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ORIGINAL ARTICLE

Alhagi Maurorum Ethanolic Extract Can Halt Potential Topiramate Neurotoxicity on Sciatic Nerve of Adult Male Albino Rats

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ABSTRACT

Background: Numerous pharmacological routes of topiramate are useful in the treatment of epilepsy and migraines. There are a lot of off-label uses for it. Because Alhagi maurorum contains phenolic and flavonoid chemicals, it has been identified as a potentially helpful medicinal plant. The study's objective was to look into the possible neuroprotective benefits of an ethanolic Alhagi maurorum extract against the probable neurotoxicity of topiramate.

Methods: The following were given orally to rats for a month in four groups: distilled water, topiramate (400 mg/kg daily), Alhagi maurorum ethanolic extract (300 mg/kg daily), and topiramate plus A. maurorum extract (AME). In the sciatic nerve, RT-qPCR was utilized to measure the levels of transcriptional mRNA of antioxidant genes, with biochemical levels of oxidative stress indicators, s100, neurospecific enolase, serum phosphorylated neurofilament-H, and acetylcholinesterase (AchE) were estimated. Histopathological examination and the expression of nitrotyrosine and myelin basic protein were assessed.

Results: Topiramate increased levels of neurospecific enolase, s100, neurotransmitter AchE, Serum phosphorylated neurofilament-H, and oxidative stress markers while decreasing antioxidant genes' transcriptional mRNA levels. Within the sciatic nerve, topiramate also resulted in a variety of neuropathological alterations, notable nitrotyrosine immune reactivity, and moderate myelin basic protein immune reactivity. Supplementing with A. maurorum extract did not cause any neurotoxicity. Antioxidant gene transcriptional mRNA levels rose in response to A. maurorum extract supplementation, and all aberrant measures were nearly normal.

Conclusion: the sciatic nerve of rats exposed to topiramate may be protected by the A. maurorum extract by preventing oxidative stress and apoptosis.

Keywords: *Alhagi maurorum*; Apoptosis; Neurotoxicity; Oxidative stress; Topiramate.

INTRODUCTION

It has been demonstrated that topiramate, an antiepileptic drug with many pharmacological mechanisms of action, is useful in treating several neurological disorders, including epilepsy and migraines. Nowadays, topiramate is bought or prescribed illegally for several illnesses, such as bipolar disorder, alcoholism, obesity, stress disorders, and cocaine dependence [1].

It results in the inhibition of voltage-gated sodium channels, the antagonization of glutamate receptor subtypes, and a rise in gamma-aminobutyric acid neurotransmitter activity [2]. Multiple adverse responses have led to the discontinuation of topiramate use. Reducing adverse effects can often be achieved,

though not always, by using small beginning dosages and progressive titration [3]. Easy fatigability, tiredness, tingling, numbness, loss of appetite with a sour taste, nausea, diarrhea, and renal-hepatic failure are among the most frequent side effects of its use. There have also been reports of memory loss, impaired cognition, suicidal thoughts, and blurred vision [4,5].

Seldom has drug-induced peripheral neuropathy been documented as a side effect of certain antiepileptic medications at large cumulative dosages or even at therapeutic drug levels. As a side effect of long-term topiramate treatment, this study outlines the clinical and diagnostic characteristics of a case study patient with peripheral neuropathy [6].

Medicinal plants are an excellent resource for complementary and alternative pharmaceuticals anywhere in the world. Unexpectedly, the adverse pharmacological effects of commonly prescribed medications have led to a surge in the use of natural sources once more [7].

Alhagi maurorum is a prospective therapeutic plant because it contains components that are flavonoids and phenolic [8]. It belongs to the Leguminosae family and goes by the popular names camel thorn and aqool [9]. A. maurorum has many uses, such as laxative, antirheumatic, analgesic, antipyretic, antiasthmatic, appetizer, and expectorant, according to Asghari et al. [10]. Numerous biological actions, including those that have antioxidant, antiviral, and anticarcinogenic qualities, are exhibited by A. maurorum [11]. Furthermore, studies conducted on animal models have demonstrated that it possesses nephroprotective and hepatoprotective qualities [12].

Oxidative stress is brought on by disruptions between reactive oxygen species, free radicals, and detoxifying processes. The neurological system is susceptible to damage by oxidation due to its quick metabolism, high oxygen requirement, and fatty acid availability [13]. The production of ROS is the primary neurotoxic mechanism for neurotoxins, according to Wang et al. [14]. There are connections between the nervous system's neuroinflammation neurodegenerative illnesses and oxidative stress activity. Neurotransmitter transport across the membrane is altered and membrane fluidity is decreased by polyunsaturated fatty acids, which are abundant in neuronal membranes and are targeted by lipid peroxidation [14].

There haven't been any current studies looking at how well A. maurorum guards against topiramate-induced sciatic nerve harm. Our goal was to ascertain the neuroprotective impact of an ethanol-based extract of A. maurorum against topiramate-mediated toxicity in adult albino rats. We assessed immune-histochemical changes, neurotransmitters, oxidative markers, and gene expression changes.

METHODS

Chemicals and Tested Compounds

A 100 mg topiramate tablet was purchased from the El-gomhoria Pharmaceutical Company located in Zagazig, Egypt. For medicinal plants, the A. maurorum leaves were acquired from Sinai Moringa Co. El-Tur, South Sinai, Egypt. Sunlong BioTech, Co. provided ELISA kits for biochemical parameters and antioxidants (Zhejiang, China). We purchased acetylcholinesterase kits from MyBioSource in the United States.

Maurorum Ethanolic Extract Preparation

Rehman et al.'s [15] method was used to manufacture the A. maurorum ethanol extract. Water was used to clean the plant's leaves, and then dried in the shadows. Before being filtered, 500 grams of powdered dried plant and 70% aqueous ethanol were mixed for a full day. The leftover ethanolic extract was concentrated by rotary evaporation (EYELA, CA-1111, Rikakikai Co., Tokyo, Japan) at 40°C. We measured the pure extract and refrigerated it at 4 C. before use.

