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RESEARCH ARTICLE

EXPLORATION OF ANTI-EPILEPTIC PROPERTY OF METHANOLIC EXTRACTION OF *OCIMUM BASILICUM*

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Abstract: The goal of the current investigation was to determine if a methanolic extract of *Ocimum Basilicum* (O.B.) leaves could protect against phenytoin induced Epilepsy. Epilepsy was studied using dosages of 400mg/kg is more effective than 200 mg/kg of methanolic extract of O.B. The extract was administered, which improved improve behavioral disturbances, oxidative stress can be reduced and antioxidant enzymes can be recovered. The results of this investigation showed that rats treated with a methanol extract of O.B. leaves have neuroprotective potential. Therefore, it can be inferred that *Ocimum basilicum* may be useful in the treatment of Epilepsy disease.

Keywords: *Ocimum Basilicum (O.B), Leaves, Phenytoin, Rat.*

INTRODUCTION

Epilepsy is the most common serious neurological disease, distributed worldwide and affecting all ages and races. The incidence is about 50 cases per 100,000 persons per year in developed societies and 100-190 per 100,000 in developing countries. Epilepsy is not a disease, but a syndrome of different cerebral disorders of the central nervous system (CNS), which is characterized by paroxysmal, excessive, and hypersynchronous discharges of large numbers of neurons [1,2].

Epilepsy can have substantial physical, psychological and social impact on patients and carries serious risks of injury, impairment of brain function and death [3]. Conceptually, epilepsy exists after at least one unprovoked seizure, when there is high risk for another, although the actual required risk is subject to debate. After a single unprovoked seizure, risk for another is 40-52 % [4, 5]. With two unprovoked non-febrile seizures, the chance by 4 years of having another is 73%, with a 95% confidence interval (CI) of 59-87 %, subsequently herein portrayed as approximately 60-90%.

The "two unprovoked seizure" definition of epilepsy has served us well, but it is inadequate in some clinical circumstances. A patient might present with a single unprovoked seizure after a remote brain insult, such as a stroke, central nervous system (CNS) infection, or trauma. A patient with such brain insults has a risk of a second unprovoked seizure that is comparable to the risk for further seizures after two unprovoked seizures [6].

Thus, there is a need for novel, more potent pharmaceutically active substances, such as plant extracts, which come from natural sources. Recent investigations have emphasized the potent neuro-protective effects of medicinal plant extracts and phytochemicals in the alleviation of Epilepsy symptoms due to their antioxidant and anti-inflammatory characteristics [7]. *Ocimum Basilicum* (OB), a member of the Lamiaceae family, is a medicinal plant that is generally referred to as "sweet basil" in English and Tulsi" in Hindi. It is one of the holy plants for Hindus on the Indian subcontinent and grows in tropical and subtropical climates [8].

Traditionally, basil has been used as medicinal plant in the treatment of headache, cough, constipation, diarrhoea, worms, warts, antiemetic, pains, diabetes and kidney malfunction. Major aroma compounds from volatile extracts of basil present anti-oxidative activity [9].

The present study was designed to assess the potential of *Ocimum Basilicum* L. methanolic extract (OBME) for the management of Epilepsy on the basis of scientific grounds by using a phenytoin-induced Epilepsy animal model [10].

MATERIALS ANDMETHODS

Collection of Plant Material

Fresh leaves of *Ocimum Basilicum* were obtained from local area in Siruli Mahavir, Puri District, Odisha (Latitude 19º 53' 13.3"N and Longitude 85º 42' 11.9" E). This leaf was authenticated by Mr. Ranjan Jena, Head of Department. Pharmacognosy, College of Pharmaceutical Sciences, Puri, Odisha. Greenish leaves of the plant were washed thoroughly with the help of tap water, and shade dried at room temperature. All dried materials were powdered and sieving under sieve number 10.

Preparation of Methanolic Extraction

The sample was extracted with methanol following a previously reported method with a slight modification [11]. 220 g of the powdered sample was extracted with 1100 ml of methanol for 6-7 h in a Soxhlet apparatus [12]. The extracts were kept in desiccators which allow the solvent is completely evaporated. The extract was filtrated; the yielding is greenish-black sticky residue. Finally, 60.64 g yield of crude drug was collected and kept in freeze.

