effects on seizure parameters are rarely investigated. This study evaluated the effect of *F. assa-foetida* hydroalcoholic extract on pentylenetetrazole (PTZ)-induced seizures in male rats. 35 adults male Wistar rats were divided into five groups (n = 7): control (saline + PTZ), three groups receiving 50, 100, and 200 mg/kg extract (i.p., 30 min before PTZ), and diazepam (1 mg/kg) as positive control. Seizures were induced by PTZ (85 mg/kg, i.p.). Parameters included latency to onset, duration of tonic, clonic, and tonic-clonic phases, total seizure duration, Racine scores, and 24-hour mortality. Data were analyzed by one-way ANOVA with LSD post-hoc test. Doses of 100 and 200 mg/kg extract significantly increased seizure latency versus control ($p < 0.001$). However, contrary to classical anticonvulsant profiles, these doses also significantly increased tonic, clonic, tonic-clonic, and total seizure durations ($p < 0.05$). The 200 mg/kg dose showed comparable latency to diazepam ($p > 0.05$) but differed significantly on seizure durations ($p < 0.05$). Racine scores were not significantly reduced by the extract. The 24-hour survival rate increased dose-dependently ($p < 0.01$). *Ferula assa-foetida* extract exhibits a mixed profile in the PTZ model: it delays seizure onset and improves survival but prolongs motor seizure durations. These findings do not support a classical anticonvulsant effect, suggesting a complex pharmacology requiring further investigation.

Keywords: *Ferula assa-foetida*, Pentylenetetrazole, Seizure, Anticonvulsant, Diazepam.

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Introduction

Epilepsy is one of the most prevalent chronic neurological disorders worldwide, affecting approximately 5.6 per 1000 individuals with active epilepsy and 6.7 per 1000 with lifetime epilepsy (Safeer et al., 2024). The global incidence rate of epilepsy is estimated at 52.5 per 100,000 person-years, with particularly high prevalence observed in West Asian countries (Safeer et al., 2024). In Iran, although comprehensive national epidemiological data are limited, available studies report a prevalence of active epilepsy ranging from 1.2 to 8.3 per 1,000 population depending on the region and study methodology. This condition is characterized by recurrent, unprovoked seizures resulting from excessive and hypersynchronous neuronal discharge in the brain, leading to significant morbidity, cognitive impairment, and reduced quality of life (Jiang et al., 2023). Despite the availability of numerous antiseizure medications (ASMs), 27-30% of patients with epilepsy develop drug-resistant epilepsy (DRE), failing to achieve sustained seizure control with existing therapeutic regimens (Jiang et al., 2023; Alghamdi et al., 2025). Among patients with idiopathic generalized epilepsy, the pooled prevalence of drug resistance has been reported at 27% (95% CI: 0.19-0.36), with psychiatric comorbidities, combined seizure types, and status epilepticus identified as significant risk factors for poor prognosis (Jiang *et al.,* 2023). Furthermore, patients with DRE face increased mortality risks, including sudden unexpected death in epilepsy (SUDEP), which occurs at an estimated incidence of 0.78-1.2 per 1000 patient-years (Wartmann et al., 2024). These limitations underscore the urgent need for novel therapeutic agents with improved efficacy and safety profiles.

The Pentylenetetrazole (PTZ)-induced seizure model remains one of the most widely used and validated preclinical paradigms for screening potential anticonvulsant compounds (Brunal et al., 2021; Samokhina and Samokhin 2018). PTZ, a tetrazole derivative, exerts its epileptogenic effects primarily through non-competitive antagonism of γ-aminobutyric acid type A (GABAA) receptors, thereby reducing inhibitory neurotransmission (Lu et al., 2022). PTZ-induced seizure activity has been detected in the 19-21 Hz beta range, which is positively correlated with generalized clonic seizures (Lu et al., 2022). While lower PTZ doses (40-50 mg/kg) are typically used for threshold tests, the higher dose of 85 mg/kg employed in the present study represents a supramaximal challenge that ensures consistent generalized tonic-clonic seizures in all animals, allowing evaluation of drug effects on severe seizure phases. This approach is particularly useful for detecting potential proconvulsant or paradoxical effects that might be masked at lower doses (Rojas et al., 2014). Additionally, PTZ administration leads to rapid downregulation of connexin 36 protein levels within 30 minutes, contributing to region-specific susceptibility to neuronal hyperactivity (Safeer *et al.,* 2024). The imbalance between inhibitory and excitatory systems results in neuronal hyperexcitability, oxidative stress, neuroinflammation, and generalized tonic-clonic seizures, mimicking key features of human generalized epilepsy (Quintans Júnior et al., 2008). The PTZ model is favored for its simplicity, high reproducibility, cost-effectiveness, and well-characterized behavioral and electroencephalographic correlates, making it an indispensable tool for antiepileptic drug discovery (Samokhina and Samokhin 2018).

In recent years, there has been a resurgence of interest in medicinal plants as alternative or adjunctive therapies for epilepsy, driven by their historical use in traditional medicine systems and potentially lower side effect profiles (Quintans Júnior et al., 2008). Plants of the genus *Ferula* have long been used in traditional medicine to treat various neurological disorders, including seizures, pain, depression, and Alzheimer's disease (Bagheri and Esmailidehaj 2024). *Ferula assa-foetida* L. (family Apiaceae), commonly known as asafoetida or "hing," is a perennial herb native to Central Asia and Iran (Bagheri and Esmailidehaj 2024). The main bioactive compounds of *Ferula* species include coumarins, monoterpenes, sulfide compounds, and polyphenols, which can improve nervous system function through multiple mechanisms (Bagheri and Esmailidehaj 2024). Studies have

demonstrated that *Ferula* plants exert protective effects on neuronal cells by reducing pro-inflammatory cytokines such as IL-6, IL-1β, and TNF-α, while also strengthening the antioxidant system and reducing oxidative stress levels in the nervous system (Bagheri and Esmailidehaj 2024; Askari et al., 2020).