Analysis of A. maurorum Ethanolic Extract by Gas Chromatography/Mass Spectrometry (GC–MS)

Agilent Technologies of Wilmington, Delaware, USA, used GC-MS to evaluate the ethanol extract of A. maurorum. The distinct components by contrasting their mass spectra with those in the Wiley and NIST Mass Spectral Library data, of the extract were identified.

Animals and ethics statement

In compliance with the National Institutes of Health's (NIH) guidelines for the handling and application of lab animals in research was applied. The care of all animals was done in compliance with the ethical regulations and the Animal Care Guidelines, as stated in " The Laboratory Animal Care and Use Guide" [16]. ZU-IACUC/3/F/279/2023 is the permission number for the protocol that we used in this work, which was authorized by the Institutional Animal Care and Use Committee at Zagazig University in Zagazig, Egypt.

In all, forty adult male albino rats weighing 150 grams each were employed in this investigation. The Zagazig University School of Medicine conducted the study. To prevent fallacies, the following surroundings were standardized in compliance with Cuschier and Backer [17]. The animal housing and cage must have enough ventilation, 50% relative humidity or less, and a 12-hour light cycle with a temperature at or below room temperature (22°C or 2°C). The light had the same duration, regularity, intensity, and character as natural light. The animal's bedding should be clean. Because noise could affect the animals' behavior, a low volume was kept up. This is where the rats were housed about two weeks before the experiment began, to allow them to adjust to any stress that might have been caused by the animal supplier's shipping procedure or by an abrupt change in the surroundings, and to remove any sick animals. The drug was given to the rats both before and after they ate a balanced diet that was full of all the nutrients they needed to stay healthy. Ad libitum is a part of it. Distiller water was supplied in distinct, pristine vessels [18]. The animals were mercifully killed by breathing ether.

Experimental design

Four groups of ten rats each were randomly assigned to be observed for a period of four weeks. Every drug is taken once daily, for six days a week, and is diluted in one milliliter of distilled water.

Group I (control group): To determine the basic performance parameters, each rat was given just standard meals and purified water. To determine the fundamental parameters.

Group II (Topiramate group) (T): Per El Makawy et al. [5], every rat was given an oral gavage with a therapeutic dose (400 mg/kg/day) of Topiramate.

Group III (A. maurorum group) (AM): A. maurorum ethanolic extract 300 mg/kg/day was gavaged into each rat [8].

Group IV (A. maurorum + Topiramate group) (AM + T): An oral gavage of A. maurorum was administered to each rat an hour before topiramate, which was given using the same dosages and procedures as before.

Excessive vehicle use was avoided to avoid gastric distention, overflow, or regurgitation, which could cause tracheal aspiration. In rats, an injection lasting one month is equal to a human injection lasting 24 months [19].

Sample Collection

Following a 24-hour experimental inquiry, after blood samples were taken from the retro-orbital plexuses of the experimental rats, they were allowed to coagulate for 30 minutes at room temperature. To separate the serum, they were spun at 3000 rpm for ten minutes. The liquid was extracted and kept in storage at -20°C until the biochemical parameters were assessed. The rats were anesthetized intraperitoneal with 50 mg/kg of pentobarbital sodium prior to their sacrifice [19]. To expose and excise segments of the sciatic nerve, each measuring approximately 10 mm in length, an incision was made longitudinally at the rear of the upper limb., and the gluteal muscles were divided **Figure 1** [20]. To facilitate further RT-PCR analyses, a portion of them were partially frozen by adding liquid nitrogen at -80°C. Additional sciatic nerve specimens were stored at -20 °C. to measure acetylcholine levels and oxidative stress markers. After being preserved in neutral buffered formalin (10%), the remaining components were handled for histopathological and immunohistochemical analysis.

Biochemical Assays

of phosphorylated neurofilament-H (PNF-H) levels in serum

An ELISA was used to measure the levels of serum phosphorylated neurofilament-H (PNF-H) according to the guidelines provided by the manufacturer. The test accuracy is 5% both within and between runs, and the detection limit is 23.5 pg/ml [21].

Neuro Specific Enolase (NSE) levels in serum

Serum NSE was tested using an Enzyme Immunometric Assay (EIA) kit following the directions provided by the manufacturer. The range of detection of the kit is 1 to 150 µg/L [22].

Levels of serum S100

Serum S100 levels were determined in accordance with the manufacturer's instructions using an ELISA kit that was purchased commercially. The detection limit of this test is 0.02 g/L [23].

Acetylcholine esterase (AChE) activity in sciatic nerve

Homogenates were prepared using an ELISA kit and the manufacturer's instructions. Examined as Tang et al. [24] state that the detection limit of this test is 0.78 to 50 ng/mL.

Sciatic nerve oxidant/antioxidant parameters

MDA levels, glutathione peroxidase (GPx), and superoxide dismutase (SOD) activities in the sciatic nerve were determined by using the protocols described by Nishikimi [25], Gross et al. [26], and Ohkawa et al. [27], in that order.

Examination of Gene Expression Associated with Oxidative Stress

Tissue homogenization of the sciatic nerve was carried out till the tissue aggregates disappeared. The The iNtRON Biotechnology, Seongnam, Korea-based RNA-spinTM Total RNA Extraction Kit was utilised to extract total RNA in compliance with the manufacturer's instructions. Utilizing a spectrophotometer (UNICO, UV2000, China), the RNA's purity and concentration were evaluated. Enzynomics Inc.'s Power cDNA synthesis Kit was used to reverse transcribe RNA in accordance with the manufacturer's instructions. (SYBR Green with low antioxidants Rox). Each real-time PCR experiment used twenty uL of cDNA, 100 pmol/uL of each primer (0.5 uL each) from Biolegio, ten uL of Jena Bioscience's EvaGreen PCR Master mix, and four uL of PCR-grade water[28,29, 30]. The primer sequences utilized are listed in **Table 1**. Using the 2CT (cycle method), the fold changes in gene expression were evaluated benchmark, with beta-actin as a housekeeping gene utilized) comparing methodology[31]. The Biochemistry Department at Zagazig University's Faculty of Medicine employed the PCR technology.

