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PHARMACOLOGICAL EVALUATION AND COMPARISON OF ANTI-EPILEPTIC ACTIVITY OF OCIMUM BASILICUM LINN AND OCIMUM SANCTUM LINN IN ALBINO RATS

Mansi Gupta*, Amrita Singh, Snehil Singh and Bhanu P. S. Sagar

IEC Department of Pharmacy, IEC College of Engineering & Technology, IEC Group of Institutions, Greater Noida, Gautam Budha Nagar, Uttar Pradesh, India.

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***Corresponding Author**
Mansi Gupta
IEC Department of Pharmacy, IEC College of Engineering & Technology, IEC Group of Institutions, Greater Noida, Gautam Budha Nagar, Uttar Pradesh, India.

ABSTRACT

Present work was envisaged to perform research on pharmacological investigations to assess and compare pharmacological activities of *Ocimum basilicum* Linn. (Sweet basil) and *Ocimum sanctum* Linn. (Shyama/holy basil) in albino rats. PPMs and SPMs such as phenolics, flavonoids, alkaloids, proteins, tannins, steroids, glycosides, terpenes, amino acids, carbohydrates, and reducing sugars were found in the qualitative analysis of EEOBL and EEOSL leaves extracts. High concentration of TFC was found in EEOBL extract in comparison of EEOBL extract. In Physico-chemical analysis, high water soluble and ethanol soluble extractive values indicated that leaves powders contain water soluble salts. The fluorescence analysis indicated that leaves powders contains high concentration of phenolic and flavonois as SPMs. EEOBL and EEOSL extracts caused no significant change blood parameters and LFTs. The EEOBL and EEOSL extracts induced a very wide safety margin (no mortality upto 2000 mg). Finally, in *in-vivo* anti-epileptic activity, EEOSL-B extract was found to be more effective than EEOSL-B but less effective than standard drug Phenytoin / Saraswat Syrup. Finally, combination of EEOBL-B and EEOSL-B very significantly decreased convulsion. Much more elaborated pharmacological research is proposed.

KEYWORDS: Anti-epileptic activity, Antioxidant, *Ocimum basilicum, Ocimum sanctum,* phenytoin, saraswat syrup, secondary plant metabolites, acute toxicity, sub-acute toxicity, shyama / holy basil, sweet basil.

INTRODUCTION

Epilepsy

Najim (2018), people with epilepsy come from all walks of life, all social classes, all geographic places, and all races. This is not a single disease; rather, it is a group of symptoms that indicate underlying brain diseases. Atypically synchronously fired neurons are produced by an aberrant dynamism of neural networks, which causes recurrent spontaneous seizures with a multifactorial aetiology. Patel and Moshe (2020), the English word epilepsy comes from the Greek word epilambanein, which meaning "to be seized." This meant both the illness and the isolated incident. The phrase alludes to the period's mystical beliefs, which contributed to the stigma attached to epilepsy because those who had it were viewed as filthy or evil. Fisher et al., 2014 reported that the epilepsy disease results from abnormal activities in the brain caused due to an inherited genetic condition, trauma to head, or diseases and developmental disorders of the brain). According to Ngugi et al., 2010, epilepsy is the second most common neurological disorder that occurs often. It has a substantial impact on patients, their families, and the healthcare system. A recent study found that nearly 90% of the 70 million epileptics worldwide live in developing countries.

Devinsky *et al*., 2018, one of the most prevalent and incapacitating long-term neurological conditions which is defined by a persistent inclination to cause epileptic seizures and the ensuing social, psychological, and cognitive ramifications. Scheffer *et al*., 2017, aetiology of epilepsy is poorly understood and encompasses a wide range genetic mutations, infectious diseases (like neurocysticercosis and cerebral malaria), autoimmune disorders, and acquired causes (like stroke).

Newton and Garcia, (2012), revealed that epilepsy has a high prevalence among people of all ages and it has been estimated that 70 million people worldwide live with epilepsy, and that >85% of this disease occurs in low-income and lower middle-income countries. Chen *et al*., 2020, as of right now, there is no known cure for epilepsy, hence pharmaceutical treatment for symptoms is still the cornerstone of treatment. Antiseizure drugs (ASMs) by definition stop or lessen the onset, spread, and intensity of epileptic seizures. Browne *et al*., 1978, most crucial goal in treating epilepsy is to achieve total control of seizures. In order to achieve this goal, patients with spontaneous recurrent seizures (SRS) are given ASMs on a continuous basis to prevent the recurrence of seizures.

Causes of Epilepsy (Singh and Trevick, 2016)

Causes of epilepsy are unknown and include the following:

- Low oxygen during birth; Stroke;
- Head injuries (during birth) or accidents during youth or adulthood;
- Brain tumors; Brain injury due to trauma;
- Brain infection, such as meningitis;
- Malformation of an area of the brain;
- Genetic factors.

Symptoms of epilepsy

Symptoms of epilepsy vary greatly between individuals and depend on the type ofseizure. Symptoms (Can be mild to severe in form) include:

- Euphoria during aura (Before the episode);
- Episodes of staring blankly; Temporary confusion;
- Uncontrollable jerking movements or twitching of the arms and legs;
- Psychological symptoms such as fear and anxiety;
- Loss of consciousness or awareness.

