RESEARCH ARTICLE

Unveiling the Multi-Targeted Mode of Action: Investigating the Antiepileptic Activity of *Murraya koengii* Extract in PTZ-induced Seizures

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ABSTRACT

Recurrent seizures are the hallmark of the neurological condition known as epilepsy. *Murraya koenigii* (curry leaves) has been traditionally used in medicine for various ailments. This study aimed at the anticonvulsant activity of HAEMK using a petylenetetrazole (PTZ) induced seizure model. Behavioral and biochemical parameters were assessed to determine the effectiveness of the extract in mitigating seizures. Albino mice were orally administered with different doses of the extract prior to PTZ administration. The behavioral parameters, including seizure latency, duration and severity, were monitored and recorded. Additionally, acetylcholine esterase, GSH, and malondialdehyde (MDA), three indicators of oxidative stress, were assessed in brain tissue homogenates. HAEMK-treated groups demonstrated a substantial increase in seizure latency and a decrease in seizure length and intensity compared to the control group. A dose-dependent MDA and acetylcholine esterase decreased concurrent GSH rise were seen in the HA-treated groups. These findings suggest that the hydroalcoholic extract of *M. koenigii* possesses antiepileptic activity, possibly mediated through its antioxidative mechanisms.

Keywords: *Murraya koenigii*, Antiepileptic activity, Behavioral parameters, Biochemical parameters, Pentylenetetrazole.

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INTRODUCTION

Millions of people worldwide suffer from epilepsy, a chronic neurological illness marked by recurring and unprovoked seizures.[1] Antiepileptic medications are available however, a sizable portion of patients have ineffective seizure control or unpleasant side effects.[2] Consequently, there is a continuous search for alternative therapies with improved efficacy and tolerability.

Alternative medicine has long been a source of potential antiepileptic agents, with numerous plants exhibiting promising pharmacological activities.[3] One such plant used frequently in ITSM (Indian traditional system of medicine for its many medicinal benefits is *Murraya koenigii*, sometimes known as curry leaves[4]. It has been suggested that it contains anti-inflammatory, neuroprotective, and antioxidant properties, which makes it a viable treatment option for epilepsy.[5,6]

Convulsant petylenetetrazole (PTZ) is widely used to cause seizures in experimental animals in order to evaluate the antiepileptic activity of various substances. PTZ-induced seizure models have been widely used due to their ability to mimic human epileptic seizures, making them valuable tools for the preclinical screening of antiepileptic agents.

This study used a PTZ-induced seizure paradigm to look into the antiepileptic properties of *M. koenigii* hydroalcoholic extract. The assessment of both behavioral and biochemical parameters was conducted to comprehensively evaluate the extract's efficacy in mitigating seizures and its underlying mechanisms of action. Gaining knowledge about possible antiepileptic qualities of *M. koenigii* may help develop new treatment approaches.

In this regard, the objective was to investigate HAEMK's antiepileptic potential utilizing behavioral and biochemical assessments in a paradigm where PTZ was used to produce seizures.

MATERIALS AND METHODS

Plant Material

M. koenigii (MK) leaves gathered from a reliable source were verified by a botanist. The leaves were cleaned, dried in the shade, and mechanically milled into a fine powder.

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Preparation of Hydroalcoholic Extract

By using maceration, the hydroalcoholic extract was created. An appropriate solvent, such as a solution of ethanol and water (1:1), was macerated with the powdered MK leaves for a predetermined amount of time (for example, 72 hours). A filter paper was used to filter the extract, and a rotary evaporator was used to concentrate the filtrate under low pressure. In order to be used later, the resulting hydroalcoholic extract (HAE-MK) was kept in airtight containers at an appropriate temperature.

Phytochemical Investigation

Phytochemical analysis of HA extract of MK was performed to determine the chemical constituents present in the extract.[7,8]

Chemicals and Reagents

Sigma Aldrich provided the AChE enzyme kit, pentylenetetrazole, and DTNB (Ellman's reagent). Centaur Pharmaceuticals Ltd. Mumbai made available Diazepam hydrochloride as a gift sample. Ethanol (Changshu Hangsheng Chemicals), tris hydrochloric acid, trichloroacetic acid, phosphate buffer (pH 7.5), sodium bicarbonate and other general chemical reagents were procured from Loba Chemie, Mumbai.

