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Research Article

Evaluation of the Anti-Epileptic Activity of *Salvia Moorcroftiana* against Pilocarpine Induced Epilepsy in Mice

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Abstract

Background: Allopathic drugs currently available to treat epilepsy have one or more inherent adverse effects when it comes to, the second most common neurological disorder. In this context, herbal drugs are considered much safer, potent, and have a better edge over synthetic drugs. This forced the research community to focus more on herbal remedies. Many Plants including some species of *salvia* have been reported to exhibit anticonvulsant activity. *Salvia moorcroftiana* is an herbaceous perennial native to the Himalayan Mountains posse's antibacterial, antitumor, and antioxidant activities.

Aims and objectives: To evaluate the antiepileptic activity of methanol extracts of *Salvia moorcroftiana*.

Materials and methods: The extract obtained was evaluated for Preliminary phytochemical screening, evaluated for *in-vitro* antioxidant and anti-epileptic activity which include behavioral, biochemical estimation, and histopathological studies.

Results: We report that SVP (300 mg/Kg) methanol extracts of *Salvia moorcroftiana* were able to reduce seizure severity score, highly significant rise in latency to seizure onset score, attenuate oxidative damage in lipids and proteins, Contrary catalase levels were strengthened as like pilocarpine control, and significant augmentation of SOD levels. Besides SVP (300 mg/Kg) methanol extracts of Salvia moorcroftiana protected against degeneration in extra-hippocampus and CA1, CA2, CA3, and DG regions compared to Pilocarpine control.

Conclusion: The results of our work Supported by strong published literature reports suggest that methanol extracts of *Salvia moorcroftiana* are potential candidates for the prevention of pilocarpine-induced epilepsy since these restrain several biomarkers of oxidative stress in an animal model and underline the potential of these extract as a natural way to treat epilepsy. These properties make them pre-clinically useful. Further detailed mechanistic studies are necessary to divulge the beneficial effects of these extracts before proceeding for clinical trials.

Keywords: Epilepsy; Salvia; Antioxidant; Antiepileptic; Pilocarpine; Methanol seizures

Introduction

Epilepsy is the second most common and frequently encountered neurological disorder identified by recurrent seizures which are the result of uncontrollable neural excitation in the brain, often accompanied by cognitive deficits and mood disorders that imposes a heavy burden on individuals, families, and also on healthcare systems [1-4]. When a person has two or more unprovoked seizures, they are considered to have epilepsy. It happens when a strong brief surge of electrical activity affects all of the brain or a part of it and lasts from few seconds to few minutes. It is assumed that one out of 10 adults will encounter seizures sometime during their lifetime. Symptoms of seizures vary from convulsions and loss of consciousness to some that are not recognized by the sufferer or even by the health care professionals this includes lip-smacking, jerking movements of arms or legs, blank staring, etc [5]. Brain tumors, traumatic head injuries, cerebrovascular disease, malformations of cortical development, infections of the central nervous system, endemic infections in resource-poor countries like malaria and neurocysticercosis, and even genetic inheritance are among the most common risk factors that increase the likelihood of developing the disease [4,6]. According to a recent study, around 70 million people worldwide have epilepsy with nearly 90% found in developing regions. In India alone a conservative estimation of more than 12 million persons that contribute to one-sixth of the global burden suffer from this neurological disorder have been reported [7] by the epidemiological studies, 70% to 80% of people developing epilepsy will go into remission, while the remaining patients will continue to have the seizures and are obstinate to treatment with all currently available therapies [8,9]. A study conducted in Kashmir [10] reported one hundred fifty-seven cases of active epilepsy, giving a crude prevalence rate of 2.47/1000 general population. The mean age for the onset of epilepsy was 5.3 years for males and 7.1 years for females with a mean duration of 6 years. Around 78.9% of cases were recorded to have generalized seizures where 74.5% cases were receiving no specific treatment. In two phases [11] prospective and

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observational study was conducted in Hazratbal Community Block Srinagar. In the first phase households of the said community were surveyed for seizure disorder using cluster sampling. Individuals with seizures were clinically evaluated with detailed history and thorough clinical examination as per protocol in the second phase. All patients were subjected to hematological, biochemical, and radiological examination. Special investigations like EEG and CSF analysis were done wherever it was required for the patient. In a sample of 15748 taken randomly out of a population of 150,000, 47 cases of seizure were found. The majority of seizures occurred in the age groups of 20-49 years. The etiological spectrum of seizures was varied and included cerebrovascular accidents, neuron infection, metabolic, tumors, idiopathic. Neuron infection and cerebrovascular accidents account for a significant number of seizures in all age groups [12].

Conventionally various chemical classes of drugs such as benzodiazepines (Diazepam), barbiturates (Phenobarbitone), Gamma-Aminobutyric Acid (GABA) analogs, succinimides (ethosuximide), hydantoins (Phenytoin), carbamazepine, etc., were extensively used in the management of epilepsy [13,14]. Recently, a much newer class of drugs such as vigabatrin, levetiracetam, topiramate, lamotrigine, zonisamide, lacosamide, rufinamide, and stiripentol have been developed, and they are considered to be comparatively safe [15,16]. However, despite copious efforts, all the currently available drugs have one or more inherent side/adverse effects such as dizziness, mental slowing, ataxia, impaired concentration, mental confusion, sleep disturbance, anorexia, somnolence, and aggression. Hence, there is greater scope for safer and potent drugs for the management of epilepsy, in this context herbal drugs are considered to have a better edge over synthetic drugs, therefore many researchers are focusing on herbal remedies, to discover better and safer medicine for epilepsy [17].