Despite the growing body of evidence on the neuropharmacological effects of *Ferula* species, the specific research gap lies in the limited and inconsistent data regarding the hydroalcoholic extract of *Ferula assa-foetida* in acute seizure models. Previous studies have primarily focused on the essential oil, gum, or methanolic extracts of this plant, often using different doses and administration routes. For instance, Kiasalari et al., (2013) investigated the anticonvulsant effect of *Ferula assa-foetida* gum extract (50, 100, and 200 mg/kg, i.p.) in a PTZ-induced kindling model in mice and reported reduced seizure severity (Kiasalari et al., 2013). Bagheri and Esmailidehaj (2024) reviewed the neuroprotective effects of various *Ferula* species, highlighting the use of different extract forms (e.g., hydroalcoholic, methanolic, essential oil) across a wide dose range (25-400 mg/kg) in different neurological conditions (Bagheri and Esmailidehaj 2024). However, most of these studies evaluated seizure latency and mortality without detailed phase-specific seizure duration analysis. Moreover, to the best of our knowledge, no previous study has systematically evaluated the hydroalcoholic extract of *Ferula assa-foetida* aerial parts in the acute PTZ-induced seizure model using a supramaximal PTZ dose (85 mg/kg) with comprehensive assessment of tonic, clonic, tonic-clonic, and total seizure durations alongside Racine scoring. Therefore, to address this research gap, the present study aimed to evaluate the effect of hydroalcoholic extract of *Ferula assa-foetida* (at doses of 50, 100, and 200 mg/kg, i.p.) on PTZ-induced seizures in male Wistar rats, assessing parameters including seizure latency, duration of tonic, clonic, and tonic-clonic phases, total seizure duration, Racine seizure scores, and 24-hour survival rate.

Materials and Methods

Animals and Housing Conditions

In this experimental study, 35 adults male Wistar rats weighing 250–300 g were used. The animals were purchased from the Animal Breeding and Holding Center of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. All rats were housed under standard laboratory conditions at a controlled temperature (22 ± 2°C) and a 12-hour light/dark cycle (lights on from 8:00 AM to 8:00 PM). The animals had free access to standard laboratory rodent chow (containing 22% protein, 4% fat, 55% carbohydrate, and 5% fiber; Behparvar Co., Iran) and tap water ad libitum. Upon arrival, the rats were acclimatized to the animal facility for 7 days before the start of the experiment. During this period, they were housed in polycarbonate cages (four rats per cage) with soft wood chip bedding, which was changed every other day. All experimental procedures were approved by the Ethics Committee of the Islamic Azad University, Izeh Branch (Ethics code: IR.IAU.ID.REC.1403.012) and followed the guidelines for the care and use of laboratory animals (National Research Council, 2011). This reference has been added to the reference list.

Preparation of Hydroalcoholic Extract of *Ferula assa-foetida.*

Ferula assa-foetida L. plants were obtained from a reputable herbal store in Izeh, Iran. The plant material was authenticated by a botanist (Dr. A. Mohammadi, Department of Botany, Islamic Azad University, Izeh Branch), and a voucher specimen (specimen No. IAU-1403-012) was deposited at the university herbarium. The aerial parts of the plant were dried at room temperature (25°C) for 10 days and then powdered using an electric grinder. The powdered material (100 g) was macerated with 800 mL of 80% ethanol (Merck, Germany) for 72 hours at room temperature on an orbital shaker at 120 rpm (Bagheri and Esmailidehaj 2024). The mixture was then filtered through Whatman No. 1 filter paper, and the solvent was evaporated using a rotary evaporator (Heidolph, Germany) at 40°C under reduced pressure. The resulting extract was completely dried in an oven at 40°C for 24 hours and stored at -20°C in airtight dark containers until use (Kiasalari et al., 2013; Jalili et al., 2022). The

extraction yield was calculated as (weight of dried extract / weight of dried plant powder) × 100.

Experimental Groups and Drug Administration

Thirty-five rats were randomly divided into five groups of seven animals each (n = 7 per group):

- **Control group:** received normal saline (1 mL/kg, i.p.) + Pentylenetetrazole (PTZ)
- **Three treatment groups:** received 50, 100, and 200 mg/kg of *F. assa-foetida* hydroalcoholic extract (i.p.) 30 minutes before PTZ injection.
- **Positive control group:** received diazepam (1 mg/kg, i.p.) + PTZ.

All injections were performed intraperitoneally using a 1 mL syringe with a 26-gauge needle. The doses of the extract were selected based on previous studies (Mandegary et al., 2012). The extract was freshly dissolved in normal saline on the day of each experiment.

Induction of Seizures

Seizures were induced by a single intraperitoneal injection of Pentylenetetrazole (PTZ, Sigma-Aldrich, USA) at a dose of 85 mg/kg (Lu et al., 2022). This dose was selected as a supramaximal challenge to ensure consistent generalized tonic-clonic seizures in all control animals, allowing evaluation of extract effects on severe seizure phases. Immediately after PTZ injection, each animal was placed individually in a Plexiglas cage (30 × 30 × 40 cm) and observed for 30 minutes for the occurrence of seizure activity (Brunal et al., 2021; Samokhina and Samokhin 2018). Behavioral observations were recorded by two independent observers who were blinded to the treatment groups.