Pathophysiology of Epilepsy (Fisher *et al*., 2017)

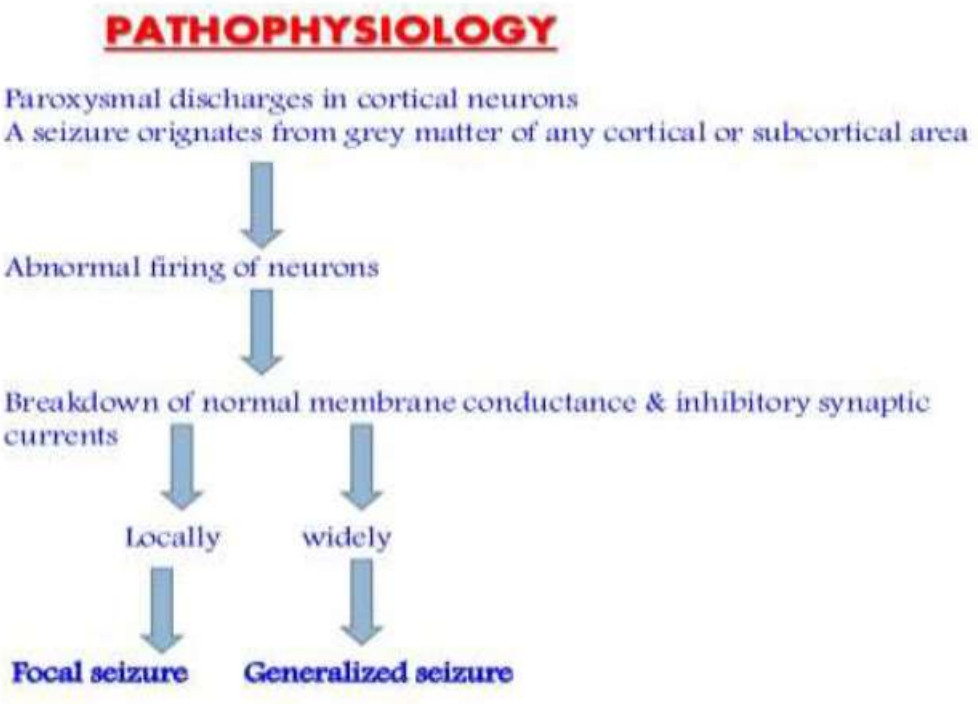

Fig. 1: Pathophysiology of Epilepsy (Galanopoulou, 2008).

Types of seizures

Common types of seizures include:

(i) Generalized Onset – Tonic- Clonic (Grand Mal)

(ii) Generalized Onset – Absence (Petit Mal)

(iii) Focal Onset - Impaired Awareness (Complex Partial)

(iv) Focal Onset - Aware (Simple Partial)

(v) Atonic (Drop Attacks)

(vi) Myoclonic

Assessment & diagnostic methods (Xu and Nguyen, 2016)