Many Plants such as *Acoruscalamus*, *Crocus sativus*, *Emblicaoffcinalis*, *Ginkgo biloba*, *Hypericumperforatum*, *Matricarirecutita*, *Panax ginseng*, *Passifloraincarnata*, etc. have been reported to exhibit anticonvulsant activity [18-20]. Some species of salvia such as *Salvia miltiorrhiza, Salvia officinalis, Salvia leriifolia, Salvia triloba* etc have also been reported to possess anticonvulsant activity. *Salvia moorcroftiana* is an herbaceous perennial native to the Himalayan Mountains from Pakistan to Western Nepal and is especially common in the Kashmir valley [21-23]. With this background, the present study has been conducted on *Salvia moorcroftiana* a widely grown herb in Kashmir that has been found to possess antibacterial, antitumor, and antioxidant activities. *Salvia moorcroftiana* in traditional medicine has been used in the treatment of cough [24] stomach pain [25] Guinea worm, boils, wounds, skin infections, hemorrhoids, colic, dysentery [26], and throat complaints.

In the present study methanol extracts of *Salvia moorcroftiana*, were evaluated in vitro antioxidant activity and its shielding effect against pilocarpine-induced behavioral, oxidative and histopathological alterations in a rodent model of epilepsy. This is a novel piece of research; such type of studies has not been conducted on this species of *Salvia* so far.

Material and Methods

Animals

Swiss albino mice (both sex's), 10-12 weeks old in the weight range of (25 g to 35 g) were obtained from central animal house facility IIIM Jammu, J&K, India. Animals were housed in polypropylene cages with dust-free rice husk as a bedding material. The animals were provided with a commercial diet (Ashirwad feed India) and water ad labium under controlled temperature (25±2°C), humidity (60±10%), and lighting (12:12 light/dark cycle) conditions. All the experimental procedures involving animals have conducted as per CPCSEA (The Committee for Control and Supervision of Experiments on Animals) guidelines after getting proper approval by Institutional Animal Ethical Committee (IAEC), PG Department of Pharmaceutical Sciences, University of Kashmir, Srinagar [No: F-IAEC(Pharm. Sc.) APPROVAL/ 2017/18].

Identification, collection, and authentication of the selected medicinal plant material

The whole plant sample of *Salvia moorcraftiana* was identified and authenticated by Dr. Akhtar, H. Malik, Taxonomist, and Centre for Biodiversity and Taxonomy (CBT), Department of Botany, University of Kashmir. A sample specimen of the plant was deposited in the center with a voucher specimen number, 2690-KASH. Around 10 kg of the whole plant of *Salvia moorcroftiana* was collected in jute bags from the Lethpora Pampore area of Pulwama district in Kashmir valley during May 2018 and was freed from dirt; air-dried and was coarsely powdered.

Preparation of plant material

For the preparation of whole plant extracts using cold maceration of the selected plant material, two different solvents were used: Methanol and Chloroform (1:1). Briefly coarsely powdered plant material 1000 g was taken in two separate maceration bottles (500 g in each). One flooded with 5.5 liters of methanol and another with 5.5 liters of Chloroform: Methanol for about 72 hours with occasional shaking. The dark green-colored filtrate was collected from both maceration bottles around 5 liters and 75 ml in each. The powdered material in both of the maceration bottles was again subjected for maceration by adding 5 liters of selected solvents in each. The light green-colored filtrate was collected again after 72 hours. The combined filtrates from the first and second phases of each maceration bottle were concentrated using a condenser. The solvents so obtained were distilled and recovered at 64.7°C. The concentrated extracts left in the round-bottomed flask were transferred to sterile Petri dishes and kept at room temperature until dried. The percentage yields were calculated and the extracts were stored at 4°C for future use.

Evaluation of antiepileptic activity

The experiment was carried out using Pilocarpine for inducing seizures in mice. In this experimental model, mice were randomly divided into seven groups consisting of 10 mice each group. Group I; Normal Control, Group II; Toxic Control, Group III; Standard Anti-Epileptic Drug (AED), Groups IV-VII were administered Methanol (200 and 400 mg/kg) and Chloroform-Methanol (200 and 400 mg/kg) extracts of *Salvia moorcroftiana*. All drugs were administered orally once daily for 21 days before the administration of Pilocarpine for inducing Status Epileptics. Experimental assessment of epileptic parameters was carried out including behavioral studies, biochemical Oxidative Stress indices, and neurodegeneration.