Seizure Parameters Recording

The following seizure parameters were recorded (Rojas et al., 2014):

1. **Latency to seizure onset:** time interval (seconds) between PTZ injection and the first clonic jerk
2. **Duration of tonic phase:** time (seconds) of tonic hindlimb extension
3. **Duration of clonic phase:** time (seconds) of clonic seizures
4. **Duration of tonic-clonic phase:** time (seconds) of tonic-clonic seizures
5. **Total seizure duration:** total time (seconds) from seizure onset to recovery (return to normal locomotor activity)
6. **24-hour survival rate:** percentage of animals surviving 24 hours after PTZ injection

Seizure intensity was scored according to the Racine scale at 5, 10, 15, 20, and 30 minutes after PTZ injection as follows (Rojas *et al.,* 2014):

- **Stage 0:** no seizure activity
- **Stage 1:** mouth and facial jerks
- **Stage 2:** head nodding
- **Stage 3:** forelimb clonus
- **Stage 4:** rearing with forelimb clonus
- **Stage 5:** rearing and falling with generalized tonic-clonic seizures.

The maximum observed score for each animal during the 30-minute observation period was recorded for analysis. All behavioral assessments were performed by an observer blinded to the experimental groups.

Statistical Analysis

Data was analyzed using SPSS software version 26 (IBM, USA). Normality of data distribution was confirmed using the Kolmogorov-Smirnov test and further verified with the Shapiro-Wilk test (recommended for sample sizes $n < 50$). Homogeneity of variances was assessed using Levene's test. For all dependent variables, homogeneity of variances was confirmed ($p > 0.05$). One-way analysis of variance (ANOVA) was used for comparisons between groups, followed by the LSD (Least Significant Difference) post-hoc test for pairwise comparisons when ANOVA was significant. The LSD post-hoc test was chosen due to its appropriate sensitivity for a limited number of pairwise comparisons (five groups) and because homogeneity of variances was confirmed. Effect sizes were calculated as eta squared (η^2), with values of 0.01, 0.06, and 0.14 considered small, medium, and large effects, respectively.

All data are expressed as mean ± standard error of the mean (SEM). Exact p-values are reported where appropriate (e.g., $p = 0.003$ rather than only $p < 0.01$). Differences were considered statistically significant at $p < 0.05$.

Results

Effect of *Ferula assa-foetida* extract on seizure latency.

As shown in Figure 1, administration of 100 and 200 mg/kg of *F. assa-foetida* hydroalcoholic extract significantly increased the latency to seizure onset compared to the control group ($p < 0.001$, $\eta^2 = 0.42$). The 50 mg/kg dose did not show a significant difference in seizure latency compared to the control group ($p > 0.05$). Although the 100 and 200 mg/kg doses increased seizure latency compared to saline ($p < 0.001$), they showed significantly lower latency compared to the diazepam-treated group ($p < 0.001$). These data indicate that the extract delays seizure onset in a dose-dependent manner.

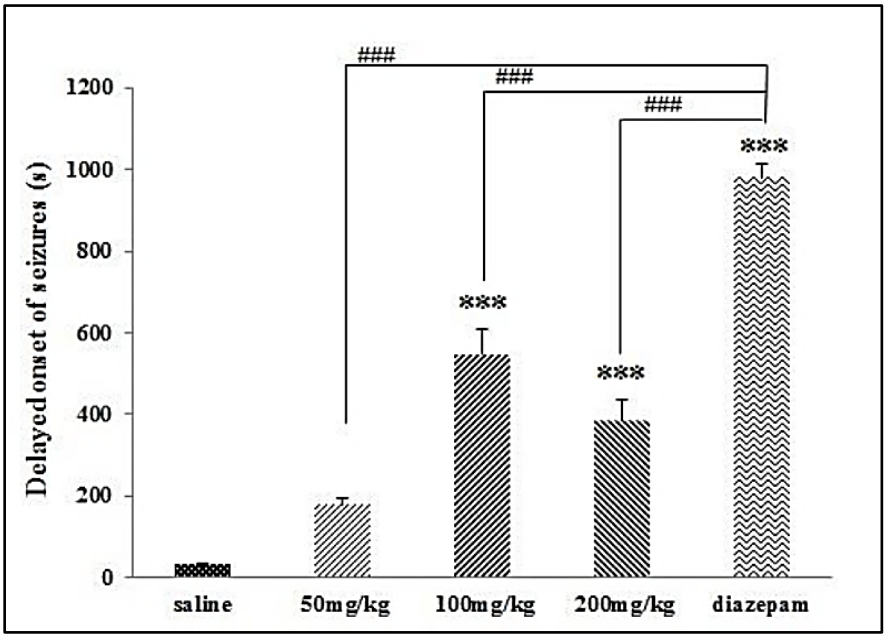

Figure 1. Comparison of seizure latency (seconds) in the control (saline) group, groups treated with different doses of Ferula assa-foetida hydroalcoholic extract (50, 100, and 200 mg/kg, i.p.), and the diazepam-treated group (1 mg/kg, i.p.) as positive control ($n = 7$). The extract at 100 and 200 mg/kg significantly increased seizure latency compared to control. Data are presented as mean ± SEM. One-way ANOVA followed by LSD post-hoc test. ***$p < 0.001$ when compared to the control group; $^{###}p < 0.001$ when compared to the diazepam group. Effect size (η^2) for treatment on latency: 0.42 (large effect).