Phytochemical Screening

There are various phytochemicals are present in the methanolic leaf extracts were screened as per standard protocols for the presence of phytochemical constituents such as glycosides, tannins, phenols, flavonoids.

Thin Layer Chromatographic Analysis

On a glass slide with pre-applied silica gel measuring 20 by 20 cm, TLC was performed. Added 10 l of *Ocimum Basilicum* extract to the surface. The extract-loaded plates were maintained in a chromatographic chamber and operated in a 7:3 chloroform: ethanol solvent system (280 ml: 120 ml) for 10 to 15 minutes [13]. It was then exposed to iodine vapour once more in a glass beaker filled with iodine crystals. The sample run spot's colour changed to indicate the presence of phenols. The ratio of the compound's or solvent's travel distance to the solvent's travel distance over a specified period of time is known as the retention factor (Rf). The sample site was then measured.

Experimental Design

Two healthy albino Wistar rats weighing 150-200 g of either sex was used for in vivo anti-Epileptic activity. The rats were acquired and placed in an animal house under proper conditions (12-hour light and dark cycle, room temperature 25°C) in polypropylene cages. The experiments were carried out as per the guideline of CPCSEA, New Delhi, India. Bearing Regd. No. 2235/PO/Re/S/23/CCSEA.

Evaluation of Anti-Epileptic Activity

Disease Induction

In rats, phenytoin (1 mg/kg) was given once daily (intraperitoneally) for twenty-one days to induce Epilepsy disease except the normal control group. It was injected before one hour of extract treatment [14].

Behavioural Analysis

The MES (Maximal Electroshock Seizure), ICES (Intermittent Cortical Electrical Stimulation) were conducted to investigate the behavioural analysis [15].

MES (Maximal Electroshock Seizure) Technique

MES, or Maximal Electroshock Seizure, is a commonly used method in animal models to induce generalized tonic-clonic seizures, which are characteristic of epilepsy. The albino Wistar rat is one of the most frequently used animal models in epilepsy research. To conduct a behavioural study of epilepsy using the albino Wistar rat model and MES, the following steps can be taken:

Animal Selection: Albino Wistar rats are chosen for their suitability in epilepsy research. These rats are widely available and have been extensively used in various studies.

Animal Housing: The albino Wistar rats

should be housed in a controlled environment with appropriate temperature, humidity, and lighting conditions. They should have access to food and water ad libitum.

Electrode Implantation: Electrodes are surgically implanted in the brain to stimulate seizures. The placement of electrodes depends on the specific research objectives and may involve the hippocampus, cortex, or other relevant brain regions associated with epilepsy.

MES Induction: Once the animals have fully recovered from surgery, the MES protocol can be initiated. The MES involves delivering a high-intensity electrical stimulation through the implanted electrodes to induce a generalized seizure. The parameters of stimulation, such as duration, frequency, and intensity, should be standardized to ensure consistency across experiments.

Observation and Recording: During and after MES induction, the behaviour of the rats should be carefully observed and recorded. This may include monitoring motor activity, convulsions, loss of consciousness, or other seizure-related behaviours.

Seizure Severity Scoring: A standardized seizure severity scale, such as the Racine scale, can be employed to quantify the intensity and progression of seizures. This allows for consistent comparison between animals and experimental groups.

Data Analysis: The recorded behavioural data can be analysed using appropriate statistical methods to assess the effects of different experimental conditions, interventions, or drug treatments on seizure severity and behaviour.

Ethical Considerations: It is crucial to adhere to ethical guidelines and regulations when conducting experiments involving animals. All procedures should be approved by an institutional animal care and use committee (IACUC) or a similar regulatory body.

By utilizing the MES technique in albino Wistar rats and conducting behavioural observations, researchers can gain insights into the pathophysiology of epilepsy, evaluate potential antiepileptic drugs, and explore various therapeutic interventions for this neurological disorder.

ICES (Intermittent Cortical Electrical Stimulation) Technique

Select a sample of albino Wistar rats and divide them into experimental and control groups. The experimental group will undergo electric shock-induced seizures, while the control group will not. Randomly assign the rats to each group to minimize bias.

Seizure Induction: Determine the appropriate intensity and duration of electric shock to induce seizures in the rats. Administer the electric shocks to the experimental group rats in a controlled manner, ensuring the safety and ethical guidelines for animal experimentation are followed.