Histopathological study

The specimens were cut into 5 m thick paraffin slices and stored in 10% formalin solution for light microscopy inspection using eosin and hematoxylin [32].

After serially slicing the slides into 5 m thick slices, immunostaining was carried out. Tissue sections were rehydrated in graded ethanol following their deparaffinization in xylene. For 10 minutes, in an effort to stop non-specific peroxidase reactions, deparaffinized tissue sections were submerged in hydrogen peroxide. Microwave antigen extraction in 0.01 M citrate buffer (pH 6.0) required twenty minutes.

Upon the slides' cleaning with PBS, they were incubated for sixty minutes at ambient temperature using monoclonal antibodies (nitrotyrosine primary antisera). The antibodies used were Abcam, UK, dilution 1:200 of anti-3-nitrotyrosine antibody ([39B6] ab61392) and Anti-Myelin Basic Protein Antibody (Millipore MAB382, Millipore Corporation Billerica, USA), both at a 1:100 concentrations. The binding position of primary antibodies was evident with the EnVisionŠ kit (Dako, Copenhagen, Denmark). Mayer's hematoxylin counterstain was subsequently applied to the portions [33]. Photomicrographs were taken using a light microscope and analyzed with Olysia Bio software [34]. Myelin basic protein (MBP) immunohistochemical dye is used to detect the brown-colored myelin sheath, while nitrotyrosine immunostaining is used to identify neural cells.

STATISTICAL ANALYSIS

The mean and standard deviation (SD) of the acquired data were shown. One-way analysis of variance (ANOVA) was used to statistically

analyze the data. The means of the control and several experimental groups were compared using the post hoc Duncan's comparison test. Version 22 of the statistical software for social sciences is produced by SPSS Inc. in Chicago, IL, USA. Each difference was statistically significant at P≤0.05.

RESULTS

GC-MS Profile of A. maurorum Ethanolic Extract

The A. maurorum extract's GC-MS analysis revealed the primary components. The GC-MS analysis of the extract of A. maurorum contained nine different components, including beta-D-glucopyranose, 1,6-anhydro-(levoglucosan) (30.91%), 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (21.24%), 3-methyl-2-(2-oxopropyl)furan (15.56%), and 3-methyl-2-dihydroxy-6-methyl- (22.24%), 3-methyl-2-(2-oxopropyl)furan (12.56%), and 3-methyl-2-(2-(2-methyl-2-butenyl)-furan (rosefuran) (12.84%).

Effect of the A. maurorum Extract on Serum Phosporylated Neurofilament-H (PNF-H), Neurospecific Enolase (NSE), and S100 Biomarkers levels in Topiramate treated Rats

PNF-H, NSE, and S100 concentrations in the sera of rats treated with topiramate were significantly (P≤0.01) greater than those of the control group. In the topiramate-treated group, concurrent supplementation with A. maurorum extract considerably (P≤0.01) reduced their level; nevertheless, in the topiramate+ Alhagi-treated group, their level did not reach up to normal control levels **Table 2**.

Effect of the A. maurorum Extract on the Sciatic Nerve Acetyle Choline Esterase Level in Topiramate-Treated Rats

Rats administered with topiramate had significantly (P≤0.01) greater levels of AChE in their sciatic nerve compared to control rats. In the topiramate-treated group, concurrent supplementation with A. maurorum extract considerably (P≤0.01) raised its level, but in the topiramate+ Alhagi-treated group, it did not approach normal control levels (Table 2).

Effect of the A. maurorum Extract on Sciatic Nerve Oxidative Stress Biomarkers in Topiramate treated Rats

When compared to control rats, topiramate-treated animals exhibited a significant (P≤0.01) reduction in SOD and GPx activity in the sciatic nerve. Conversely, in the topiramate + Alhagi-treated rats, concurrent supplementation with A. maurorum extract significantly (P≤0.01) improved the decreased levels of such indicators and nearly normalized them. Moreover, animals treated with topiramate had significantly greater (P≤0.01) MDA concentrations in their sciatic nerve compared to control rats. Rats treated with topiramate + Alhagi, however, exhibited noticeably lower sciatic nerve MDA levels than rats treated with topiramate (Table 2).

Effect of the A. maurorum Extract on the Sciatic Nerve mRNA Expression of MBP and nitrotyrosine Genes in Topiramate-treated Rats

The mRNA transcription of MBP and nitrotyrosine-associated genes was significantly ($p < 0.05$) downregulated and elevated in the sciatic nerve of topiramate-treated rats compared to control rats after topiramate therapy respectively. These transcriptional levels were normalized in the topiramate+Alhagi-treated group by contemporaneous supplementation with the A. maurorum extract (Figure 7A, B).

Effect of the A. maurorum Extract on the Sciatic Nerve mRNA Expression of Antioxidant Genes in topiramate-treated Rats

Topiramate treatment led to a significant ($p < 0.05$) downregulation of the sciatic nerve mRNA transcription of oxidative stress-associated genes (SOD, GPx, and CAT) compared to control rats. Concurrent supplementation with the A. maurorum extract normalized these transcriptional levels in the topiramate + Alhagi-treated group (Figure 7C, D, E).

Histopathological and Immunohistochemical Investigations

Rats treated with Alhagi and control groups had sciatic nerve slices stained with Eosin and Haematoxylin (H&E), which revealed a normal histological structure of nerve fiber bundles with each bundle having a small layer of perineurium, a connective tissue. A collection of nerve fibers

encircled by perineurium was known as the sciatic nerve fascicle. An unstained region of dissolved myelin and axoplasm surrounds myelinated nerve fibers. Nuclei of Schwann cells began to appear between the nerve fibers. Moreover, nerve fibers that were not myelinated were observed (Figure 2A, B, C). The topiramate-treated group lost the typical architecture of the sciatic nerve and the majority of the nerve fibers disintegrated and split off from the perineurium. The perineurium and the majority of nerve fibers were significantly separated from one another and enveloped them, revealing dilated blood vessels (Figure 3 A, B, C).