Effect of *Ferula assa-foetida* extract on tonic phase duration.

Figure 2 demonstrates that all treatment groups receiving different doses of *F. assa-foetida* extract, as well as the diazepam-treated group, showed a significant increase in tonic phase duration compared to the control group ($p < 0.01$ and $p < 0.001$; $\eta^2 = 0.38$). Furthermore, the groups treated with 50 and 200 mg/kg exhibited a significant increase in tonic phase duration compared to the diazepam-treated group ($p < 0.001$ and $p < 0.05$, respectively). Thus, contrary to a classical anticonvulsant effect, the extract prolonged the tonic phase of PTZ-induced seizures.

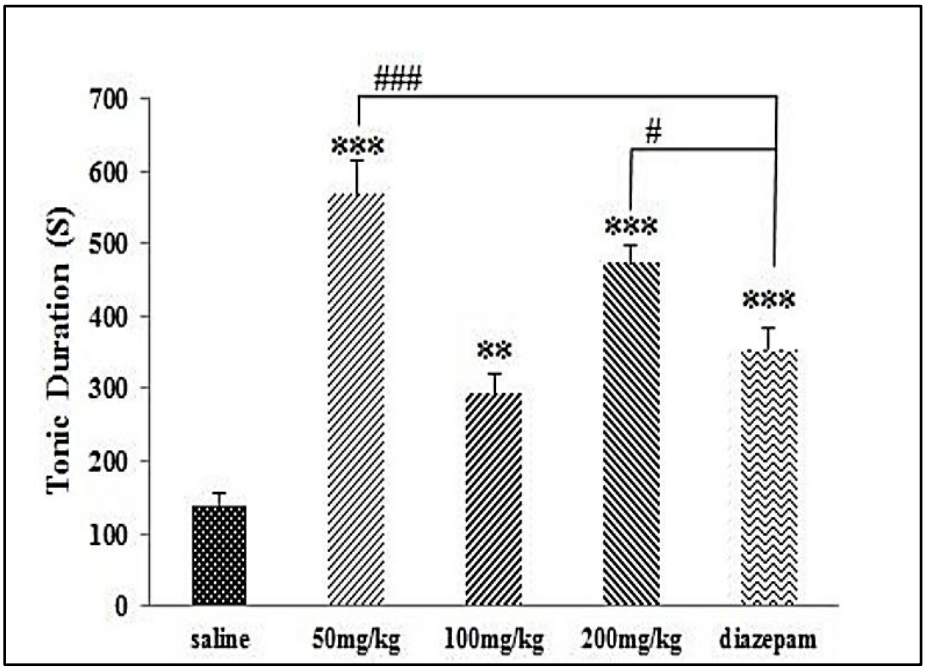

Figure 2. Comparison of tonic phase duration (seconds) in the control (saline) group, groups treated with different doses of *Ferula assa-foetida* hydroalcoholic extract (50, 100, and 200 mg/kg, i.p.), and the diazepam-treated group (1 mg/kg, i.p.) (n = 7 per group). All doses of the extract significantly increased tonic phase duration compared to the control group. Data are presented as mean ± SEM. One-way ANOVA followed by LSD post-hoc test. $^{**}p < 0.01$, $^{***}p < 0.001$ when compared to the control group; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ when compared to the diazepam group. Effect size (η^2) = 0.38 (large effect). Note: Increased tonic duration indicates that the extract did not produce a classical anticonvulsant effect on this parameter.

Effect of *Ferula assa-foetida* extract on clonic phase duration.

Treatment with 50 mg/kg of *F. assa-foetida* extract significantly increased clonic phase duration compared to the control group ($p < 0.001$; $\eta^2 = 0.31$). Additionally, the groups treated with 50 and 100 mg/kg of the extract showed a significant increase in clonic phase duration compared to the diazepam-treated group ($p < 0.001$ and $p < 0.05$, respectively). The 200 mg/kg dose did not significantly differ from the control group ($p > 0.05$). These findings indicate that lower doses of the extract paradoxically prolonged clonic seizure activity.

Effect of *Ferula assa-foetida* extract on tonic-clonic phase duration.

While the diazepam-treated group showed a significant decrease in tonic-clonic phase duration compared to the control group ($p < 0.01$; $\eta^2 = 0.44$), all groups treated with different doses of *F. assa-foetida* extract exhibited a significant increase in tonic-clonic phase duration compared to the diazepam-treated group ($p < 0.05$ and $p < 0.01$). Compared to the control group, the extract-treated groups showed no significant reduction in tonic-clonic duration. This further supports that the extract does not produce a classical anticonvulsant effect.