Experimental Animals

The Swiss Albino mice used in this study were procured from Shree Farms in Bhandara, India. All the experimental animals were housed at the animal house facility of the institute at a constant temperature of 23 ± 2°C. Swiss albino mice (20–25 g), were acclimatized for 12-hour dark and 12-hour light cycles. Every study began with animals that had never been exposed to medicine or experimentation. IAEC had approved the experimental protocol. The animals were fed a standard laboratory feed and unrestricted access to water was provided.

Toxicity study in mice

The OECD guidelines (No 423) were followed in the oral acute toxicity research conducted on mice to assess the toxicity or unfavorable effects of MK extracts.[9]

Induction of Seizures

PTZ was used to induce seizures in the mice.

Treatment Schedule

With six animals in each group, the animals were divided into six groups at random. Everyday group I (NCG) received double-distilled water intravenously (10 mL/kg). Double distilled water pretreatment and benzodiazepine (18 mg/kg, p.o.) were given to group II (Diazepam, PCG-Positive control group) each day. PTZ (90 mg/kg, p.o.) was administered daily to group III (PTZ group, NgCG-Negative Control Group) coupled with double-distilled water pretreatment. In groups IV (HA 125), V (HA 250), and VI (HA 500), the extract was administered 60 minutes prior to PTZ. After 24 hours from the last PTZ treatment, cognition was evaluated. Animals were quickly killed by decapitation at the conclusion of the cognitive exam, and the brains were taken out for biochemical analysis.

Antiepileptic Activity

The antiepileptic activity was followed with little modification during the present study. Swiss Albino mice were used as experimental animals for the present study.[10]

Principle

PTZ induces convulsions by interfering with glycine-mediated postsynaptic inhibition. Glycine is an inhibitory transmitter that is essential for spinal cord interneurons and motor neurons. In higher CNS areas, glycine also serves as a modulator of PTZ-sensitive postsynaptic inhibition. Several compounds have been shown to possess antiepileptic properties. PTZ is believed to produce convulsive effects identical to those of petitmal convulsions in humans.

Requirements

Diazepam hydrochloride, PTZ, hydroalcoholic extracts, double distilled water.

Procedure

We employed groups of six mice, each weighing between 20 and 25 g, of either sex. Every day for ten days, the test mice got their test dose; however, on the tenth day, 90 mg/kg p.o. of PTZ was administered one hour after the planned dose of MK extracts. The diazepam 18 mg/kg p. o. dose was administered to Group II mice 15 minutes before administering PTZ.

Step 1: Administration of DDW to Group I experimental animals
Step 2: Administration of Diazepam pretreatment to Group II experimental animals.
Step 3: Administration of PTZ to Group III experimental animals.
Step 4: Administration of HA 125 mg/kg to Group IV experimental animals.
Step 5: Administration of HA 250 mg/kg to Group V experimental animals.
Step 6: Administration of HA 500 mg/kg to Group VI experimental animals.

Behavioral Assessments

Seizure latency

The time that passed between the first seizures occurring and PTZ being administered was noted.

Seizure duration

The duration of each seizure episode was noted.

Percent protection

The percent of animal protected from convulsions was calculated for evaluating antiepileptic activity.

Biochemical Parameters

Oxidative stress

Malondialdehyde (MDA) was measured using thiobarbituric acid reactive substances (TBARSs), an indicator of lipid peroxidation.[11,12]

Principle

The thiobarbituric acid (TBA) technique of malondialdehyde (MDA) quantification works on the basis of an acidic interaction between MDA and TBA that forms a colored complex. This technique is frequently employed to evaluate the degree of oxidative stress and lipid peroxidation.