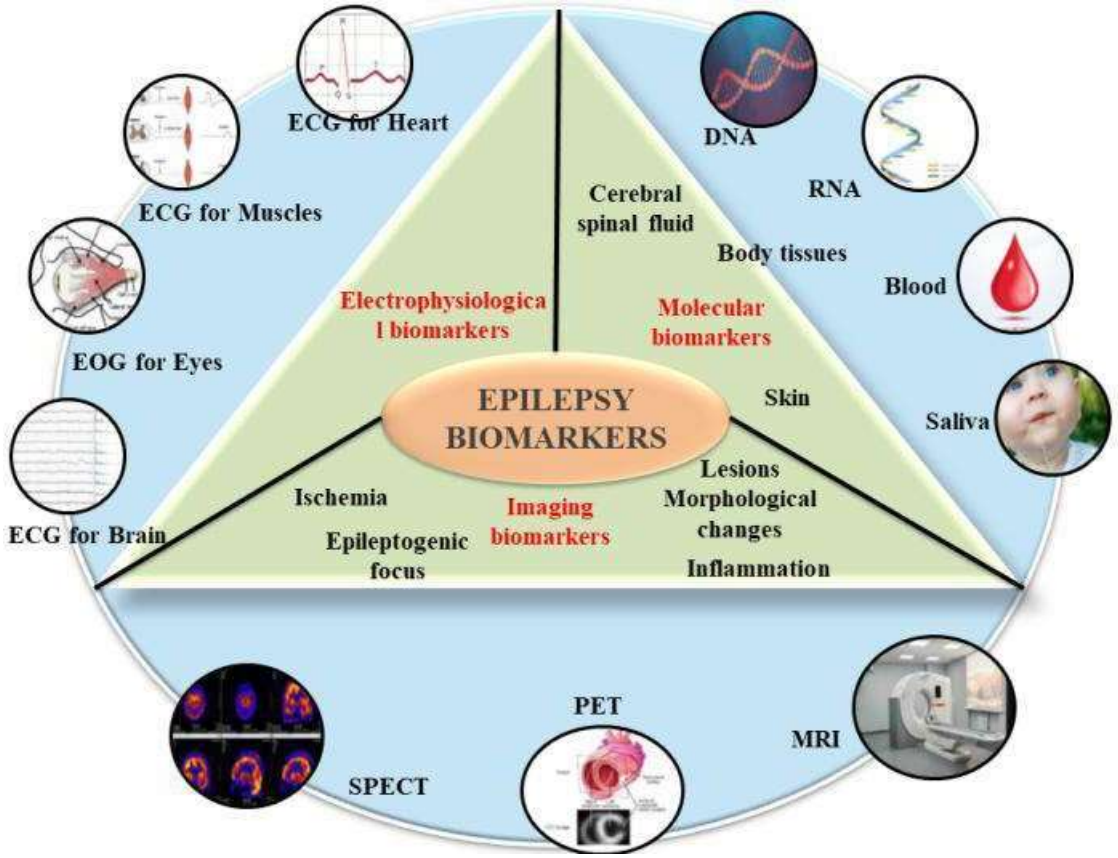

Fig. 2: Biomarkers for diagnosis and treatment of Epilepsy.

Tiwari *et al*., 2019, epilepsy biomarkers can be categorised using genetic biomarkers, electrophysiological signals, and neuroimaging. The study of electrical activity, including as interictal spikes, and known as ophysiology.

The brain, heart, and muscles are examined with electrocorticography (ECoG), electrocardiography (ECG), electromyography (EMG), and electroencephalography (EEG), in that order. Imaging biomarkers can be used to diagnose lesions, traumas, or epileptiform

abnormalities utilising imaging technologies as well as the levels of metabolites in blood or tissues, such as proteins, neuropeptides, enzymes, etc., in a manner that correlates with different elements of the disease.

Treatment of epilepsy

Stephen and Brodie (2009), the phrases "anticonvulsant" and "antiepileptic" are synonymous. An antiepileptic medication is one that is administered to treat the 32 epilepsies, whereas an anticonvulsant is a chemical that keeps lab animals from having seizures during tests. Principles of management any brain tumours or other conditions that trigger epilepsy must be treated. Patients must be made aware of the condition, the duration of the treatment plan, and the need of adhering to it. Avoiding triggers including alcohol, sleep deprivation, and mental stress is advised. It's reasonable to expect some natural variation; for instance, women may experience fits more frequently or only during their periods. There are now roughly 30 ASMs / CSMs available for the treatment of epilepsy.

According to Forsgren *et al*., 2005, antiepileptic dugs (AEDs) can reduce epileptic seizures. There are a plethora of allopathic medications available for epilepsy; however, each drug comes with adverse effects (Vyawahare *et al*., 2007). There are a plethora of allopathic medicines available to treat epilepsy, but they all have a plethora of negative side effects. Olufunmilayo *et al*., 2007 revealed that there are so many allopathic medicine available to treat epilepsy but all are having numerous side effects.

There are three main processes involved in action of antiepileptic drugs:

(i) Reducing the voltage-dependent sodium channels hence decreasing the electrical excitability of cell membranes

(ii) Improving GABA-mediated synaptic inhibition by inhibition of GABA transaminase or the use of drugs that directly agonist GABA receptors (causing a decrease in cell excitability due to increased membrane permeability to chloride ions);

(iii) Inhibiting T-type (glutamate);