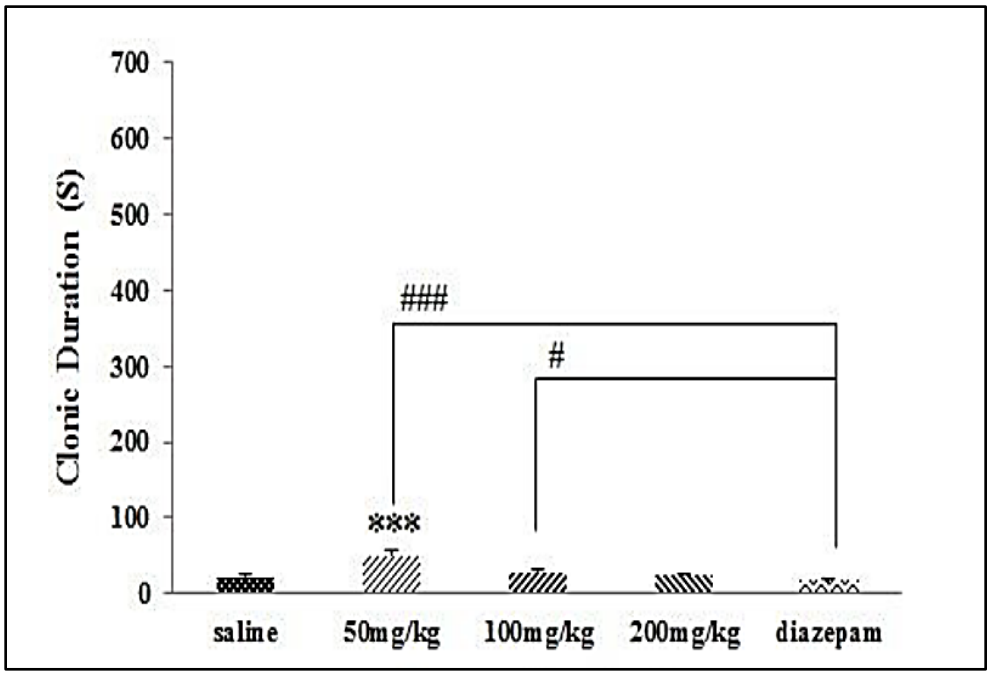

Figure 3. Comparison of clonic phase duration (seconds) in the control (saline) group, groups treated with different doses of *Ferula assa-foetida* hydroalcoholic extract (50, 100, and 200 mg/kg, i.p.), and the diazepam-treated group (1 mg/kg, i.p.) (n = 7 per group). The 50 mg/kg dose of the extract significantly increased clonic phase duration compared to the control group. Data are presented as mean ± SEM. One-way ANOVA followed by LSD post-hoc test. ***$p < 0.001$ when compared to the control group; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ when compared to the diazepam group. Effect size (η^2) = 0.31 (large effect).

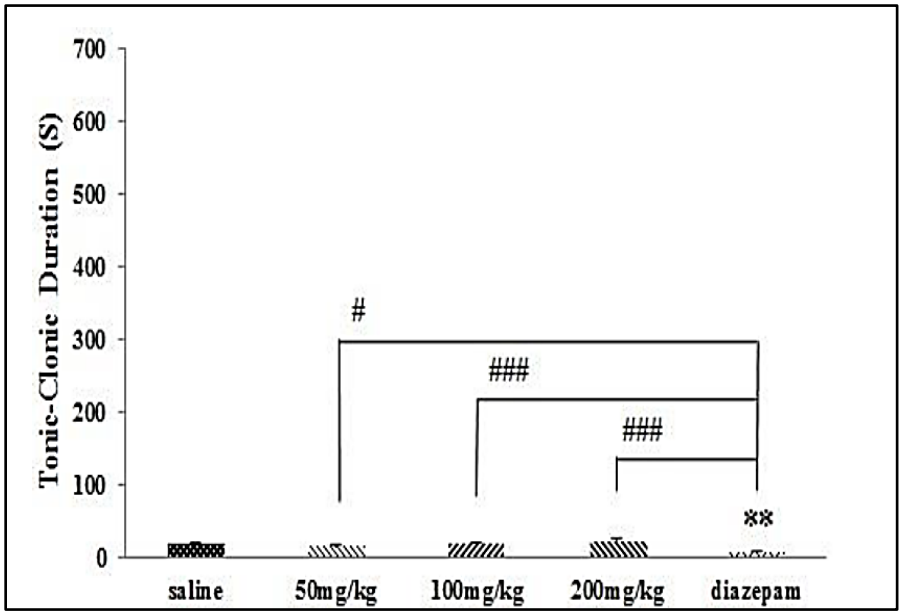

Figure 4. Comparison of tonic-clonic phase duration (seconds) in the control (saline) group, groups treated with different doses of *Ferula assa-foetida* hydroalcoholic extract (50, 100, and 200 mg/kg, i.p.), and the diazepam-treated group (1 mg/kg, i.p.) (n = 7 per group). Diazepam significantly reduced tonic-clonic duration compared to control, whereas all doses of the extract increased tonic-clonic duration compared to diazepam, with no significant reduction compared to control. Data are presented as mean ± SEM. One-way ANOVA followed

by LSD post-hoc test. **p < 0.01 when compared to the control group; #p < 0.05, ###p < 0.001 when compared to the diazepam group. Effect size (η^2) = 0.44 (large effect).

Effect of *Ferula assa-foetida* extract on total seizure duration.

As illustrated in Figure 5, the groups treated with 100 and 200 mg/kg doses of *F. assa-foetida* extract, as well as the diazepam-treated group, showed a significant increase in total seizure duration compared to the control group ($p < 0.01$; $\eta^2 = 0.36$). This indicates that the extract, rather than abbreviating seizure activity, prolonged the overall seizure episode.