Behavioral studies

Pilocarpine Model of Status Epilepticus (SE) was used for inducing epilepsy. The dose of Pilocarpine (260 mg/kg I.P) was standardized for induction of SE in 100% of normal mice which was found in conformity with that of Lenz [27]. Groups (II-VIII) were administered a single dose of Pilocarpine (260 mg/kg; I.P.) for induction of SE. To

avoid side effects induced by peripheral cholinergic activation, mice were treated with atropine sulfate monohydrate (1 mg/kg, IP) 30 minutes before pilocarpine injection [27], while diazepam (10 mg/kg) was used to reduce pilocarpine-induced mortality in mice [28]. Thestandard AED i.e., Sodium Valproate was administered to Group III for the comparison, while extracts were administered to different groups (IV-VII) respectively. The prophylactic administration of the drug was continued orally once daily for 21 days before the administration of a single dose of Pilocarpine. The mice were then placed in Plexiglas chambers for behavioral monitoring of seizure progression for 60 min. The continuous seizure activity was recorded as per slight modification [29] with the addition of stage 6th. Stage 1: Immobilization and Staring Stage, Stage 2: Head Nodding Stage, Stage 3: Rearing accompanied by Forelimb Clonus and Wet Dog Shakes Stage, Stage 4: Falling and Wobbling Stage, Stage 5: Jumping, Circling, or Rolling Stage, Stage 6: Severe Tonic-Clonic Seizures. The highest score was recorded at intervals of every 3 minutes. The behavioral monitoring was done for 60 minutes. A substance was considered to possess anticonvulsant activity if it decreased seizure severity score and increased its onset time when compared to score in mice treated with Pilocarpine alone.

Biochemical estimations

Animals from each group were sacrificed by cervical dislocation, brains removed from the skull, Cleaned of blood in normal saline, hippocampus harvested from six of the isolated brains, and four brains preserved in formalin solution for conduction of histopathological studies. The biochemical estimations were performed on homogenized tissue. Hippocampus obtained from six animals was blotted dry, weighed, and homogenized in 0.01 mM phosphate-buffered saline (PBS) PH 7.4 (10% w/v). Homogenates were centrifuged to obtain post mitochondrial fluid (PMF/PMS) of the hippocampus which was subjected to biochemical estimations that included Protein levels [30], Thiobarbituric Acid Reactive Substance (TBARS) [31], Catalase [32] Superoxide dismutase [33].

Histopathological studies (Mice Hippocampus)

The tissue for micro-sectioning was processed by the paraffin embedding technique. Briefly, the brain sections with hippocampal regions were sliced into thin sections (3 min), Placed in perforated Cassettes, marked for identification, and washed with running water for 2 hours for removal of fixative. Dehydration by passing the tissue through graded alcohols, beginning with 70% alcohol and finally absolute alcohol. After dehydration, the alcohol was cleared out and replaced by a solvent in which paraffin is miscible. Acetone and Benzene were used as cleaning solvents. Followed by Paraffin impregnation/embedding, It was achieved by giving four changes one hour each, in the molten paraffin bath. The first change in a mixture of clearing agent and paraffin (1:1) hastens the process. The process was carried out in a thermostat paraffin oven maintained at a temperature slightly (1-2°C) above the melting point of paraffin wax i.e., 54-55°C. The impregnated tissue was submerged in molds containing molten paraffin wax, which was allowed to cool and marked for identification. 8-µm thick coronal sections, every 100 -µm from 2.3 to 4.3 mm posterior to the bregma with the help of microtome and mounted the sections on clean marked glass slides. Slides were examined under low (10X) and high power (40X) respectively to assess neuronal, cellular, and terminal degeneration. Neuronal damage or loss after 24 hours post pilocarpine administration was assessed by determining the frequency of pyknotic cells [34].

Statistical analysis

The quantitative data obtained from *in-vitro* antioxidant tests, Seizure Severity, latency to onset of status epilepticus, and Biochemical estimations was represented as Mean ± SEM and analyzed using one-way Analysis of Variance (ANOVA) followed by Dunnet comparison test. The toxic group was compared with the normal group and all other groups were compared with the toxic group. **p< 0.01, *p<0.05 were considered highly significant and significant in all cases, while the value of P >0.05 was considered as non-significant (ns). The data were analyzed using SPSS computer software package (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA)

Results

Physical characteristics and percentage yield of extracts

The percentage yield of extracts was calculated according to the below formula

$$\%\ yield = \frac{\text{Weight of extract obtained}}{\text{amount of material (WP)}\,taken} \times 100$$

Bel

Seizure severity: The seizure severity score revealed a highly significant (p<0.01) fall (3.26±0.27) in mice of group III as compared to that observed in toxic control (receiving pilocarpine only).The seizure severity score for Group I (Normal Control) was taken as zero. Pre-treatment with Methanol extract (200 and 400 mg/Kg) and Chloroform: Methanol extract (200 mg/Kg) in groups IV, V, and VI showed a significant fall in seizure severity while group VII receiving Chloroform: Methanol (400 mg/Kg) revealed highly significant(P<0.01) fall in the score (3.29±23) (Figure 1).

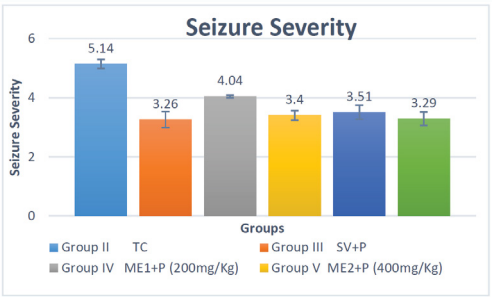

Figure 1: Effect of extracts of the whole plant of *Salvia moorcroftiana* and Sodium Valproate against pilocarpine-induced Seizure Severity in mice.