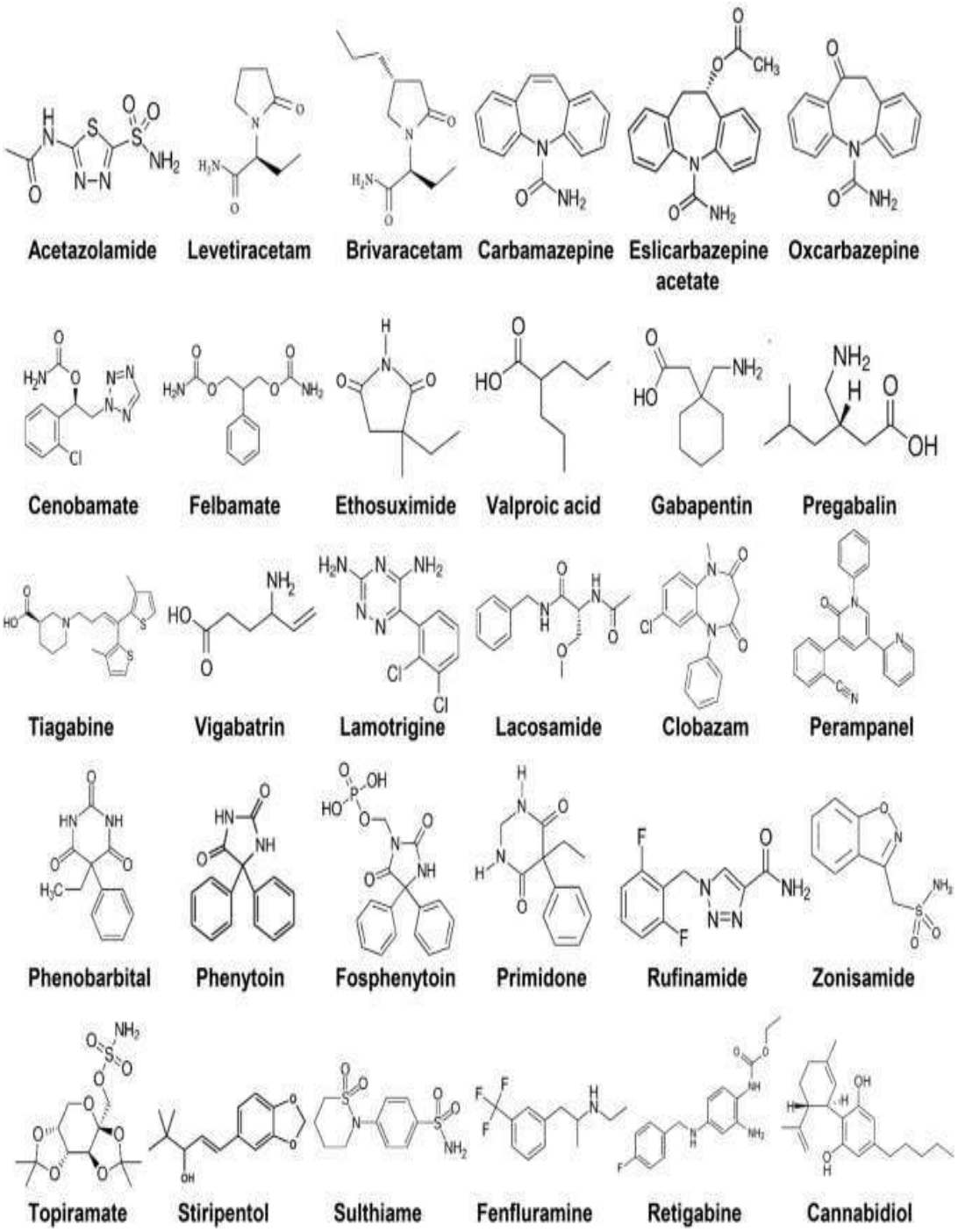

Fig. 3: Clinically used anti-seizure drugs.

Herbal medicines in treatment of epilepsy

Kupiec & Raj (2005), there are so many allopathic medicine available to treat epilepsy but all are having numerous side effects. Plant sources may be a good substitute to treat epilepsy because they do not have side effects.

Plant sources may be a good substitute to treat epilepsy because they comparatively do not have side effects. Herbal formulations present in market to treat epilepsy are very limited.

Since plant sources don't have any negative side effects, they might be a good alternative for treating epilepsy. There are very few herbal remedies available on the market to treat epilepsy. *Ananas camosus* is a plant that raises serotonin levels in the brain. Serotonin helps GABA, a neuroinhibitory transmitter in the brain, attach to its receptors. So far, scientific comparative pharmacological investigations for anti-epileptic activity (synergistic / antagonizing / indifference) of EEOBL and EEOSL against seizures induced in rats by maximal electroshock method (MES) is not reported in literature. Herbal formulations present in market to treat epilepsy is very limited. *Ananas camosus* is a plant which increase serotonin level in brain and serotonin facilitate binding of GABA to its receptors, which is a neuro inhibitory transmitter in brain. So the extract of leaves of this plant may be useful to treat epilepsy (Lee *et al*., 2005). The use of herbal medicine is restricted to extract level. Standardized protocols regarding the phytopharmaceuticals, their doses, dosing schedule, special precaution etc. have not been developed.

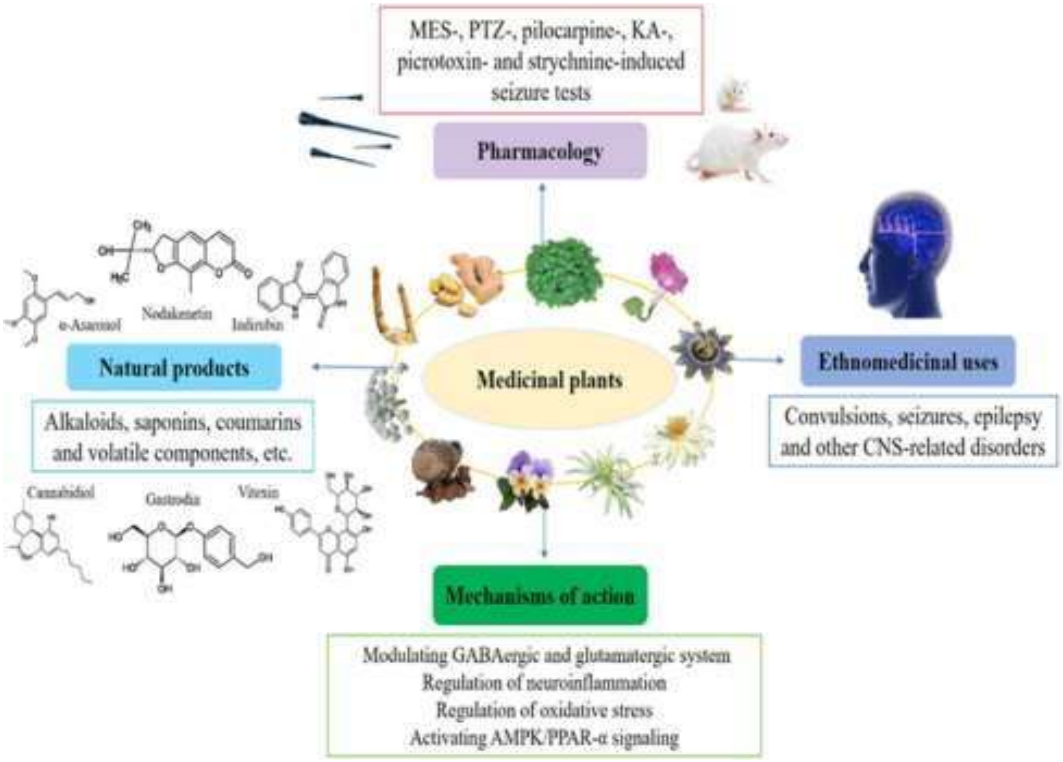

Fig. 4: Herbal medicines in treatment of epilepsy.