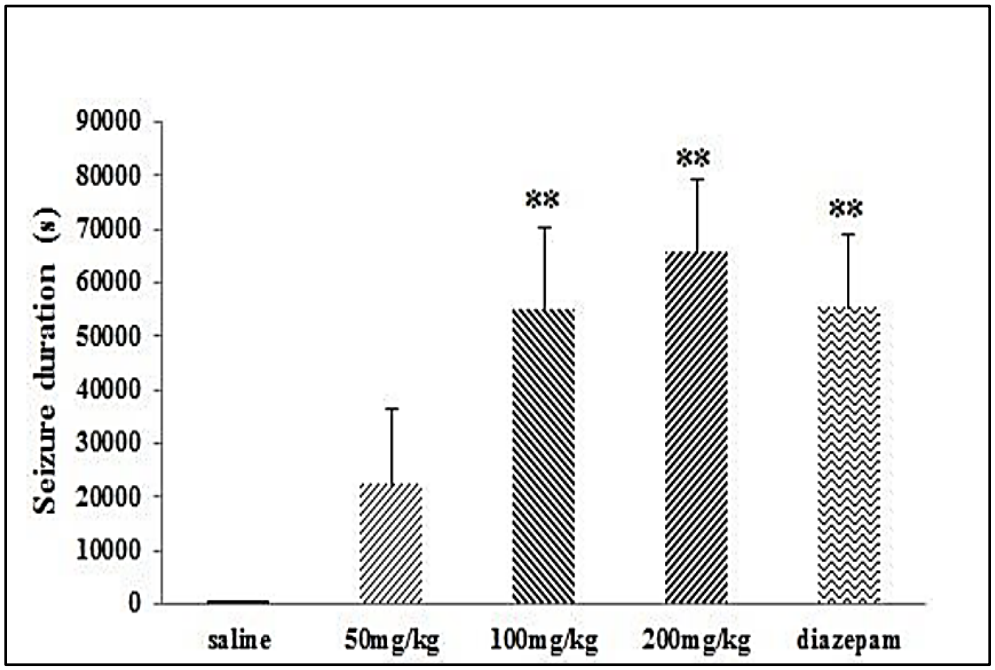

Figure 5. Comparison of total seizure duration (seconds) in the control (saline) group, groups treated with different doses of *Ferula assa-foetida* hydroalcoholic extract (50, 100, and 200 mg/kg, i.p.), and the diazepam-treated group (1 mg/kg, i.p.) (n = 7 per group). The extract at 100 and 200 mg/kg significantly increased total seizure duration compared to the control group. Data are presented as mean ± SEM. One-way ANOVA followed by LSD post-hoc test. **p < 0.01 when compared to the control group. Effect size (η^2) = 0.36 (large effect). Note: Prolongation of total seizure duration is opposite to the effect expected from a classical anticonvulsant.

Effect of *Ferula assa-foetida* extract on 24-hour survival rate.

Figure 6 shows that the 24-hour survival rate in groups treated with 100 and 200 mg/kg doses of *F. assa-foetida* extract, as well as the diazepam-treated group, significantly increased compared to the control group in a dose-dependent manner ($p < 0.01$; $\eta^2 = 0.29$). This survival benefit occurred despite the prolongation of seizure durations, suggesting that the extract may protect against seizure-induced mortality through mechanisms independent of seizure duration.

Effect of *Ferula assa-foetida* extract on seizure intensity (Racine scale)

Racine scores were recorded at 5, 10, 15, 20, and 30 minutes post-PTZ injection (Figure 7). The control group reached a mean maximum Racine score of 4.8 ± 0.2. The extract at 50, 100, and 200 mg/kg produced mean maximum Racine scores of 4.6 ± 0.3, 4.4 ± 0.3, and 4.1 ± 0.4, respectively. None of these differences reached statistical significance compared to control ($p > 0.05$ for all comparisons; $\eta^2 = 0.11$, small effect). The diazepam group showed a significantly lower Racine score (2.1 ±

0.3, $p < 0.001$ vs. control; $\eta^2 = 0.51$, large effect). Thus, the extract did not significantly reduce seizure intensity as measured by the Racine scale.