Because of the reorganization of the sciatic nerve and its seeming normal architecture, the majority of the aforementioned lesions were diminished in the group that had topiramate + Alhagi treatment. Nerve fascicles were primarily formed by myelinated nerve fibers and Schwann cell nuclei in endoneurial spaces. Significant nerve fiber separation persisted (Figure 4 A, B, C).

The immunoexpression of MBP in the sciatic nerve tissues of different study groups is shown in Figure 5A, B, and C. The myelin sheath tissues of rats treated with Alhagi and control groups both exhibited high levels of positive MBP immunoexpression. Rats fed topiramate had tissues with weak positive immunoexpression of MBP. The A. maurorum extract co-treatment considerably reduced the rats' exposure to topiramate compared to raising the MBP immunoexpression to a staining level that was somewhat positive, but it did not return it to the control range. The immunological sciatic nerve staining area (%) of MBP expression in treated rats across different experimental groups is displayed in Figure 5D. This figure showed a high percentage of MBP expression in the control group that is statistically higher in comparison to the topiramate-treated group. The topiramate+Alhagi group showed a high percentage of its expression in comparison to the topiramate-treated group.

The immunoexpression of nitrotyrosine in the sciatic nerve tissues of the different research groups is shown in Figures 6A, B, and C. The tissues of the rats receiving Alhagi treatment and the control group exhibited negligible to moderate levels of nitrotyrosine immunoexpression. The tissues of rats administered topiramate exhibited a notable increase in nitrotyrosine immunoexpression. The co-administration of A. maurorum extract significantly decreased this immunoexpression to a moderate degree, but it did not reach the control values. The immunological staining area (%) of nitrotyrosine expression in the sciatic nerve of rats receiving treatment from several experimental groups is demonstrated in Figure 6D. This figure showed a low percentage of nitrotyrosine expression in the control group that is statistically lower in comparison to the topiramate-treated group. The topiramate+Alhagi group showed a low percentage of its expression in comparison to the topiramate-treated group.

Table 1: Oligonucleotide primer sequences used for real-time PCR

Genes	Forward Primer (5'→ 3')	Reverse Primer (5' → 3')	Product Size/bp	GenBank Accession Numbers
β-Actin	GCA CCA CAC CTT CTA CAA TG	TGC TTG CTG ATC CAC ATC TG.	619	AH003498.3
MBP	GACTGAGTACCTGA ACCGGCATC	CTGAGCAGCGTCTTCA GAGACA	135	NM_016993.1
Nitrotyrosine	GAGACAGACAGTGG AACTGACGATG	GGCGCAAAGTGACTG GATGA	147	NM_012922.2

Genes	Forward Primer (5'→ 3')	Reverse Primer (5' → 3')	Product Size/bp	GenBank Accession Numbers
CAT	GTCCGATTCTCCAC AGTCGC	CGCTGAACAAGAAAG TAACCTG	272	AH004967.1
SOD	ATGGGGACAATACA CAAGGC	TCATCTTGTTTCTCGTG GAC	225	Z21917.1
GPx	CACAGTCCACCGTG TATGCC	AAGTTGGGCTCGAACC CACC	292	S50336.1

MPB: myelin basic protein. CAT: catalase. SOD: superoxide dismutase. GPx: glutathione peroxidase.

Table 2: Statistical comparison of the biochemical parameters in the sera and sciatic nerve of different studied groups using one-way ANOVA and posthoc Duncan's comparison test

parameters	Control	Alhagi	Topiramate	topiramate + Alhagi	F value	*P* value
PNF-H (ng/ml)	0.49 ± 0.04	0.55 ± 0.08	6.19 ± 0.20***	0.56 ± 0.08###	0.456	0.001
NSE (µg/L)	8.54 ± 0.32	9.61 ± 1.22	31.55 ± 0.86***	9.66 ± 1.82###	0.582	0.001
S100 (µg/L)	0.095 ± 0.003	0.100 ± 0.007	0.353 ± 0.036***	0.101 ± 0.009###	0.578	0.001
AchE (ng/g tissue)	2.51± 0.01	5.9± 0.11	19.9± 0.31***	6.1± 0.02###	0.790	0.001
MDA (nmol/g tissue)	60.33 ± 0.33	59.00 ± 0.58	92.33 ± 1.45 ***	73.00 ± 1.16 ###	0.328	0.001
SOD (U/g tissue)	40.69 ± 0.43	40.71 ± 0.23	24.86 ± 0.81 ***	39.19 ± 0.41 ###	0.591	0.001
GPx (U/g tissue)	21.07 ± 0.38	21.05 ± 0.53	14.00 ± 0.58 ***	19.52 ± 0.29 ###	0.486	0.001

Data were expressed as mean ± SE, $n = 10$ for each group. *** Significantly different compared to the control group at $p < 0.001$. ### Significantly different compared to the topiramate-treated group at $p < 0.001$. PNF-H: serum phosphorylated neurofilament-H. NSE: neurospecific enolase. AchE: acetylcholinesterase. MDA: malondaldehyde. SOD: superoxide dismutase. GPx: glutathione peroxidase.