Latency to the onset of status epilepticus: The latency to seizure onset score revealed a highly significant (p<0.01) rise (50.5±2.66) in mice of group III as compared to that observed in toxic control (receiving pilocarpine only). Pretreatment with Methanol extract (200 and 400 mg/Kg) and Chloroform: Methanol extract (200 mg/Kg) in groups IV, V, and VI showed a significant rise in the latency to seizure onset score while group VII receiving Chloroform: Methanol (400 mg/Kg) revealed highly significant(P<0.01) rise in the score (45.10±3.10) (Figure 2).

Effect on biochemical parameters

Protein levels: The protein levels decreased (6.63±0.40) significantly (p<0.01) in mice of group II that had received the only pilocarpine as compared to the levels in control mice (Group I). Pretreatment with Methanol extract (200 and 400 mg/Kg) and Chloroform: Methanol extract (200 mg/Kg) in groups IV, V, and

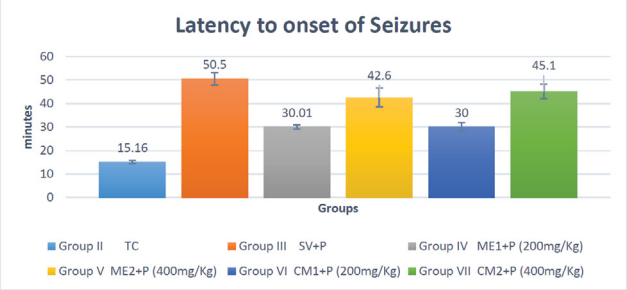

Figure 2: Effect of extracts of the whole plant of *Salvia moorcroftiana* and Sodium Valproate on Latency to the onset of Status Epileptics.

VI showed a significant rise in protein levels while group VII receiving Chloroform: Methanol (400 mg/Kg) revealed highly significant($P<0.01$) rise protein levels (9.86±0.20) (Table 2).

Thiobarbituric acid reactive substance (TBARS): The MDA levels increased (4.00±0.13) significantly ($p<0.01$) in mice of group II that had received the only pilocarpine as compared to the levels in control mice (Group I). Pretreatment with Methanol extract (200 and 400 mg/Kg) and Chloroform: Methanol extract (200 mg/Kg) in groups IV, V, and VI showed a significant decrease in MDA levels while group VII receiving Chloroform: Methanol (400 mg/Kg) revealed highly significant($P<0.01$) decrease in MDA levels (2.53±0.12) (Table 3).

Catalase (CAT): The catalase levels increased (28.21±1.10) significantly ($p<0.01$) in mice of group II that had received the only pilocarpine as compared to the levels in control mice (14.07±0.85) (Group I). Pretreatment with Methanol extract (200 and 400 mg/Kg) and Chloroform: Methanol extract (200 mg/Kg) in groups IV, V, and VI showed a significant increase in catalase levels while group VII receiving Chloroform: Methanol (400 mg/Kg) revealed highly significant($P<0.01$) increase in catalase levels (34.17±0.20), compared to the levels in the toxic group (Table 4).

Superoxide Dismutase (SOD): The Superoxide Dismutase SOD levels curtailed (1.86±0.09) insignificantly in mice of group II that had received the only pilocarpine as compared to the levels in control mice (2.41±0.20) (Group I). Pretreatment with Methanol extract (200 and 400 mg/Kg) and Chloroform: Methanol extract (200 mg/Kg and 400 mg/Kg) in groups IV, V, VI, and VII showed insignificant augmentation in SOD levels (Table 5).

Neurodegeneration: Two of the brains from each group preserved in 10% Formalin were subjected to histopathological procedures. Transverse section of normal mice brain of group I (Figure 3), administered normal saline for 21 days revealing normal morphology of neurons (H & E × 40X). The Histopathology of Brain Hippocampal and Cortical areas revealed condensation of pyramidal neurons in mice of group II (Figure 4). Transverse section of mice brain of group III (Figure 5), administered Sodium Valproate (300 mg/Kg) followed by a single dose of pilocarpine, revealing normal morphology of neurons**.** Pretreatment with Methanol extract (200 and 400 mg/Kg) and Chloroform: Methanol extract (200 mg/Kg) in groups IV (Figure 6), V (Figure 7), and VI (Figure 8) showed very mild condensation and degeneration of neurons. While group VII (Figure 9) receiving Chloroform: Methanol (400 mg/Kg) revealed almost normal morphology of neurons.

Table 1: Physical features and percentage yield of extracts observed in the whole plant of *Salvia moorcroftiana*.

S. No.	Type of extract	Color	Consistency	% age yield
1.	Methanol	Dark brown	sticky	7.99
2.	Chloroform+Methanol	Dark brown	sticky	7.3

Discussion

The extraction of the whole plant of *Salvia moorcroftiana* was performed by cold maceration using solvents Methanol and Chloroform: Methanol (1:1). Previous studies have reported that 4.2 kg of the whole plant of *Salvia moorcroftiana* gives 220 g of a crude extract with a percentage yield of 5%. Our studies have revealed that for methanol extract 500 g of the whole plant of *Salvia moorcroftiana* gives a percentage yield of 7.99% and for Chloroform: Methanol extract 500 g of the whole plant of *Salvia moorcroftiana* gives a percentage yield of 7.3% (Table 1).