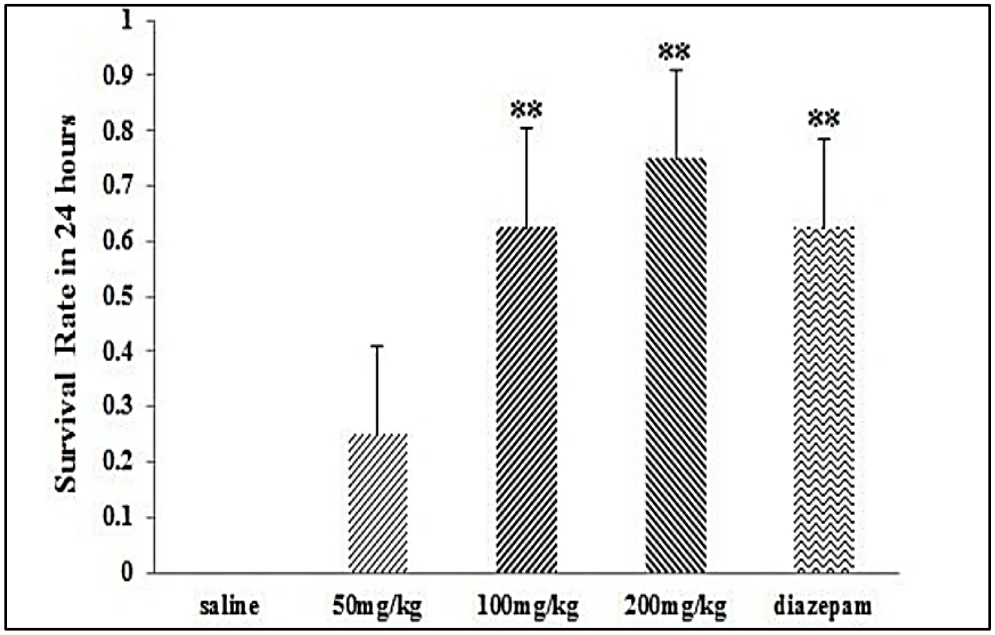

Figure 6. Comparison of 24-hour survival rate (%) in the control (saline) group, groups treated with different doses of *Ferula assa-foetida* hydroalcoholic extract (50, 100, and 200 mg/kg, i.p.), and the diazepam-treated group (1 mg/kg, i.p.) (n = 7 per group). The extract at 100 and 200 mg/kg significantly increased survival rate in a dose-dependent manner. Data are presented as mean ± SEM. One-way ANOVA followed by LSD post-hoc test. **$p < 0.01$ when compared to the control group. Effect size (η^2) = 0.29 (large effect).

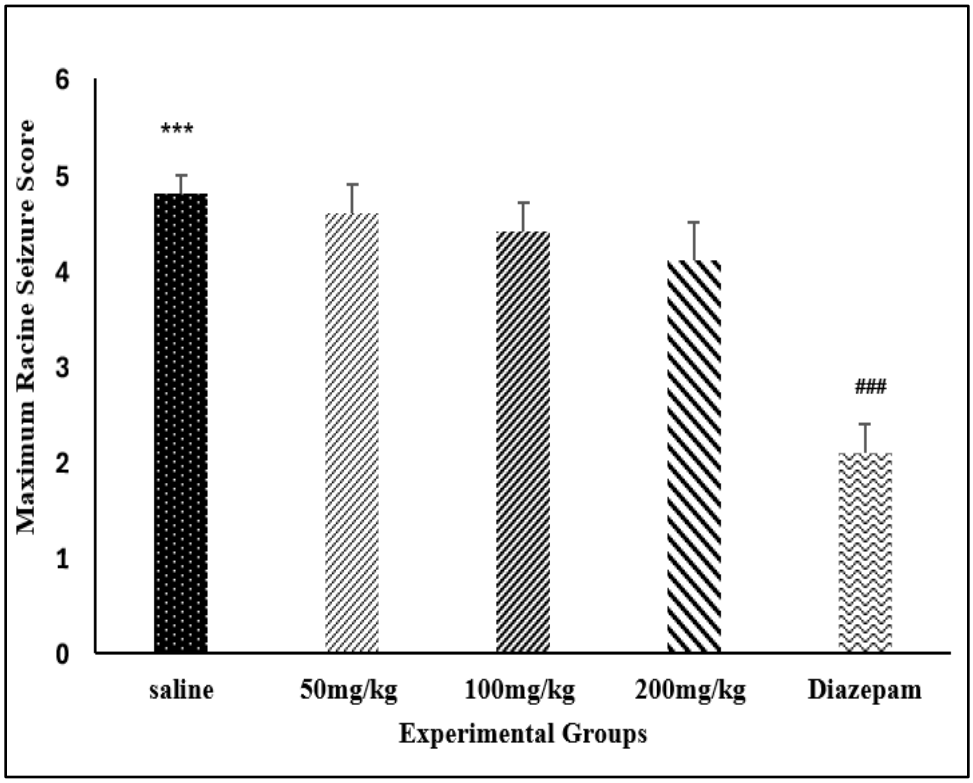

Figure 7. Comparison of maximum Racine seizure scores in the control (saline) group, groups treated with different doses of *Ferula assa-foetida* hydroalcoholic extract (50, 100, and 200 mg/kg, i.p.), and the diazepam-treated group (1 mg/kg, i.p.) (n = 7 per group). Scores were recorded at 5, 10, 15, 20, and 30 minutes after PTZ injection, and the maximum score for each animal is presented. The extract did not significantly reduce Racine scores at any dose. Only diazepam produced a significant reduction. Data are presented as mean ± SEM. One-way ANOVA followed by LSD post-hoc test. ***$p < 0.001$ when compared to the control group; ###$p < 0.001$ when compared to the diazepam group. Effect size (η^2) = 0.51 (large effect for diazepam, non-significant for extract).

Phytochemical characterization of the extract (limitation statement)

As acknowledged in the Discussion section, no phytochemical analysis (e.g., total phenolic content, total flavonoid content, HPLC, or GC-MS) was performed on the specific batch of extract used in this study. This limitation is fully described in the Discussion.

Discussion

The findings of this study demonstrated that intraperitoneal administration of PTZ (85 mg/kg) successfully induced generalized seizures in adult male Wistar rats, which is consistent with previous reports (Esmaeili et al., 2017; Güneş et al., 2014). The present study evaluated the effects of different doses (50, 100, and 200 mg/kg) of *Ferula assa-foetida* hydroalcoholic extract on PTZ-induced seizures and revealed a complex and mixed pharmacological profile (Alimohammadi et al., 2014). Specifically, the extract significantly increased the latency to seizure onset and improved 24-hour survival in a dose-dependent manner (Figure 1 and Figure 6). However, contrary to classical anticonvulsant agents such as diazepam, the extract significantly increased the duration of tonic, clonic, and tonic-clonic phases, as well as total seizure duration (Figures 2-5). Racine seizure scores were not significantly reduced (Figure 7). This pattern – delayed onset but prolonged motor seizures – is unusual and warrants careful interpretation. A true anticonvulsant should shorten seizure duration; therefore, the present extract does not meet the criteria for a classical anticonvulsant agent (Stafstrom and Carmant, 2015).