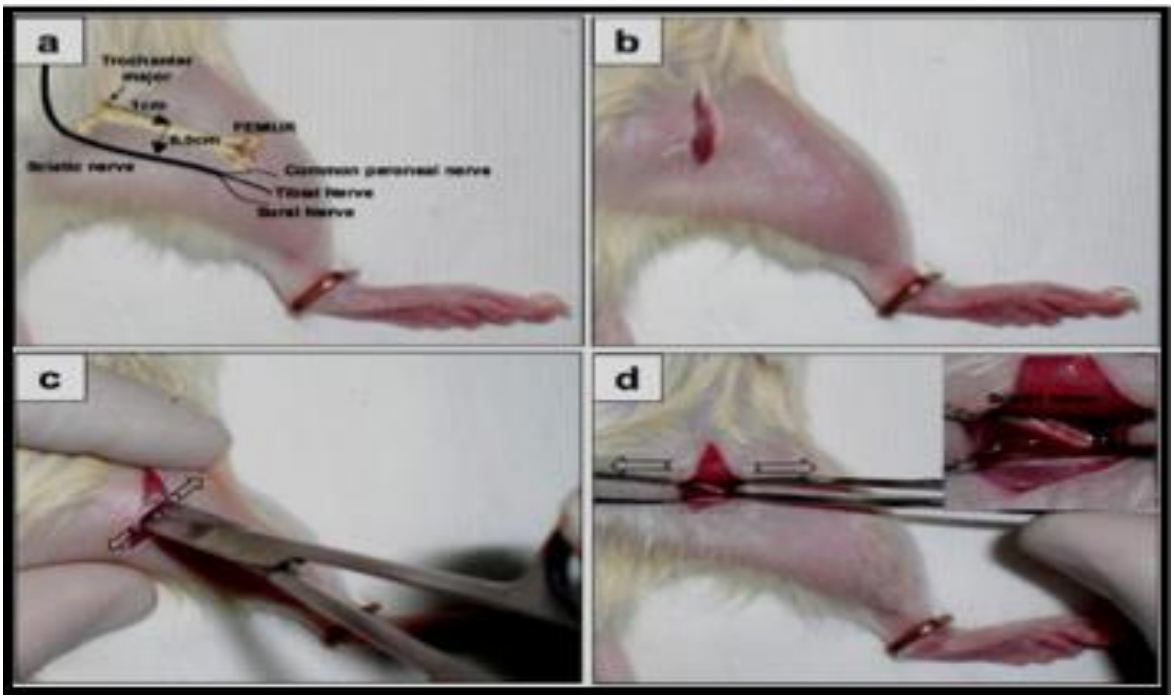

Figure (1): Rhombic dissection between the maximum gluteus and quadriceps muscles was carried out allowing the complete vision of sciatic nerve **[20].**

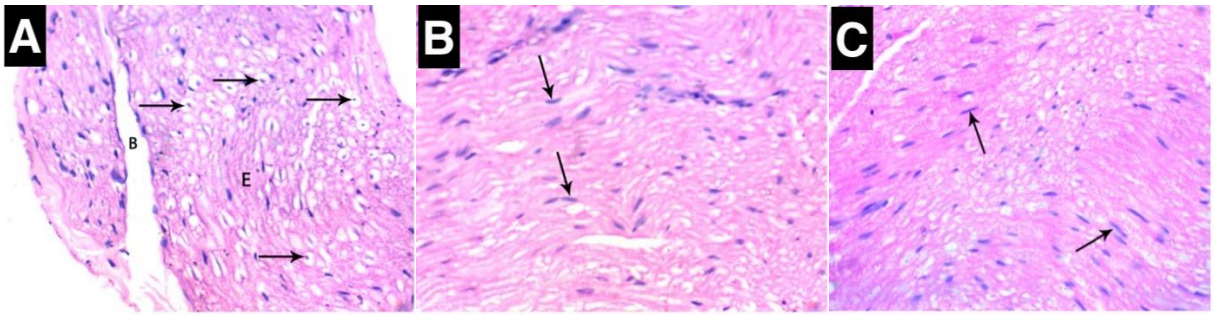

Figure (2): Photomicrograph of H&E stained sections of sciatic nerve from control group showing: (A) closely packed nerve fibers with endoneurium in between (E) and sciatic nerve fascicle containing group of nerve fibers surrounded by perineurium(B). Each individual nerve fiber consists of central axon surrounded by myelin sheath. (B,C) the elongated wavy Schwann cell nuclei (→) (H&E x 400).

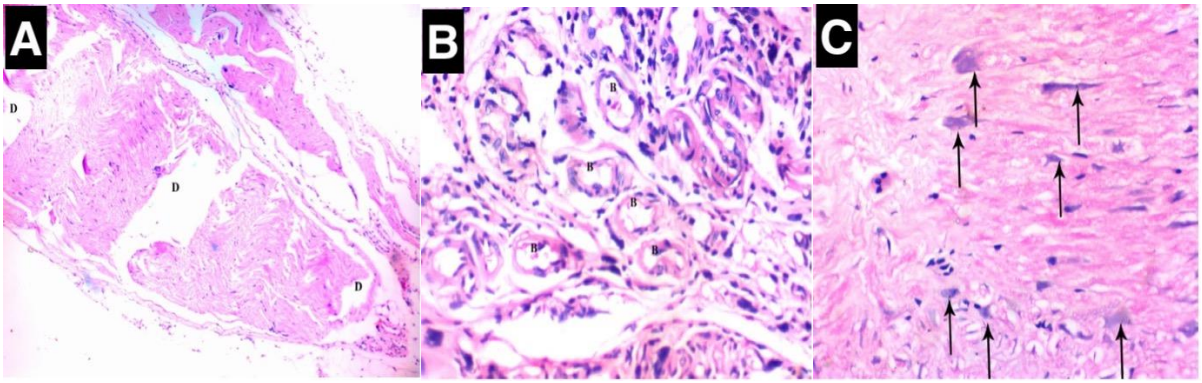

Figure (3): Photomicrograph of H&E stained sections of sciatic nerve from topiramate trated group showing: (A) disorganization of nerve fascicles. Most of nerve fibers are widely separated from each other and from overlying perineurium with degeneration (D). (B)Inflammation of sciatic nerve tissue on background of demyelination and degeneration with increased number of blood vessels (B). (C) Mild, Moderate, Severe demyelination and degeneration of myelin sheath in some fibers with remnant and disturbed Schwann cells nuclei ( ) (H&E x 400).