In the current study, the preventive efficacy of extract was investigated for the amelioration of Pilocarpine induced Status Epilepticus and epilepsy-related neuronal damage. Pilocarpine is a muscarinic acetylcholine receptor agonist. Systemic or intracerebral injection of pilocarpine causes seizures that build up into a limbic SE. Structural damages and subsequent development of spontaneous recurrent seizures resemble those of human complex partial seizures [28]. Antiepileptic Drugs (AEDs) that are effective against complex partial seizures in humans can also halt spontaneous seizures in the pilocarpine model. Using the epilepsy model obtained by systemic administration of pilocarpine in mice, seizure severity, latency to onset of the seizure, protein estimation, lipid peroxidation (TBARS), catalase (CAT), and Superoxide Dismutase (SOD) activities and histopathological analysis were investigated in mice brain. Pretreatment with Methanol extract (200 and 400 mg/Kg) and Chloroform: Methanol extract (200 mg/Kg) in groups IV, V, and VI showed a significant fall in seizure severity while group VII receiving Chloroform: Methanol (400 mg/Kg) revealed highly significant fall in the score (Figure 1).

Furthermore, the latency to seizure onset score revealed a highly significant rise in mice of group III as compared to that observed in toxic control (receiving pilocarpine only). Pretreatment with Methanol extract (200 and 400 mg/Kg) and Chloroform: Methanol extract (200 mg/Kg) in groups IV, V, and VI showed a significant rise in the latency to seizure onset score while group VII receiving Chloroform: Methanol (400 mg/Kg) revealed a highly significant rise in score (Figure 2).

Protein estimation is based on the principle that under alkaline conditions Cu^{2+} forms a complex with the peptide bonds of protein and becomes reduced to Cu^{+}. The Cu^{+} as well as the groups of tyrosine, tryptophan, and cysteine residues, then reacts with folins reagent. The reagent reacts first by producing an unstable product which is slowly reduced to become molybdenum/tungsten blue and the blue-colored complex shows maximum absorption at 700 nm. Amino acids from proteins are used to synthesize the neurotransmitters that allow the brain to network and communicate properly [30]. In the present study, the protein levels were decreased in the hippocampus of mice administered pilocarpine (260 mg/Kg i.p) alone as compared to normal mice. Pretreatment with Methanol extract (200 and 400 mg/Kg) and Chloroform: Methanol extract (200 mg/Kg) in groups IV, V, and VI showed a significant rise in protein levels while group VII receiving Chloroform: Methanol (400 mg/Kg) revealed highly significant raise protein levels (Table 2). The damage caused by free radicals produced as a consequence of recurring seizures measured by lipid peroxidation assay. As an index of lipid peroxidation, the

Table 2: Effect of *Salvia moorcroftiana* and Sodium Valproate on Protein levels (g/dl) against Pilocarpine induced Status Epilepticus in mice.

S. No.	Group I NC	Group II TC	Group III SV+P	Group IV ME1+P (200 mg/kg)	Group V ME2+P (400 mg/kg)	Group VI CM1+P (200 mg/kg)	Group VII CM2+P (400 mg/kg)
1	16.35	8.25	13.15	7.85	9.15	9.25	9.75
2	15.15	6.15	11.15	7.15	9.75	9.15	10.15
3	13.25	7.25	12.35	8.25	8.65	9.2	9.85
4	15.25	6.15	12.65	9.05	7.95	8.75	8.95
5	17.15	6.5	14.15	8.15	9.25	9.15	10.25
6	14.55	5.45	11.95	7.95	8.15	9.25	10.25
Mean ± SEM	**15.28 ± 0.55****	**6.63 ± 0.40****	**12.56 ± 0.41****	**8.06 ± 0.25****	**8.81 ± 0.28****	**9.13 ± 0.07****	**9.86 ± 0.20****
p-value		p<0.01 (I *vs.* II)	p<0.01 (II *vs.* III)	p<0.01 (II *vs.* IV)	p<0.01 (II *vs.* V)	p<0.01 (II *vs.* VI)	p<0.01 (II *vs.* VII)

Table 3: Effect of extracts of whole plant of *Salvia moorcroftiana* and Sodium valproate on Hippocampal Lipid Peroxidation levels against Pilocarpine induced Status Epilepticus in mice.

S. No.	Group I NC	Group II TC	Group III SV+P	Group IV ME1+P (200 mg/kg)	Group V ME2+P (400 mg/kg)	Group VI CM1+P (200 mg/kg)	Group VII CM2+P (400 mg/kg)
1	1.15	4.52	1.75	3.85	3.15	3.25	3.05
2	1.34	4.13	2.05	3.45	2.95	3.05	2.65
3	1.52	3.85	1.96	4	2.06	4	2.55
4	1.91	4.05	2.45	3.25	4.04	2.95	2.4
5	1.56	3.55	2.35	3.05	3.52	2.1	2.45
6	1.54	3.95	2.05	4.15	2.65	3.55	2.1
Mean ± SEM	**1.50 ± 0.10**	**4.00 ± 0.13****	**2.10 ± 0.10****	**3.62 ± 0.17ns**	**3.06 ± 0.28****	**3.15 ± 0.26***	**2.53 ± 0.12****
p-value		p<0.01 (I *vs.* II)	p<0.01 (II *vs.* III)	p>0.05 (II *vs.* IV)	p<0.01 (II *vs.* V)	p<0.05 (II *vs.* VI)	p<0.01 (II *vs.* VII)

Table 4: Effect of extracts of whole plant of *Salvia moorcroftiana* and Sodium Valproate on Hippocampal Catalase levels against Pilocarpine induced Status Epilepticus in mice.