Advantages of Herbal Medicine (Balandrin *et al*., 1993)

i. he low cost of the herbal products make it economically feasible;
ii. Easy availability of herbal medicine products for consumption;
iii. Herbal medicines are relatively harmless, digest easily, with minimumor no side effects and safe.

Table 1: Herbal Drugs to treat epileptic seizures.

S. No.	Plant & Family	Pharmacological Reference
1.	*Brahmi Ghrita* (Scrophulariaceae)	Achaliya *et al*., 2005;
2.	*Cissus Sicyoides* (Vitaceae)	De Almeida *et al*., 2009;
3.	Passion Flower **(**Passifloraceae)	Nassiri *et al*., 2007;
4.	Rosa Domescana **(**Rosaceae) Flower	Moghimi *et al*., 2008;
5.	*Argyreia Speciosa* (Convolvulaceae)	Vyawhare *et al*., 2009;
6.	*Drosera Burmannii*: (droseraceae)	Hema *et al*., 2009;
7.	*Glycerrhiza glabra* **(**Fabaceae)	Nassiri *et al*., 2007;
8.	*Oscimum sanctum* (Leaves)	Pemminati *et al*., 2007;
9.	*Pongamia pinnata* (Fabaceae)	Manigauha *et al*., 2009;
10.	*Berberis vulgeris* (Berberidaceae)	Bhutada *et al*., 2010;
11.	*Clerodendrum infortunatum* (Verbenaceae)	De Smet *et al*., 1997 ; Pal *et al*., 2009;
12.	*Echium amoenum* (Boraginaceae)	Heidari *et al*., 2006;
13.	*Boerhaavia diffusa* (Nyctaginaceae)	Heidari *et al*., 2006;
14.	*Butea Monosperma* (Fabaceae)	Zhizhen *et al*., 2009
15.	*Valeriana officinalis* (Valerianaceae)	Rezvani *et al*., 2010,
16	*Saussurea lappa* (Asteraceae)	Shirishkumar *et al*., 2009
17	*Cymbopogon winterianus* (Poaceae)	Quintans *et al*., 2008,
18	*Taxus wallichiana* (Taxaceae)	Nisar *et al*., 2008;
19.	*Nardostachys Jatamansi* (Family: (Valerianaceae)	Rao *et al*., 2005; Debjit *et al*., 2009; Arun *et al*., 2012;
20.	*Dorstenia arifolia* (Moraceae)	Giselezapata *et al*., 2010;
21.	*Scutellaria lateriflora* (Lamiaceae)	Zhizhen *et al*., 2009;
22.	*Sutherlandia frutescens* (Fabaceae)	John & Ojewole 2008; Chauhan *et al*., 2018;

***Ocimum basilicum* (Tulsi Leaves; Holy basil; Lamiaceae)**

Putievsky *et al*., 2005, "Basil" is derived from the Latin word "Basilius, because it was thought that the plant was used to make royal perfumes, basil was known as the "royal plant" in Greek. About 150 species of *Ocimum* are found in the tropics of both the old and new Worlds, according to Paton (1992). Certain species, which have culinary and medicinal uses, are frequently grown in more temperate climates. *Ocimum basilicum* (also Known as sweet basil; family Lamiaceae) also called great basil or Saint-Joseph's-wort. India and other tropical parts of Asia are the original home of basil. It grows well in areas of temperate climate.

Fig. 5: *Ocimum basilicum* plant parts.

Chemistry of *Ocimum basilicum* (Abhay *et al*., 2014)

Whole plant: Kruger *et al*., 2002, camphor, cubenol, eugenol, methyl eugenol, citral; Tateo (1989), α-cubebene, methyl eugenol; Ozcan and Chalchat (2002), α-muurolene, P-cymen, β-cubebene, geranyl acetate, α-pinene, myrcene, limonen; Benedec *et al*., 2009, triterpenic acids, linalool, phytosterols, guaiene, farnesene, epibicycloses; Kruger *et al*., 2002, methyl chavicol , citral, thymol, camphor, eugenol;

Leaves and Seeds: Bunrathep *et al*., 2007, citral, methyl chavicol, β-myrcene, β- ocimene. fenchone, camphor, linaloo, α-bulnesene; Lee *et al*., 2005, camphene, β- pinene, sabinene, borneol, terpinolene, eugenol, β-bourbonene, β-elemene, α-guaiene, limonene, caryophyllene oxide, α-cadinene, cadinol, phytol, α-bisabolol;

Flower: Jamal *et al*., 2002, rosemeric acid;

Traditional and Medicinal uses of *Ocimum basilicum*

Yamani *et al*., 2016, Immunno-stimulatory; Enhance secretion of phlegm (relieve cough); Anti-inflammatory; Anti-microbial (viruses, bacteria, fungi and protozoa); Adaptogenic; Improve Digestion; Reduce Stress; Antioxidant; Anti-microbial; Cure Respiratory diseases, immune system booster.