Procedure

Tris HCl (0.5 mL) and post-mitochondrial supernatant (0.5 mL) were combined for two hours at 37°C. The mixture was centrifuged for 10 minutes at 1000 rpm after adding 1-mL of 10% trichloroacetic acid. One ml of 0.67% thiobarbituric acid was added to 1-mL of supernatant, and the tubes were then immersed in boiling water for 10 minutes. After adding 1-mL of double-distilled water and allowing it to cool, the absorbance was measured at 532 nm. Using an extinction coefficient of 1.56 × 105 M^{-1} cm-^{1}, the reactive chemicals for thiobarbituric acid were measured. The biuret method was used to determine tissue protein.

Step 6: The following equation was used to calculate the MDA content:

$$\text{Content MDA (nMol per mg)} = \left[\frac{Sample\ absorbance - Blank\ Absorbance}{MDA\ absorbance\ coefficient}\right] \times \frac{Sample\ Volume}{Protein\ Concentration}$$

Here,

Sample absorbance

The absorbance value obtained from the spectrophotometer for the sample after the reaction between MDA and TBA. This absorbance represents the color intensity of the MDA-TBA complex.

Blank absorbance

The absorbance value obtained from the spectrophotometer for the blank sample, which contains all the reagents except the sample. The blank absorbance accounts for any non-specific reactions or background absorbance.

MDA absorbance coefficient

The molar absorption coefficient or extinction coefficient represents the molar absorptivity of the MDA-TBA complex at the specific wavelength used for measurement. This coefficient can be obtained from literature or determined experimentally.

Sample volume

The volume of the sample used in the assay, typically expressed in milliliters (mL).

Protein concentration

The presence of protein in the sample, typically expressed in milligrams per milliliter (mg/mL).

Glutathione Level

Ellman's method was used for estimating glutathione level.[13-15]

Principle

DTNB and free thiol groups in glutathione combine to generate a yellow-colored product whose absorbance at 412 nm is proportional to the glutathione content. This reaction is the basis for Ellman's technique.

Procedure

Step 1: Briefly, 2 mL of Ellman's reagent (10 mM, $NaHCO_3$ 15 mM) was combined with 160 µl of supernatant.

Step 2: at room temperature, the reaction mixture was incubated for 5 minutes.

Step 3: A spectrophotometer was used to read the absorbance at 412 nm.

Step 4: The formula below is used to estimate glutathione levels,

$$\text{Glutathione concentration} = \left[\frac{\text{Absorbance of sample} - \text{Absorbance of Blank}}{\text{Absorbance coefficient}}\right] \times \text{Dilution factor}$$

Here,

Absorbance of sample

The absorbance value obtained from the spectrophotometer at the specific wavelength used for measuring the glutathione-DTNB complex. This absorbance is typically obtained after the reaction between the sample and the DTNB reagent.

Absorbance of blank

The absorbance value obtained from the spectrophotometer for the blank sample, which contains all the reagents except the sample. This blank absorbance accounts for any non-specific reactions or background absorbance.

Absorbance coefficient

The molar absorption coefficient or extinction coefficient representing the molar absorptivity of the glutathione-DTNB complex at the wavelength used for measurement.

Acetylcholinesterase Enzyme (AChE) Activity Test

Ellman technique was followed for estimating the level of AChE activity.[16,17]

Principle

Thiocholine, which AChE produces, combines with DTNB to form a colorimetric product at 412 nm that is proportionate to the level of AChE activity. The Ellman technique is the foundation of this test.

Procedure

The brains of three animals from each group were isolated after sacrificing them. The isolated brain samples were homogenized in 0.1M phosphate buffer and the resulting mixture was centrifuged at 14000 rpm. The assay was conducted using the supernatant. At 412 nm, the absorbance was measured.

AChE activity was measured using the following equation-

$$\text{AChE activity}\frac{\text{units}}{\text{L}} = \frac{\text{A2 min} - \text{A10 min}}{\text{A calib} - \text{A blank}} \times \text{n} \times 200$$

Where

n = is the dilution factor,

A2min = calibrator's absorbance at 2 minutes

A10min = calibrator's absorbance at 10 minutes

Acalib= calibrator's absorbance at 10 minutes

Ablank = blank's absorbance at 10 minutes.