S. No.	Group I NC	Group II TC	Group III SV+P	Group IV ME1+P (200 mg/kg)	Group V ME2+P (400 mg/kg)	Group VI CM1+P (200 mg/kg)	Group VII CM2+P (400 mg/kg)
1	16.25	30.15	34.12	30.35	32.64	34.12	33.92
2	12.14	26.95	32.72	32.91	33.12	33.05	34.01
3	14.27	27	34.12	32.12	35.04	32.92	34.65
4	16.12	30.05	35.35	30.72	33.05	33.01	33.55
5	11.05	24.01	36.65	30.05	31.91	31.53	34.9
6	14.63	31.15	36.79	36.02	32.21	37.01	34.01
Mean ± SEM	**14.07 ± 0.85**	**28.21 ± 1.10****	**34.95 ± 0.65****	**32.02 ± 0.91****	**32.99 ± 0.45****	**33.60 ± 0.75****	**34.17 ± 0.20****
p-value		p<0.01 (I *vs.* II)	p<0.01 (II *vs.* III)	p<0.01 (II *vs.* IV)	p<0.01 (II *vs.* V)	p<0.01 (II *vs.* VI)	p<0.01 (II *vs.* VII)

Table 5: Effect of extracts of the whole plant of *Salvia moorcroftiana* and Sodium Valproate on Hippocampal Superoxide Dismutase (SOD) levels against Pilocarpine induced Status Epilepticus in mice.

S.No.	Group I NC	Group II TC	Group III SV+P	Group IV ME1+P (200 mg/kg)	Group V ME2+P (400 mg/kg)	Group VI CM1+P (200 mg/kg)	Group VII CM2+P (400 mg/kg)
1	2.45	2.1	2.1	2.05	2.15	2.19	2.25
2	1.95	1.85	2.25	2.15	2.43	2.3	2.33
3	2.86	1.47	2.32	2.01	2.2	2.05	2.15
4	2.25	1.84	2.3	2.03	2.09	2.13	2.15
5	3.12	2.05	2.41	1.95	2.1	2.21	2.37
6	1.88	1.86	1.95	1.85	2.02	2.02	2
Mean ± SEM	**2.41 ± 0.20**	**1.86 ± 0.09****	**2.22 ± 0.06****	**2.00 ± 0.04 ns**	**2.16 ± 0.05****	**2.15 ± 0.04***	**2.20 ± 0.05****
p-value		p<0.01 (I *vs.* II)	p<0.01 (II *vs.* III)	p>0.05 (II *vs.* IV)	p<0.01 (II *vs.* V)	p<0.05 (II *vs.* VI)	p<0.01 (II *vs.* VII)

formation of TBARS was measured, which is widely adopted as a sensitive method for the measurement of lipid peroxidation? The MDA levels increased significantly in mice of group II that had received the only pilocarpine as compared to the levels in control mice (Group I). Pretreatment with Methanol extract (200 and 400 mg/Kg) and Chloroform: Methanol extract (200 mg/Kg) in groups IV, V, and VI showed a significant decrease in MDA levels while group VII receiving Chloroform: Methanol (400 mg/Kg) revealed a highly significant decrease in MDA levels (Table 3).

Catalase enzyme affords protection to neuronal cells by removing free radicals. It reacts with H_2O_2 to form water and molecular oxygen and with H donors (ethanol, formic acid, methanol, or phenols) with peroxidase activity. It protects cells against H_2O_2 generated inside them. In the UV range, H_20_2 shows a continuous increase in absorption with decreasing wavelength. The decomposition of H_20_2 can be followed directly by an increase in absorbance at 240 nm. The difference in absorbance (δA) per unit time is a measure of Catalase activity [32]. In the present study, catalase levels increased significantly in mice of group II that had received the only pilocarpine as compared to the levels in control mice (Group I). Pretreatment with Methanol extract (200 and 400 mg/Kg) and Chloroform: Methanol extract (200 mg/

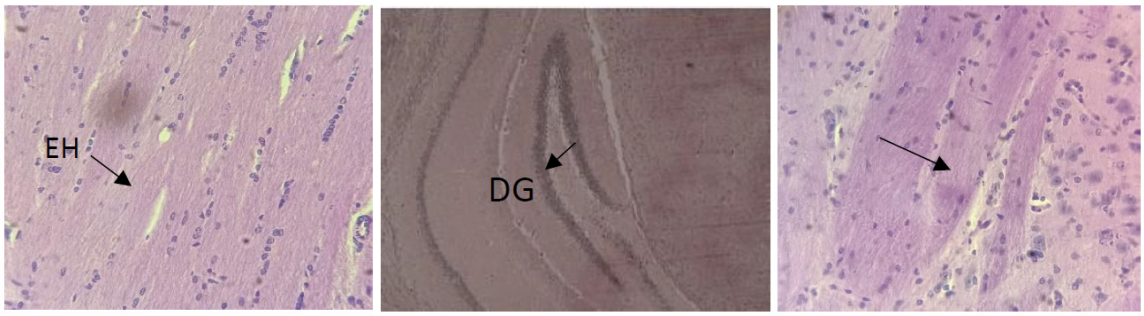

Figure 3: Transverse section of normal mice brain of group I, administered normal saline for 21 days revealing normal morphology of neurons (H & E × 40X) (EH: Extra Hippocampal; DG: Dantal Gyrus).