Seizure Monitoring: Use video recording or other monitoring techniques to document the occurrence and duration of seizures in the experimental group. Establish a seizure rating scale to classify and quantify the severity of each seizure based on observable behavioural manifestations.

Behavioural Assessments: Conduct pre- and post-seizure assessments on both the experimental and control groups to compare changes in behaviour. Implement a battery of behavioural tests such as open field test, elevated plus maze, Morris water maze, and novel object recognition to evaluate changes in locomotion, anxiety-like behaviour, spatial memory, and recognition memory. Perform the assessments at regular intervals following the induction of seizures to capture short-term and long-term effects.

Data Analysis: Analyse the collected data using appropriate statistical methods. Compare the behavioural outcomes between the experimental and control groups to determine the effects of electric shock-induced seizures. Consider factors such as seizure severity, frequency, and duration in relation to behavioural changes.

Ethical Considerations

Ensure the study adheres to ethical guidelines for animal research and has

received approval from the relevant institutional animal care and use committee. Minimize any potential harm or distress to the animals during the study. Implement appropriate measures for animal welfare, including housing, feeding, and veterinary care.

RESULTS AND DISCUSSION

Phytochemical Analysis

The results of the preliminary phytochemical screening of methanolic leaf extracts of *Ocimum basilicum* as summarized in Table 1 revealed the presence of phenols, flavonoids, tannins, glycoside and saponins.

Table 1: Phytochemical screening of methanolic leaf extracts of Ocimum Basilicum

Phytochemical compound	Method	Ocimum Basilicum
Flavonoids	Lead acetate solution Test	+
Tannins	Ferric chloride Test	+
Glycosides	Keller Killiani Test	-
Phenolics	Ferric chloride Test	+
Saponins	NaOH Test	+

Behavioural Analysis

MES (Maximal Electroshock Seizure) Technique

The electrical stimulus (50 mA, 50 Hz, 1-s duration) was applied through ear clip electrodes using a stimulator apparatus. Six groups of 10 albino wister rat each pre-treated i.e. with the extract (200 & 400 mg/kg), the solvent of the extract (10 mg/kg, as control), phenytoin (25 mg/kg, as positive control). After 30 min, the animals received trans-auricular electroshock. Abolition of Hind Limb Tonic Extension within 10 s after delivery of the electroshock was the criterion for anticonvulsant effect. when compared to phenytoin treated group as shown Whereas, 400mg/kg is more effective than 200 mg/kg.

Table 2: Effect of O. Basilicum on MES test in phenytoin treated rat

	Mean	SD
Control	51.5	4.46
Phenytoin	8.83	1.6
OCE 200	15	3.16
OCE 400	8.83	1.6

ICES (Intermittent Cortical Electrical Stimulation) Technique

Electroshock seizures were induced by application of electrical stimulation via earclip electrodes. The initial stimulation was a 100 Hz, 5 ms square wave with an intensity of 10 mA; the intensity of successive stimulations was linearly increased by 1 mA/1 s, with successive stimulations separated by 1 min [16-19]. This procedure was repeated until tonic hind limb flexion was observed, allowing for the identification of a seizure current threshold for each individual rat. *Ocimum Basilicum* 200 mg/kg and 400 mg/kg groups showed significant decrease. when compared to phenytoin treated group as shown in Table 3. Whereas, 400mg/kg is more effective than 200mg/kg.

Table 3 : Effect of O. Basilicum on ICES test in phenytoin treated rat

	Mean	SD
CONTROL	85	7.74
PHENYTOIN	137.5	11.29
OCE 200	85	7.74
OCE 400	107.5	11.29

DISCUSSION

Epilepsy is a chronic non-communicable disease of the brain that affects around 50 million people worldwide. It is characterized by recurrent seizures, which are brief episodes of involuntary movement that may involve a part of the body (partial) or the

entire body (generalized) and are sometimes accompanied by loss of consciousness and control of bowel or bladder function. Seizure episodes are a result of excessive electrical discharges in a group of brain cells. Different parts of the brain can be the site of such discharges. Seizures can vary from the briefest lapses of attention or muscle jerks to severe and prolonged convulsions. Seizures can also vary in frequency, from less than one per year to several per day.

Characteristics of seizures vary and depend on where in the brain the disturbance first starts, and how far it spreads. Temporary symptoms occur, such as loss of awareness or consciousness, and disturbances of movement, sensation (including vision, hearing and taste), mood, or other cognitive functions.