200 is the equivalent activity (units/L) of the calibrator when assayed and read at 2 minutes and 10 minutes.

Statistical analysis

The findings were presented as mean ± S.D followed a one- or two-way ANOVA. Dunnett's multiple comparison test was followed thereafter. $p < 0.05$ was used to evaluate statistical significance in each instance.

Phytochemical investigation

HAEMK had shown the presence of proteins, carbohydrates, sterols, alkaloids, and phenolic compounds.

Toxicity study in mice

Since no mortality or unusual toxicity was seen at the dose of 5000 mg/kg, it was determined that the HA extract was safe up to this point. Animals given the aforementioned doses did not exhibit any anomalous behavior or significantly alter their behavior patterns. In accordance with OECD recommendations[8], 1/10th of the maximum safe dose may be chosen for the study. Therefore, three dosages of HAEMK were used for the investigation.

Antiepileptic activity

MK at the test doses significantly improved the convulsion protection percentage as compared to the untreated group. Further MK treatment significantly improved the latency of tonic convulsions and duration of seizures in mice (Table 1: Figures 1 and 2).

Biochemical Parameters

Effect of HAEMK on MDA levels

MDA levels in mice brain hippocampus tissue significantly increased in PTZ-treated mice compared to DDW-controlled mice. When compared to mice treated with PTZ, HA (125, 250, and 500 mg/kg) significantly decreased MDA levels (Figure 3).

Effects of HAEMK on glutathione level

When compared to DDW control animals, GSH levels in the hippocampus tissue of mice after PTZ treatment significantly decreased. When compared to PTZ-treated mice, treatment with HA extracts of MK (125, 250, and 500 mg/kg) significantly raised GSH levels (Figure 4).

Figure 5 displays the AChE test results. When compared to the PTZ-treated group, the HA-500 mg/kg showed significantly reduced acetyl-cholinesterase enzyme (AChE) activity ($p <$ 0.005). The lowest AChE activity was seen in the diazepam-treated group (1421 ± 23.75 U/L), followed by MK-HA 500 mg/kg (2542 ± 18.12 U/L). AChE activity was seen to be considerably lower in the group treated with diazepam than in the group treated with PTZ (p <0.005).

DISCUSSION

The antiepileptic efficacy of *M. koenigii* hydroalcoholic extract was investigated in the present study. An extract was subjected to phytochemical screening, revealing the presence of alkaloids, sterols, terpenoids and phenolic compounds. To determine the maximum safe dose to be used for the study, acute toxicity experiments were conducted. As a result, three doses were chosen.

The antiepileptic activity was evaluated for the HA extracts of MK leaves by PTZ-induced convulsion in mice. Parameters like percent protection, latency of clonus and duration of seizure were observed in each group of animals as per the protocol to assess the effectiveness of the extract. HA extract at all doses was found to exhibit antiepileptic potential against the PTZ-induced convulsions in mice. Diazepam-