The effects observed in this study are partially consistent with and partially contradictory to previous reports on *Ferula* species. Several studies have reported anticonvulsant or neuroprotective effects for different *Ferula* species. For example, Bagheri and Esmailidehaj (2024) reviewed the neuroprotective effects of the *Ferula* genus and reported anticonvulsant activity for some species, including *Ferula gummosa* and *Ferula sinkiangensis*, in various seizure models (Bagheri and Esmailidehaj, 2024). However, those studies primarily evaluated seizure latency and mortality, not detailed phase-specific seizure durations (Bagheri and Esmailidehaj, 2024). In agreement with our finding of increased seizure latency, previous studies have also reported that *Ferula* species can delay seizure onset, likely through GABAergic modulation (Ghanbari et al., 2021; Quintans Júnior et al., 2008).

In contrast, our finding of prolonged seizure durations contradicts some previous reports. Kiasalari et al., (2013) reported that *Ferula assa-foetida* gum extract reduced seizure severity in a PTZ-induced kindling model in mice, which appears inconsistent with our finding of prolonged seizure durations (Kiasalari et al., 2013). This discrepancy may be explained by differences in the extraction method (gum vs. aerial parts), animal species (mouse vs. rat), seizure model (kindling vs. acute), or the PTZ dose used (lower doses in kindling vs. supramaximal 85 mg/kg in the present study) (Rojas et al., 2014). Similarly, some studies on other medicinal plants have reported paradoxical proconvulsant effects at certain doses, which has been attributed to the presence of multiple bioactive compounds with opposing actions (Quintans Júnior et al., 2008). To our knowledge, no previous study has reported a paradoxical prolongation of motor seizure phases by *Ferula assa-foetida* extract, making our finding novel but also highlighting the need for replication.

The present study was not designed to elucidate the molecular mechanisms underlying the observed effects. However, based on existing literature on *Ferula* species,

several hypotheses can be proposed for future investigation. Phytochemical analyses have identified numerous bioactive constituents in *Ferula* species, including sesquiterpene coumarins (farnesiferol A, ferocolicin), flavonoids (luteolin, quercetin, kaempferol), volatile sulfur compounds, and phenolic acids (Kartal et al., 2006). Flavonoids have been shown to exert benzodiazepine-like effects by binding to GABAA receptors, enhancing chloride channel opening, and promoting neuronal hyperpolarization (Ghanbari et al., 2021). Studies have demonstrated that flavonoids can increase the seizure threshold and reduce seizure severity through GABAergic mechanisms (Okoye, Akah, and Omeke, 2010). The structural similarity between certain flavonoids and benzodiazepines supports their potential as natural anticonvulsant agents (Quintans Júnior et al., 2008). These mechanisms could potentially explain the observed delay in seizure onset in our study.

The present study revealed that the hydroalcoholic extract of *Ferula assa-foetida* exerts a mixed pharmacological profile in the PTZ-induced seizure model. Although the extract significantly delayed seizure onset and improved survival, it prolonged motor seizure durations and did not reduce Racine scores, indicating that it does not possess a classical anticonvulsant effect comparable to diazepam. The paradoxical prolongation of seizure phases might be attributed to sulfur-containing compounds or biphasic effects of certain phytochemicals at higher doses, though these remain speculative hypotheses requiring experimental verification (Bagheri and Esmailidehaj, 2024; Quintans Júnior et al., 2008).

The study probably suggests that lower PTZ doses (40-50 mg/kg) might be required to detect potential anticonvulsant effects masked by the supramaximal dose (85 mg/kg) used here (Rojas et al., 2014; Samokhina and Samokhin, 2018). Overall, it is imperative to conduct additional research, specifically examining phytochemical characterization of the extract, adverse effects (motor coordination, sedation, behavioral changes), sex differences, plasma/brain concentrations of bioactive compounds, and antagonist studies (e.g., flumazenil for GABAA receptors) (Bagheri and Esmailidehaj, 2024; Kartal et al., 2006; Stafstrom and Carmant, 2015; Ghasemi and Zahediasl, 2012; Nassiri-Asl, Shariati-Rad, and Zamansoltani, 2008; Ghanbari et al., 2021). Understanding the role of these factors can provide valuable insights into the mechanisms underlying the paradoxical profile of *Ferula assa-foetida* extract and facilitate the development of potential therapeutic interventions.

Conclusion

The results of this study demonstrate that the hydroalcoholic extract of *Ferula assa-foetida* has a mixed effect in the PTZ-induced seizure model in male Wistar rats. While it significantly increased seizure latency and reduced seizure-induced mortality in a dose-dependent manner, it prolonged the duration of tonic, clonic, tonic-clonic, and total seizure phases without significantly reducing Racine scores. Therefore, the extract does not exhibit a classical anticonvulsant profile comparable to diazepam. Instead, its effects suggest a complex pharmacological action that may involve simultaneous modulation of multiple targets. These findings do not support the use of *F. assa-foetida* as a standalone anticonvulsant based on this study alone. Further studies – including EEG monitoring, receptor binding assays, toxicity assessments, fractionation to identify individual active compounds, and testing across a range of PTZ doses – are essential to clarify the mechanisms underlying this paradoxical profile and to assess whether any therapeutic potential exists.

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Conflicts of interest

There are no conflicts of interest.

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