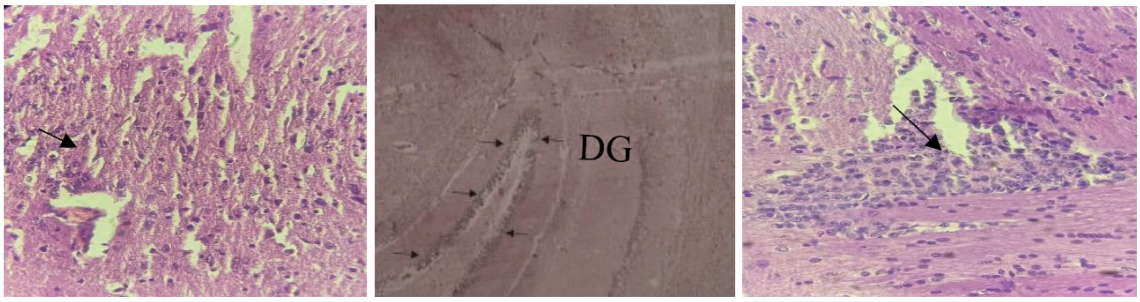

Figure 4: Transverse section of mice brain of group II, administered only Pilocarpine (260 mg/Kg, i.p) revealing condensation of pyramidal neurons (H&E × 40X) (DG: Dantal Gyrus).

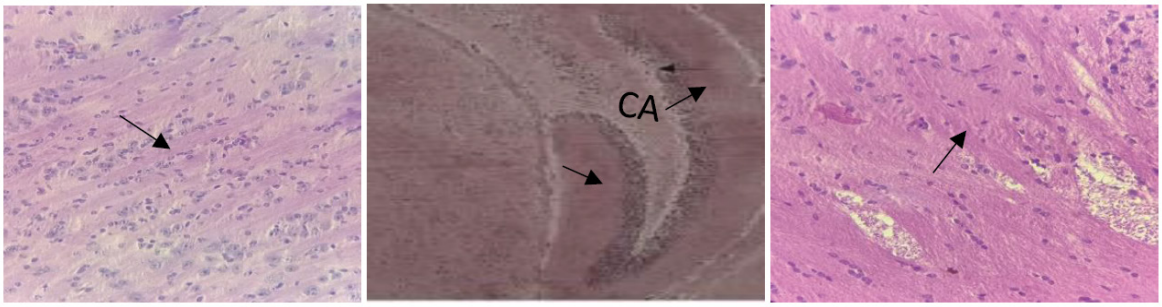

Figure 5: Transverse section of mice brain of group III, administered Sodium Valproate (300 mg/Kg) followed by a single dose of pilocarpine, revealing normal morphology of neurons (H & E × 40X) (CA: Cornu ammonis).

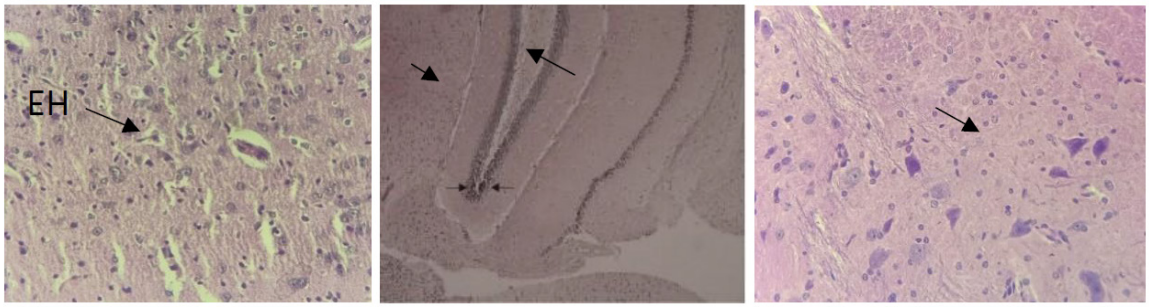

Figure 6: Transverse section of mice brain of group IV, administered methanol extract (200 mg/Kg) followed by a single dose of Pilocarpine, revealing mild condensation and degeneration of neurons (H & E × 40X) (EH: Extra Hippocampal).

Kg) in groups IV, V, and VI showed a significant increase in catalase levels while group VII receiving Chloroform: Methanol (400 mg/Kg) revealed a highly significant increase in catalase levels, compared to the levels in a toxic group (Table 4).

Superoxide ($O^{\cdot -}$), one of the reactive oxygen species generated by mitochondrial respiration, is involved in a variety of biological processes in the Central Nervous System. The superoxide dismutases (SODs) are enzymes that catalyze the conversion of ($O^{\cdot -}$) to hydrogen

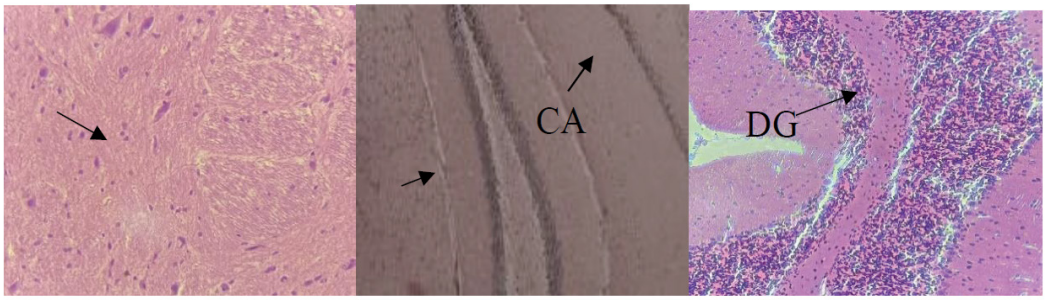

Figure 7: Transverse section of mice brain of group V, administered methanol extract (400 mg/Kg) followed by a single dose of Pilocarpine, revealing very mild condensation and degeneration of neurons (H & E × 40X).
CA: Cornu Ammonis; DG: Dentate Gyrus