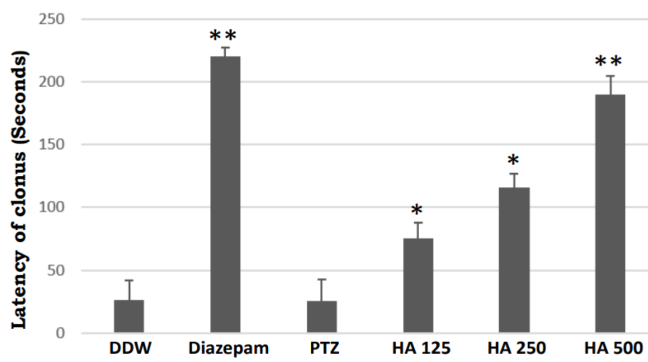

Figure 1: Effect of HAEMK on latency of clonus

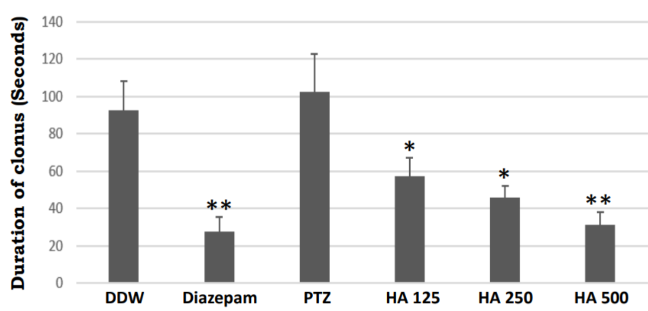

Figure 2: Effect of *HAEMK* on the duration of seizures

Table 1: Effects of HAEMK on parameters evaluated for antiepileptic activity

Groups	*Dose (mg/Kg)*	*No. of animals convulsed/No. of animals used*	*% Protection*	*Latency of clonus (Seconds)*	*Duration of seizures (Seconds)*
DDW	10 ml/Kg	6/6	0	26.67 ± 15.28	92.5 ± 15.87
Diazepam	18	1/6	83.33	220.15 ± 7.02**	27.56 ± 7.78**
PTZ	90	6/6	0	25.67 ± 17.06	102.45 ± 20.33
HA	125	5/6	16.67	75.83 ± 11.29*	57.33 ± 9.87*
HA	250	4/6	33.3	115.83 ± 11.70*	45.75 ± 6.24*
HA	500	3/6	50	189.67 ± 15.33**	31.33 ± 6.65**

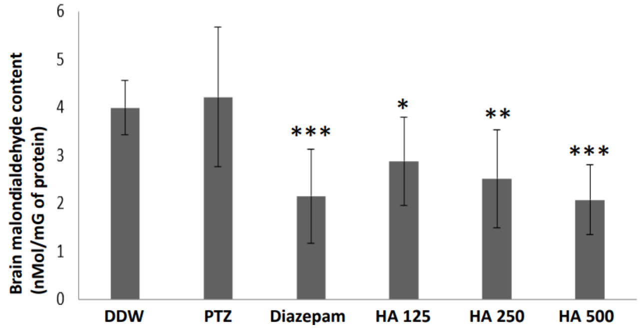

Figure 3: Effect of *M. koenigii* HA extract on brain malondialdehyde content

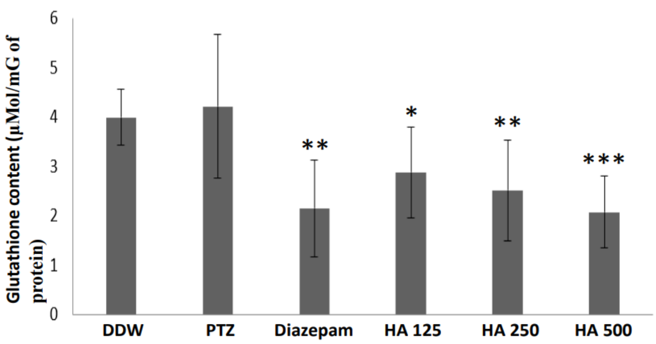

Figure 4: Effect of *M. koenigii* HA extract on glutathione content Acetyl cholinesterase Enzyme Activity Test

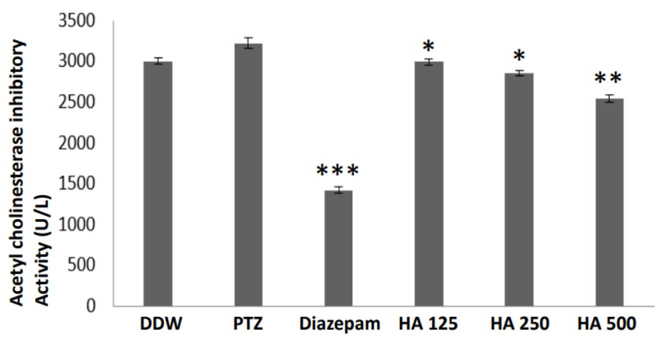

Figure 5: Acetylcholinesterase inhibitory activity of *M. koenigii* HA extracts

treated groups offered maximal protection of 83.33 percent with 220.15 ± 7.02 seconds latency of clonus, found to be the most significant. Seizure duration was found to be shorter in the diazepam-treated group ($p < 0.01$). The dose-dependent antiepileptic activity was observed within the groups treated with HA extract. HA 500 group exhibited more significant results statistically, providing percent protection of 50, latency of clonus (189.67 ± 15.33 seconds) and duration of seizures (31.33 ± 6.65 Seconds).