Ocimum *basilicum* (Tulsi) (Lamiaceae; Eugenol; methyl cinnamate, linalool)	• Reduce pain
	• Relieve cough, congestion and difficulty in breathing.
	• Suppress inflammation (swelling).
	• Improve the process of digestion and food absorption.
	• Improve the secretion of phlegm in the respiratory tract.
	• Strengthen immune system / modify immune function.
	• Active against microbial growth and actions.

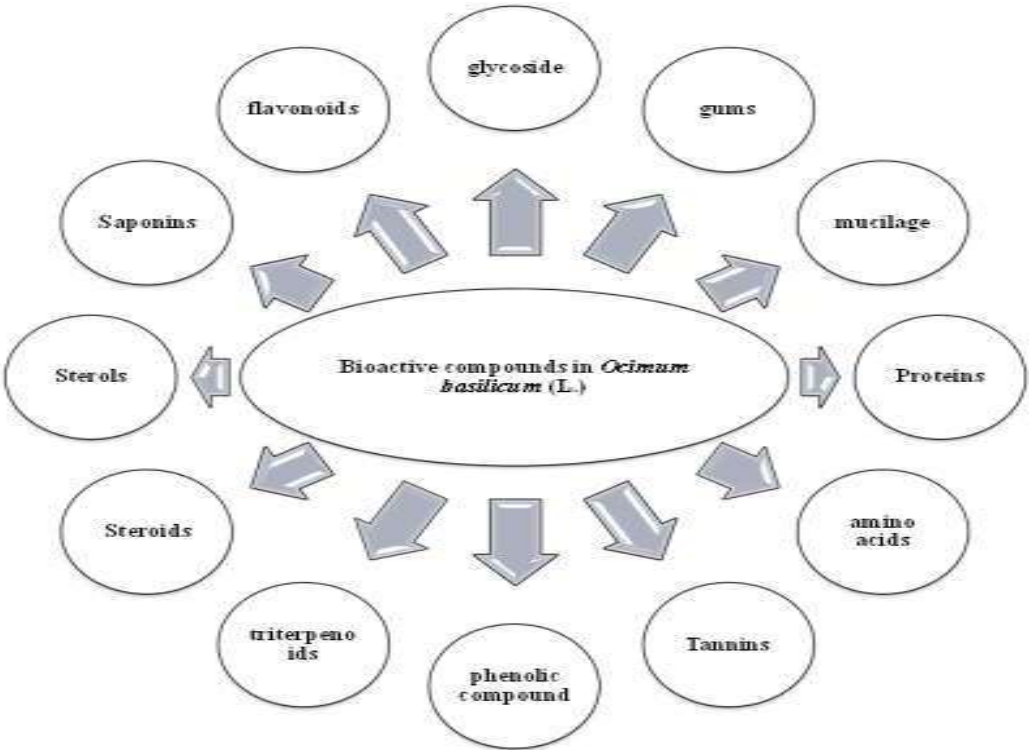

Fig. 6: Bioactive compounds of *Ocimum basilicum*.

***Ocimum basilicum*:** Pharmacological Activities

Table 2: *Ocimum basilicum* Linn. Medicinal activities.

Part	Biological Activity
Plant Leaves (PL) / Leaves Extract (LE)	Antifungal and insect repelling
	Insecticidal
	Antimicrobial (Ilhan *et al*., 2008)
Aerial parts (AP)	Anti convulsant and Hypnotic
	Anti-convulsant (Oliveira *et al*., 2009)
Whole Plant (WP)	Anti-oxidant (Politeo *et al*., 2007)
	Antimicrobial and Antioxidant
	Anti-viral (Chiang *et al*., 2005)
	Potent cytotoxicity (Kathirvel and Ravi, 2012)
	Anti-gardial
	Antiseptic
	Phytotoxicity and Haemagglutination

***Ocimum sanctum / Ocimum tenuiflorum* (Shyma Tulsi; Krishna Tulsi)**

Bast *et al*., 2014, numerous species of basil are among the many herbs and shrubs thatbelong to the genus Ocimum. *Ocimum sanctum*, also known as *Ocimum tenuiflorum*, is a perennial plant that is used for both decorative and medicinal purposes. Sundaramurthi *et al*., 2012, *Ocimum sanctum*, also known as tulsi, is considered to be one of the most precious and sacred of the various Orient's healing and health-promoting herbs. It is known as the "Queen

of herbs," the fabled "Incomparable one," and "The Mother medicine of nature." For ages, people have used Tulsi for its medicinal, religious, and culinary purposes. These usages have been documented in China, India, and other Asian countries as well as North Africa and Australia. Five species were identified in Linnaeus' 1753 description of the genus *Ocimum*. One of the main reasons Hindus pray to Tulsi, a plant with significant spiritual and medicinal qualities, is that she is known in Pauranic mythology as Vishnu Priya, or "Beloved of Lord Vishnu."