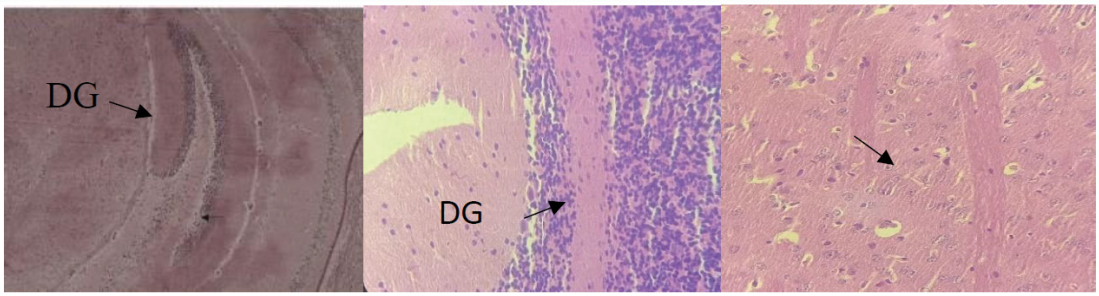

Figure 8: Transverse section of mice brain of group VI, administered Chloroform+Methanol extract (200 mg/Kg) followed by a single dose of Pilocarpine, revealing very mild condensation and degeneration of neurons (H & E × 40X) (DG: Dantate Gyrus,).

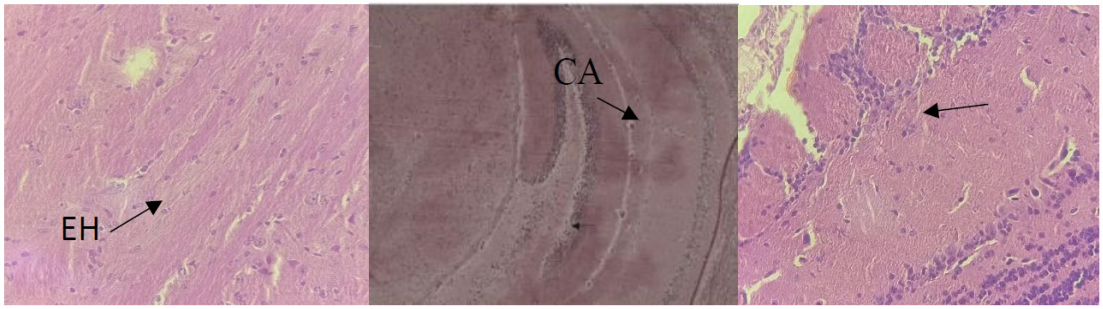

Figure 9: Transverse section of mice brain of group VI, administered Chloroform+Methanol extract (400 mg/Kg) followed by a single dose of Pilocarpine, revealing almost normal morphology of neurons (H & E × 40X). (EH: Extra hippocampal; CA: Cornuammonis).

peroxide and help prevent the build-up of toxic ($O^{\circ-}$) (Maier CM and Chan PH, 2002). The SOD levels decreased insignificantly in mice of group II that had received the only pilocarpine as compared to the levels in control mice (Group I). Pretreatment with Methanol extract (200 and 400 mg/Kg) and Chloroform: Methanol extract (200 and 400 mg/Kg) in groups IV, V, VI, and VII showed an insignificant increase in SOD levels, compared to the levels in a toxic group (Table 5).

Histopathological changes have been observed in different hippocampal as well as Extra-Hippocampal (EH) areas as a consequence of pilocarpine-induced SE. The hippocampus, one of the important parts of the brain consists of complex interfolded layers of the Dentate Gyrus (DG) and Cornu Ammonis (CA). Each region of the hippocampus includes the various subfields and lamellae which play role in the storage and retrieval of episodic memory. The main target of hippocampal damage by pilocarpine is CA3 and CA1 region of the dorsal hippocampus. In the present study. The destruction of neurons along with neuronal condensation and necrosis in DG, CA1, CA2, and CA3 was observed in the mice treated with pilocarpine alone as compared to normal mice. Pretreatment with Methanol extract (200 and 400 mg/Kg) and Chloroform: Methanol extract (200 mg/Kg) in groups IV, V, and VI showed mild condensation and degeneration of neurons. While group VII receiving Chloroform: Methanol (400 mg/Kg) revealed almost normal morphology of neurons.

Conclusion

The present study concludes that Methanol and Chloroform: methanol extracts of *Salvia moorcroftiana* are potential candidates against chemically induced seizures and neurodegeneration since these extracts restrain important biomarkers of oxidative stress in an animal model of epilepsy. These properties make this plant pre-clinically useful. Further detailed mechanistic studies are necessary to divulge the beneficial effects of these extracts before proceeding with clinical trials.

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