People with epilepsy tend to have more physical problems (such as fractures and bruising from injuries related to seizures), as well as higher rates of psychological conditions, including anxiety and depression. Similarly, the risk of premature death in people with epilepsy is up to three times higher than in the general population.

Epilepsy is not contagious. Although many underlying disease mechanisms can lead to epilepsy, the cause of the disease is still unknown in about 50% of cases globally. The causes of epilepsy are divided into the following categories: structural, genetic, infectious, metabolic, immune and unknown. Examples include: brain damage from prenatal or perinatal causes (e.g. a loss of oxygen or trauma during birth, low birth weight),congenital abnormalities or genetic conditions with associated brain.

The use of anti-epileptic drugs (AEDs) is the standard medical treatment for epilepsy and it is effective in controlling seizures in about two-thirds of people with epilepsy. AEDs act by suppressing the over excitability of cortical neurons either by directly stabilising the nerve membrane, enhancing the activity of inhibitory transmitter Gamma aminobutyric acid (GABA) or a combination of both mechanisms. Some drugs mimic, neutralise or prolong the effect of neurotransmitters such as GABA (Brown, 2016) identified three ways by which AEDs exert their effects:

By mainly targeting sodium and calcium channels to modulate the intrinsic membrane conducting activities to inhibit excessive firing of neurons, Inhibit GABA metabolism or block GABA transport, Inhibit the excitatory mechanism, mainly the glutamatergic system.

There is a wide range of effective AEDs which are available in tablet, capsule, injection or as syrup for children. However, the strategy of treatment should be individualised based on the type and severity of symptoms, co-medication and co-morbidity, previous response to drug and patient's preference (NICE 2016).

According to the British National Formulary (BNF) (2017), drugs used to control epilepsy include Oxcarbazepine, primidone, clobazam, retigabine, phenytoin, topiramate, valporic acid, vigabatrin, carbamazepine, ethosuximide, phenobarbital, and levetiracetam In the UK, initiation and subsequent drug management (first line treatment and adjunctive) is informed by NICE guidance.

Oxidative stress is known to be present in epilepsy as a cause or/and a consequence of epileptogenesis process. Furthermore, it was also shown that long-term treatment with AEDs can cause elevated free radical production in neuronal cells and consequently increased oxidative damage, leading to neurodegeneration. Several conventional AEDs are known to be extensively metabolized in the body.

Through their metabolic transformation, numerous reactive metabolites, which are capable of covalent binding to biological macromolecules, are formed. Thus, the AEDs may, besides their main inhibitory activity on the epileptic focuses, also provoke systemic toxicity, either through increased oxidative

damage or covalent binding of their reactive metabolites to biological macromolecules. Hence, it is of particular importance to find out the impact of AEDs on oxidative stress. Ocimum Basilicim having anti-oxidant properties it reduce the oxidative stress and other symptoms which are shows by epilepsy disease.

CONCLUSION

In this study, the therapeutic potential of *Ocimum Basilicum* in Epilepsy disease was investigated.

The antioxidant potential of *Ocimum Basilicum* was evaluated through behavioural studies and its anti-Epilepsy activity was confirmed. It has been found to improve behavioural disturbances in a phenytoin induced Epilepsy rat model. It has been observed that, oxidative stress can be reduced and antioxidant enzymes can be recovered. Therefore, it can be concluded that this Ocimum Basilicum might have a potential in treatment of Eplilespy.