Biochemical parameters were evaluated post-epileptic study. HA extract post antiepileptic activity was subjected to evaluation on the basis of biochemical parameters. PTZ-induced kindling changes in lipid peroxidation were assessed. A significant rise was observed in malondialdehyde content (Indicative of oxidative stress in brain) of the PTZ-treated group. All the test groups receiving HA extracts were found to be reducing the malondialdehyde content significantly compared to PTZ treated group. Glutathione content was found to be low in PTZ-treated group, while test groups with HA extract and diazepam were found to increase the glutathione levels. The results of increased glutathione levels by HA extracts were found significant when compared to PTZ-treated group. Further, memory impairment is related to the anticholinesterase activity of the molecules. HA 500 group showed a significant reduction in acetylcholinesterase activity. However, diazepam-treated group had shown the lowest acetylcholinesterase activity (1421 ± 23.75U/L). As a result, in the current investigation, *M. koenigii* extract protected against epilepsy-related memory impairment by lowering oxidative stress and cholinesterase activity in mice given PTZ. Using a PTZ-induced seizure paradigm, the current study assessed the antiepileptic effect of HAEMK extract.

The presence of numerous chemical components in the extract may be responsible for the antiepileptic actions that have been found. Bioactive compounds are known to be present in *M. koenigii* and have a range of pharmacological actions, including anti-inflammatory, antioxidant, and neuroprotective benefits.[4,5] One important class of compounds found in *M. koenigii* is alkaloids. Alkaloids, such as murrayacine and mahanine, have been isolated from the plant and have shown promising antiepileptic potential and are known to modulate neurotransmitter release and exhibit anticonvulsant effects by interacting with various receptors in the central nervous system.[18-20]Additionally, the presence of flavonoids and phenols in *M. koenigii* may contribute to its antiepileptic activity. By lowering oxidative stress and inflammation in the brain, flavonoids, including kaempferol and quercetin, have been said to exhibit neuroprotective properties.[4,21] Phenolic compounds, including caffeic acid and rutin, have also demonstrated antiepileptic effects by modulating neurotransmission and reducing excitotoxicity.[4] Furthermore, terpenoids present in *M. koenigii*, such as β-caryophyllene and α-humulene, exhibit anti-inflammatory and neuroprotective activities.[5] These compounds can suppress inflammatory responses and regulate cytokine levels, thereby reducing neuroinflammation, which is implicated in the pathogenesis of epilepsy. Iridoids (isomahanine) identified in *M. koenigii*, have been reported to modulate neurotransmitter release and inhibit seizures in experimental models.[16, 18]

The collective presence of these chemical constituents in the hydroalcoholic extract of *M. koenigii* may contribute to its observed antiepileptic activity. The extract's ability to increase seizure latency, reduce seizure duration and severity, and modulate oxidative stress markers suggests its potential in managing epilepsy.

CONCLUSION

Additional research is required to locate and isolate the key ingredients that are responsible for the antiepileptic actions of *M. koenigii*. Additionally, investigations into the underlying mechanisms of action, such as the modulation of neurotransmitter systems, ion channels, or signaling pathways, would provide a deeper understanding of its antiepileptic potential.

In the PTZ-induced seizure model, the hydroalcoholic extract of *M. koenigii* showed notable antiepileptic efficacy. The hydroalcoholic extract's alkaloids, flavonoids, phenols, terpenoids, and other compounds can be implicated in the symptoms that were seen.

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