Fig. 7: Leaves of *Ocimum sanctum*.

Difference between *Ocimum basilicum* and *Ocimum sanctum*

Table 3: Difference between *Ocimum basilicum* and *Ocimum sanctum.*

Parameter	***Ocimum basilicum***	***Ocimum sanctum/ Ocimum tenuiflorum***
Plant Height	Sweet basil is small (upto 2 feet)	Krishna basil is large (2 to 3 feet tall)
Soil	soil acidity between 5.1 and 8.5	a narrower pH range of 6.5 to 7.5.
Flower	Basil's white flower spikes only appear in summer / White Flower	Holy basil blooms year-round, cream to white or pink / purplish.
Inflorescence	Verticellaster	Verticellaster
Leaves	Sweet Basil has fragrant, green to reddish leaves / Green leaves.	Holy basil's leaves smooth, elliptical, green to purple / leafpurplish white.
Leaf Margin	Slightly undulate	Serrate
Seed Color	Black	Black

Phytochemistry of *Ocimum sanctum / Ocimum tenuiflorum*

Whole Plant (WP): Methyleugenol; β-caryophyllene; L-Asparaginase; Bioactive polyketides and alkaloids (Daowan *et al*., 2013)

Plant Leaves (PL): octane, α- Thujene, β-pinene, citronellal, sabinene, 1,8,-cineole, terpiniolene, α-cubebene, linalool, β-elemene, isocaryophyllene, α-amorphene, 4,11-seinadiene, carvacrol, germacrene-d, myrtenylformat, cadinene, geraneol, nerolidol,

humulene oxide, α-bisbolol, cissesquisainene hydrate, 14-hydroxy-α-humulene; apigenin, apigenin-7-oglucuronide, isorientin, molludistin (Arenal *et al*., 2012)

Traditional Uses of *Ocimum sanctum*

Singh *et al*., 2012, because of its capacity to extend life, *Ocimum sanctum* is known as "the elixir of life." Traditionally, the plant's parts have been used to treat a variety of ailments, including the common cold, headache, flu, earache, fever, colic pain, sore throat, bronchitis, asthma, hepatic disorders, malaria fever, and as a countermeasure for snake and scorpion stings.

Medicinal and Pharmacological Uses of *Ocimum sanctum* (Das *et al*., 2012)

Table 4: Pharmacological uses of *ocimum sanctum*.

Part	Pharmacological/Biological Activity
Whole Plant	Hypoglycemic and anti-hyperglycemic activity
	Fibroblast and keratinocyte gene expression activity
	Standardization for increasing eugenol distribution activity
	Hyperglycemic activity
	Analgesic activity
	Anti-arthritic activity, Anticancer activity
	Anticoagulant activity Anticataract activity
	Antidiabetic
	Wound healing effect
	Anti-oxidant activity
	Anti-microbial activity

Ngugi *et al*., 2010, illustrated that there are so many allopathic medicine available to treat epilepsy but all are having numerous side effects. Plant sources may be a good substitute to treat epilepsy because they comparatively do not have side effects. Herbal formulations present in market to treat epilepsy are very limited (Vyawahare *et al*., 2007).

- *Ocimum basilicum* and *Ocimum sanctum* have phytotherapeutics agents;
- Comparative anti-epileptic studies of Sweet basil and Shyama basil is not reported;
- Research undertaken comparative anti-epileptic activity of *Ocimum basilicum* and *Ocimum sanctum*.

In the present investigation, seizures were induced in wistar albino rats by maximal electroshock method (MES). This research investigation was an attempt to perform research on pharmacological investigations to assess and compare antioxidant activity and anti-epileptic activity of *Ocimum basilicum* and *Ocimum sanctum* and undertaken with following objectives:

- For Standardisation of Sweet basil and Krishna basil;
- Analysis of *O. basilicum* **(**Sweet basil) and *O. sanctum* (Krishna basil)*;*
- *In-vitro* investigations of EEOBL and EEOSL for anti-oxidant activity;
- Anti-epileptic activities of EEOBL and EEOSL;
- Comparison of anti-epileptic of EEOBL and EEOSL with Phenytoin /Saraswat Syrup (Standard drugs);

Procurement and Authentication of leavescollection of plant raw materials

Fresh leaves of *Ocimum basilicum* Linn. and *Ocimum sanctum* Linn. were procured locally from plants growing in Herbal Garden of Institute Campus during October-November 2023 and authenticated by pharmacognostical analysis by a Botanist (Dr. K R Sharma, Former Professor, Delhi University) / Pharmacognosist. Procured fresh plant material of Sweet basil and Shyma basil were assessed morphologically, microscopically and chemically.

Fig. 8: Inflorescence of *Ocimum sanctum*.

Fig. 9: Leaves of *Ocimum sanctum*.