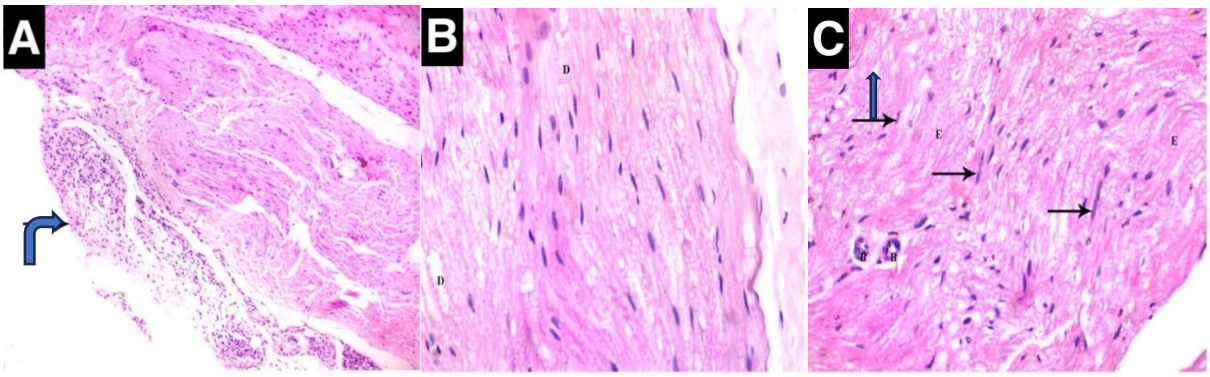

Figure (4): Photomicrograph of H&E stained sections of sciatic nerve from topiramate +A. maurorm trated group showing:(A) apparently normal nerve fascicle formed mostly of mylinated nerve fibers ( ). (B) Nerve fibers are still widely separated (D). and (C) Schwann cells nuclei in endoneurial spaces normal myelinated nerve fibers, Schwann cell nuclei (→) with endoneurium (E) in between and occasional blood vessels(B) (H&E x 400).

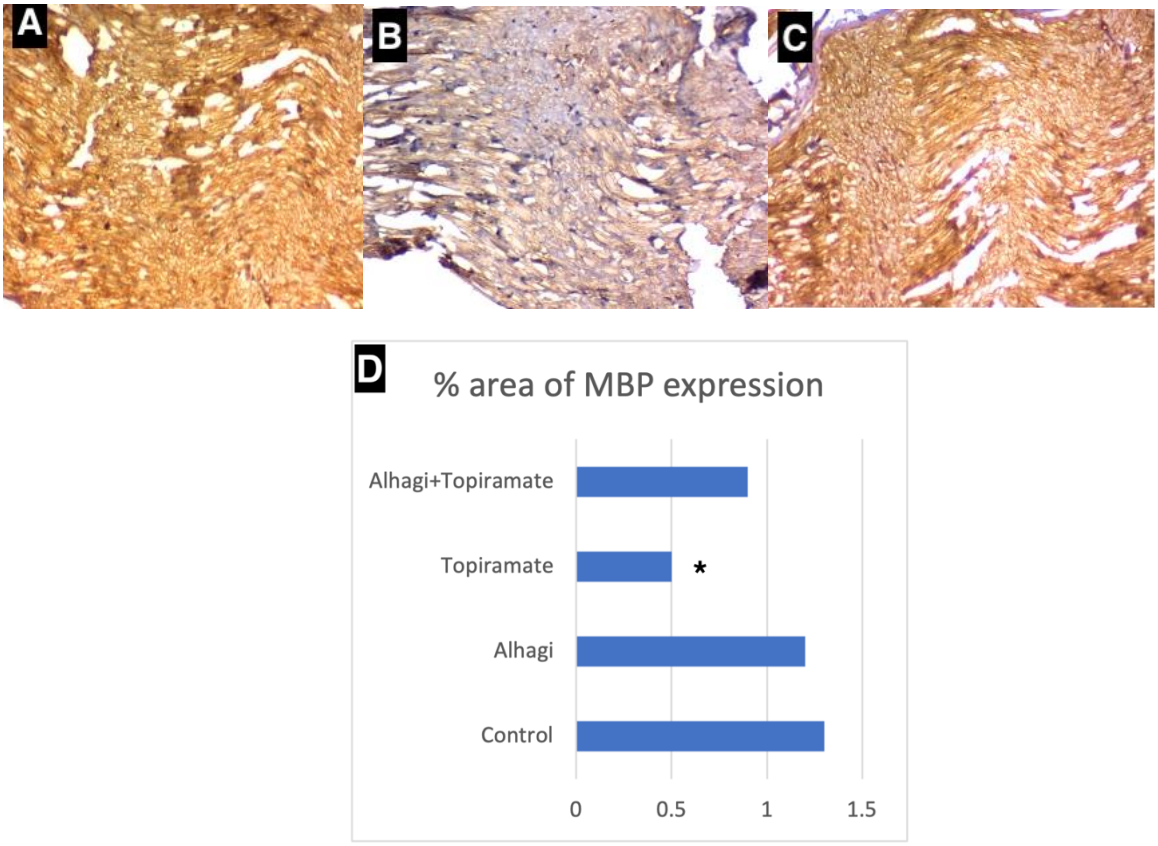

Figure (5): Immunohistochemical MBP stained sections of sciatic nerves showing (A): Control group with strong positive staining for MBP in the myelin sheath. (B): Topiramate treated group showing weak positive reaction for MBP in the myelin sheath. While, (C): Alhgai and toipramate treated group showing moderate positive reaction for MBP. (Immunohistochemical for MBP ×400). (D): Immune staining area (%) of MBP expression in the sciatic nerve of treated rats from different experimental groups.