REFERENCES

1. Abdullatif BM, Asiri NA. Effect of Deficit Irrigation on Photosynthesis Pigments, Proline Accumulation and Oil Quality of Sweet Basil (Ocimum basilicum L.) at Flowering and Seed Setting Stages. Int J Biol Pharm Allied Sci 2012;1(3):271-284.
2. Baykan B, Altindag EA, Bebek N, Ozturk AY, Aslantas B, Gurses C, et. al. Myoclonic Seizures Subside in the Fourth Decade in Juvenile Myoclonic Epilepsy. Neurology 2008; 70:2123-2129.
3. Baykan B, Martinez-Juarez IE, Altindag EA, Camfield CS, Camfield PR. Lifetime Prognosis of Juvenile Myoclonic Epilepsy. Epilepsy Behav. 2013;28 (Suppl 1):S18-24.
4. British Epilepsy Association (BEA) (2003) Seizures explained. BEA, Leeds. Blundell J. (2006) Neurologic health breakdown. In Chang E, Daly J, Elliott D. (eds). Pathophysiology: Applied to Nursing Practice. Elsevier, Australia: 225-241
5. Camfield CS, Camfield PR. Juvenile Myoclonic Epilepsy 25 Years after Seizure Onset: A Population-based Study. Neurology 2009;73:1041-5.
6. Crespel A, Gelisse P, Reed RC, Ferlazzo E, Jerney J, Schmitz B, Genton P. Management of Juvenile Myoclonic Epilepsy. Epilepsy Behav 2013; 28 (Suppl 1):S81-6.
7. Dejanovic B, Lal D, Catarino CB, Arjune S, Belaidi AA, Trucks H et. al. Exonic Microdeletions of the Gephyrin Gene Impair GAB Aergic Synaptic Inhibition in Patients with Idiopathic Generalized Epilepsy. Neurobiol Dis 2014;67:88-96.
8. Deppe M, Kellinghaus C, Duning T, Möddel G, Mohammadi S, Deppe K. Nerve Fiber Impairment of Anterior Thalamocortical Circuitry in Juvenile Myoclonic Epilepsy. Neurology 2008; 71:1981-1985.
9. Dodson WE, Definitions and Classification of Epilepsy, in: Shorvon, SD, Fish, DR, Perruca E, Dodson WE (Eds.), The Treatment of Epilepsy. Blackwell Science Ltd, Oxford, pp. 3-20.
10. Javed H, Nagoor Meeran MF, Azimullah S, Adem A, Sadek B, Ojha SK. Plant Extracts and Phytochemicals Targeting α-Synuclein Aggregation in PD Models. Front Pharmacol 2018;9:1555.
11. Hossain, MA, Kabir M, Salehuddin S, Rahman SM, Das A, Singha SK et. al. (2010) Antibacterial Properties of Essential Oils and Methanol Extracts of Sweet Basil (Ocimum Basilicum) Occurring in Bangladesh. Pharm. Biol. 48;504-511.
12. Soran ML, Cobzac Codruta S, Varodi C, Lung I, Surducan, E, Surducan V. The Extraction and Chromatographic Determination of the Essential Oils from *Ocimum Basilicum L.* by Different Technique. J Phys Conf ser 82;012-016.
13. Divisha R, Ranganathan V, Vijayakaran K, Elamaran A. Evaluating *Ocimum Basilicum* and *Ocimumte Tenuiflorum* Leaf Extracts for the Presence of Phenolic Compounds. J Pharmacogn Phytochem 2018;7(6):2453-2456.
14. Hanif AM, Al-Maskari YM, Al-Maskari A, Al-Shukaili A, Al-Maskari YA, Al-Sabahi, NJ. Essential Oil Composition, Antimicrobial and Antioxidant Activities of Unexplored Omani Basil. Journal of Medicinal Plants Research 2011;5(5):751-757.
15. Jaliwala YA, Panda PK, Patro VJ, Chourasia N, Bhatt NK, Amit P et. al. Pharmacognostic Preliminary

Phytochemical Screening of Ficusarnottianamiq. Journal of Current Pharmaceutical Research 2011;6(1):21-27

16. Lin K, Carrete H, Lin J, Peruchi MM, de Araújo Filho GM, Guaranha MSB et. al. Magnetic Resonance Spectroscopy Reveals an Epileptic Network in Juvenile Myoclonic Epilepsy. Epilepsia 2009a; 50:1191-200.
17. O'Muircheartaigh J, Vollmar C, Barker GJ, Kumari V, Symms MR, Thompson P *et. al.* Abnormal Thalamocortical Structural and Functional Connectivity in Juvenile Myoclonic Epilepsy. Brain 2012; 135:3635-44.
18. Dunham NW, Miya TS. A Note on a Simple Apparatus for Detecting Neurological Deficit in Rats and Mice. J Am Pharm Assoc Am Pharm Assoc (Baltim) 1957; 46(3):208-9.
19. Kashyap CP, Ranjeet K, Vikrant A, Vipin K. Therapeutic Potency of Ocimum Kilimandscharicum Guerke -A Review. Global J Pharmacol 2011;5(3):191-200.