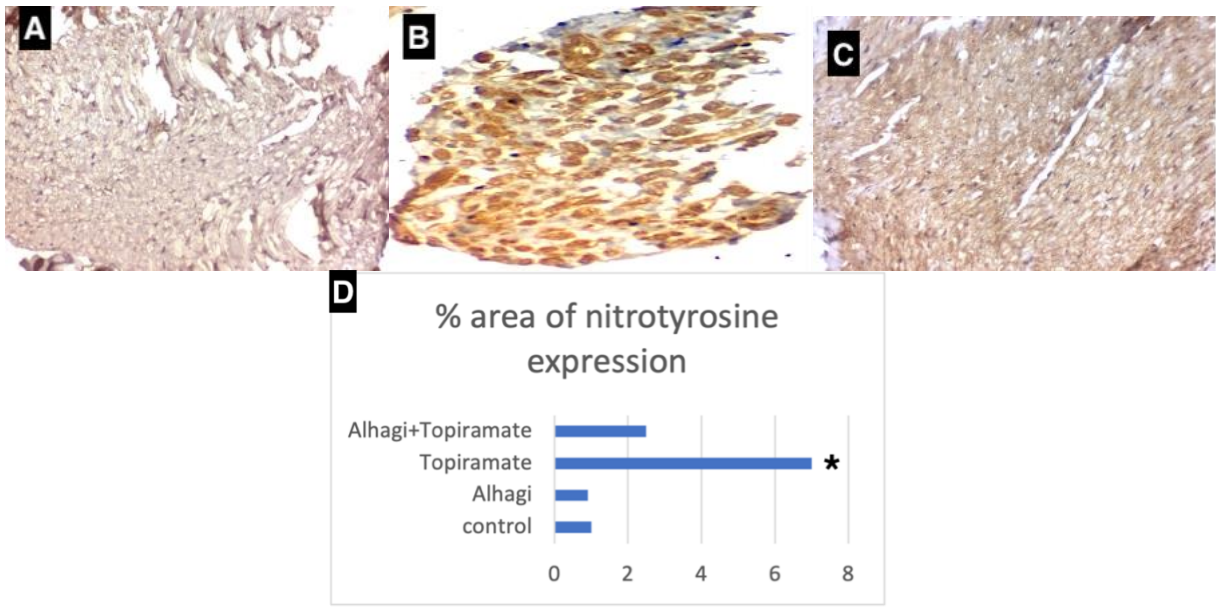

Figure 6: Nitrotyrosine immunohistochemical stained sections of sciatic nerves showing (A): Control group with weak positive staining for nitrotyrosine in the myelin sheath. (B): Topiramate-treated groups showed a strong positive reaction for nitrotyrosine in the myelin sheath. While (C): Alhagi and topiramate treated group showing weak positive reaction for nitrotyrosine. (Immunohistochemical for nitrotyrosine ×400) (D) Immune staining area (%) of nitrotyrosine expression in the sciatic nerve of treated rats from different experimental groups.

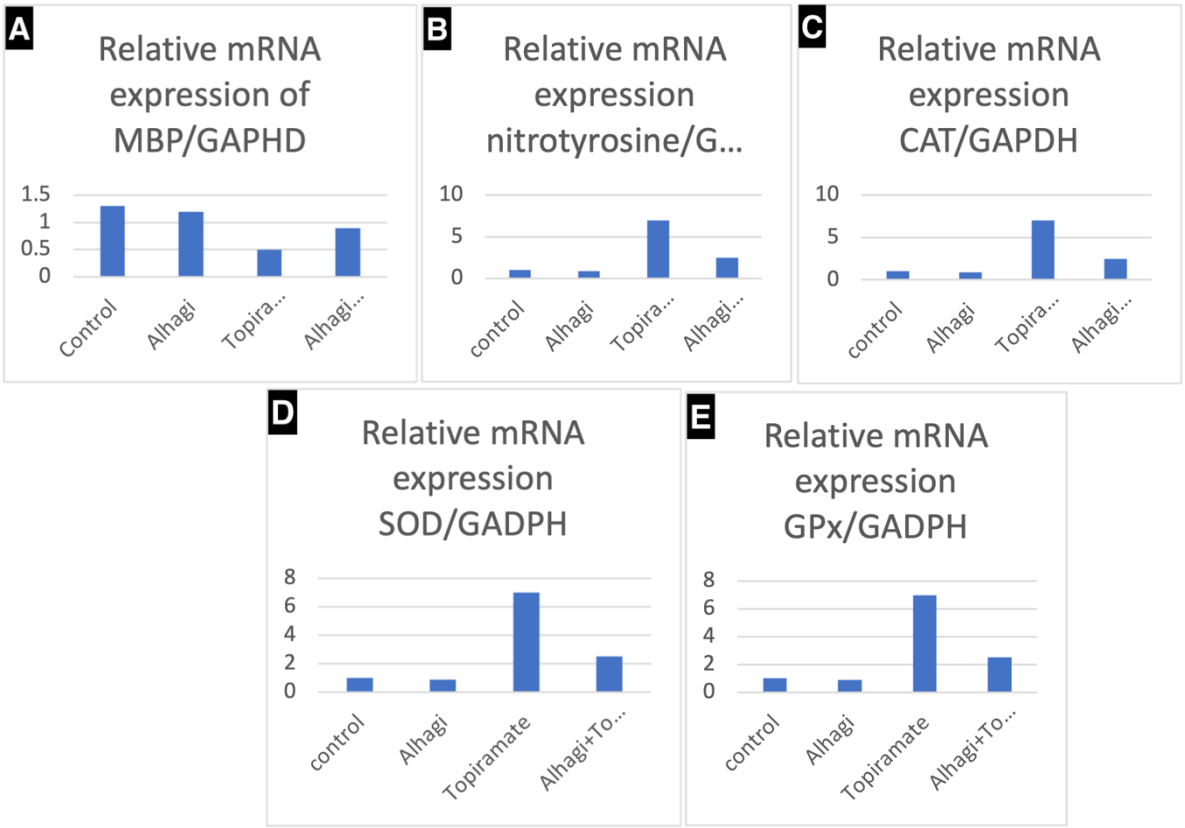

Figure 7: Effect of the Alhagi maurorum extract on the mRNA levels of genes of myelin basic protein (MBP), nitrotyrosine, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx).

DISCUSSION

As far as we know, there are no reports, experimentally, on the effect of topiramate on the sciatic nerve and the protective effect of A. maurorum extract on the sciatic nerve.

Research has demonstrated that a range of natural products are extremely successful in lowering the dangers connected to the abuse of some medications [35]. Thus, the purpose of the current investigation was to determine whether an A. maurorum extract could lessen the various biochemical and histological alterations that topiramate induces in the sciatic nerve. Our results showed that the sera of rats treated with topiramate had increased levels of PNF-H, NSE, and S100.

These results are in line with those of Weise et al. [36], who discovered that A 7-year-old girl receiving valproate–topiramate therapy exhibited elevated NSE levels. Calik et al. [37] discovered Serum S-100 levels were considerably elevated in patients with focal intractable epilepsy receiving antiepileptic medication. One protein indicator of glial activation or injury is S-100. In some cases, it may serve as a trustworthy peripheral biomarker for neuronal injury. Rat sciatic nerves' levels of S-100 protein and enolase isozymes were measured during the degeneration and regeneration phases. Kato and Satoh [38] concluded that variations in the levels of S-100 protein and enolase isozymes in a study that used rat sciatic nerve modeling to show degeneration and regeneration.

Our findings demonstrated that rats given topiramate had higher levels of AChE in their sciatic nerves.

This result concurs with Mabrouk et al. [39]. ACh is one of the neurotransmitters that is necessary for proper brain function. It synchronizes neuronal firing, alters synaptic transmission and plasticity, and changes the excitability of neurons. It has been shown that aberrant acetylcholine levels are linked to neurodegenerative disorders [40]. Despite the fact that AChE does not cause apoptosis, research has revealed that cells that overexpress AChE are vulnerable to it [38]. According to the results of Al-Kawaz et al. [41], topiramate decreased the levels of ACh in the sciatic nerve, but according to the current study, topiramate elevated the sciatic nerve's AChE activity. It seems that the action of AChE is connected to topiramate's neurotoxicity, which is in line with the previously described findings [42].

Our results showed that the sciatic nerve's oxidative stress markers (MDA) were raised while the GPx and SOD activity were lowered. These outcomes align with research by Sarangi et al. [43] which discovered that in brain tissue, topiramate lowered glutathione, GPx, and SOD activity while increasing MDA levels.

Reactive oxygen species (ROS) overproduction and their detoxification are out of balance, which leads to oxidative stress. Because of its rapid rate of oxygen consumption, weak antioxidant protection, and the existence of redox-active metals, the nervous system is particularly vulnerable to oxidative stress [44]. Moreover, lipid peroxidation targets polyunsaturated fatty acids (PUFA), which are widely distributed in neuronal membranes [45]. It's been proven that excessive reactive oxygen species (ROS) production damages neural cells, resulting in necrosis or apoptosis [46].

A helpful indicator for determining how much lipid peroxidation has occurred is malonaldehyde (MDA), a result of PUFA's peroxidative breakdown. A significant element causing damage to neurons [47].

The proapoptotic protein nitrotyrosine is triggered by signaling processes in cells moving through apoptosis. It is essential for the death of neurons in several circumstances, such as embryonic apoptosis, ischemia, hypoxia oxidative stress, loss of nerve growth factor, and nerve damage, as the principal neuronal executor [48].

Therefore, The hypothesis was made that neural death was a process resulting in the adverse effects of antiepileptic medications [49]. In the current investigation, the sciatic nerves of rats treated with topiramate showed strong expression of nitrotyrosine protein. Furthermore, rats with traumatic nerve injury had higher levels of nitrotyrosine protein and

mRNA, which were assumed to be indicators of neuronal cell death [50].

The present study's results showed that topiramate exposure caused neuroapoptosis, as evidenced by the notable downregulation of MBP mRNA transcriptional levels and the notable upregulation of pro-apoptotic gene mRNA levels (nitrotyrosine) in the sciatic nerve of rats given topiramate. Additionally, the sciatic nerve rats in the topiramate-treated group had decreased MBP immunoexpression relative to the group under control. These results were consistent with previous research [51,52,53]. The malfunctioning of mitochondria is worsened by the activation of these proteins [52].

The findings that were previously published were supported by the histology observations made here. Histopathological analysis of the sciatic nerve in the topiramate-treated group indicated major architectural abnormalities and severe damage, in line with previous research [52,53].

Our research revealed a connection between the oxidation process, neuroinflammation, and death and topiramate's evident harm to neurons. Faulty connections between neurons, neuroinflammation, oxidative damage, and neurons death are examples of potential pathogenic features [54].

The remarkable finding that the A. maurorum extract is similar to the antiepileptic drug and can effectively cure migraine attacks was made possible by the simultaneous administration of the extract and topiramate [55]. The results showed that alhagi did not present a neurotoxic danger. Along with showing a significant reduction in apoptosis in the sciatic nerve tissues as indicated by the marked upregulation of the MBP genes and downregulation of the nitrotyrosine genes, additionally, the recent study revealed the neuroprotective benefits of the interaction between alhagi and topiramate, as evidenced by the overall improvement in the studied sciatic nerve with regressed neuropathic lesions and improved the histopathological lesion. The oxidative stress levels and markers improved, according to the biochemical analysis.

This aligns with the research findings of Saber et al. [45], which established that alhagi shields the tissues of the brain and central nervous system from the apoptotic, histological, and neurochemical harm brought on by exposure to lead. Similarly, the A. maurorum extract lessened the oxidative damage to the liver brought on by norfloxacin [56] and carbon tetrachloride [57]. The A. maurorum extract's antioxidant benefit could be the cause of this soothing effect. The observed activity may be explained by the elevated concentration of many bioactive constituents [58]. For example, 1,6-anhydro-beta-D-glucopyranose (levoglucosan) (28.91%), was discovered to have antioxidant and free radical scavenging capabilities in the extract of Barleria noctiflora leaves [59]; The primary bioactive ingredient in the mixture, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, has been shown to have strong antioxidant properties. A. maurorum extract: The main active component of the extract is 3-(2-methyl-2-butenyl)-2-methyl-2-furan, which has exhibited hydroperoxyl radical harvesting properties [60].

Nevertheless, concomitant administration of the A. maurorum extract corrected these injuries. This implied that the extract from A. maurorum provides histological defense against topiramate-induced sciatic nerve damage. These results are substantiated by past research demonstrating that the extract from A. maurorum has a protective effect against the histological damage to the liver caused by carbon tetrachloride [57].

CONCLUSIONS

The current research indicates that the rats were shielded from the neurotoxicity of topiramate by contemporaneous supplementation of the extract from A. maurorum. The potential correlation between the ameliorative action of A. maurorum extract and its ability to harvest free radicals, abrogate lipid peroxidation, and exhibit antioxidant and anti-apoptotic characteristics was approved. Further research is needed to investigate the A. maurorum extract's attenuating effect on the nerve tissue damage resulting from other antiepileptic drugs.

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