Qualitative chemical analysis of leaves

Different extracts of leaves (dried under shade, coarsely pulverized separately) of sweet basil like *O. basilicum* petroleum ether extract (OBPEE), *Ocimum basilicum* chloroform extract (OBCE), *O. basilicum* methanol extract (OBME), *O. basilicum* ethanol extract (OBEE) and *O. basilicum* aqueous extract (OBAE) (non-polar to polar and solvent) extracts. Similarly, different extracts of leaves (shade dried, coarsely pulverized separately) of Shyama / Krishna basil like *Ocimum sanctum* petroleum ether extract (OSPEE), *Ocimum sanctum* chloroform extract (OSCE), *Ocimum sanctum* methanol extract (OSME), *Ocimum sanctum* ethanol extract (OSEE) and *Ocimum sanctum* aqueous extract (OSAE) (non-polar to polar and solvent) extracts. These extracts were subjected to preliminary phytochemical screening for

PPMs and SPMs (chemical groups) using standard procedures described by Harborne (1973); Trease and Evans (1985); Sofowora (1993); Khandelwal (2008); Kokate (2005).

Estimation of Total Phenolic Content in EEOBL and EEOSL Extracts

In this experiment, TPC of EEOBL and EEOSL extracts was determined by a spectrophotometric method reported by Jeong *et al*., 2010. Appearance of blue colour on addition of few drops of alcoholic ferric-chloride in extract of leaves of *O. basilicum* and *O. sanctum* indicated phenolic compounds which were spectrophotometrically analysed.

Estimation of TFC in EEOBL and EEOSL Extracts

Kamtekar *et al*. 2009 reported that the TFC determination of EEOBL extract of *Ocimum basilicum* Linn and EEOSL extracts of *Ocimum sanctum* Linn. Absorbance of standard and sample extracts by spectroscopic / colorimetric (pink colour of TFC content).

Physico-chemical Analysis of *Ocimum basilicum* and *Ocimum sanctum* Linn. Physico-chemical Parameters of Analysis

Table 5: Physicochemical analysis of leaves of sweet basil.

Quantitative parameter	% w/w
Ethanol Soluble Extractive Value (ESEV; % w/w)	7.20
Water Soluble Extractive Value (WSEV; mg/g)	22.2
Acid Insoluble Ash Value (AIAV; % w/w)	1.68
Total Ash Value (TAV; % w/w)	1.86
Water Soluble Ash Value (WSAV; % w/w)	2.16
Acid Soluble Ash Value (ASAV; % w/w)	1.12
Loss on Drying (LOD; % w/w)	1.48
pH (1% and 10 % aqueous solution)	7.3 and 6.64

Table 6: Physicochemical analysis of leaves of Shyama / Krishna basil.

Quantitative parameter	% w/w
ESEV	8.54
WSEV	25.4
Acid Insoluble Ash Value (AIAV; %w/w)	1.76
Total Ash Value (TAV; %w/w)	1.94
Water Soluble Ash Value (WSAV; %w/w)	2.32
Acid Soluble Ash Value (ASAV; %w/w)	1.18
Loss on Drying (LOD; % w/w)	1.52
pH (1% and 10 % aqueous solution)	7.4 and 6.62

Fluorescence analysis

Fluorescence analysis helps in identification of *Ocimum basilicum* and *Ocimum sanctum* Linn leaves powders (mesh 40) were carried as per SOPs.

Table 7: Fluorescence of leaves of sweet basil (*Ocimum basilicum* Linn).

Treatment	Observations	
	Day Light	UV Light
Sweet basil Powder (40 mesh)	Green	Green
Powder + 1 ml Sodium hydroxide soln.	Green	Dark Green
Powder + 1 ml Picric acid soln.	Yellow	Dark Yellow
Powder + 1 ml Acetic acid soln.	Greenish Brown	Dark Brown
Powder + 1 ml Hydrochloric acid	Light Green	Dark Green
Powder + 1 ml Nitric acid	Light Green	Dark Green
Powder + 1 ml Iodine soln.	Light Brown	Dark Brown
Powder + 1 ml ferric chloride soln.	Green	Blue

Table 8: Fluorescence of leaves of Shyama basil (*Ocimum sanctum* Linn).

Treatment	Observations	
	Day Light	UV Light
Sweet basil Powder (40 mesh)	Green	Green
Powder + 1 ml Sodium hydroxide soln.	Green	Dark Green
Powder + 1 ml Picric acid soln.	Yellow	Dark Yellow
Powder + 1 ml Acetic acid soln.	Light Brown	Dark Brown
Powder + 1 ml Hydrochloric acid	Green	Dark Green
Powder + 1 ml Nitric acid	Green	Dark Green
Powder + 1 ml Iodine soln.	Light Brown	Dark Brown
Powder + 1 ml ferric chloride soln.	Blue	Purple

Table 9: Fluorescence of leaves of sweet basil (*Ocimum basilicum* Linn).

Solvent	Observations		
	Visible Light	Long Wavelength	Short Wavelength
n-Hexane	Yellowish	Green	Red
Pet-ether	Yellowish	Green	Dark Red
Methanol	Dark Green	Blue	Dark Purplish
Ethanol	Dark Green	Dark Blue	Purplish Black
Water	Yellow	Yellowish Green	Dark Yellow

Table 10: Fluorescence of leaves of Krishna basil (*Ocimum sanctum* Linn).

Solvent	Observations		
	Visible Light	Long Wavelength	Short Wavelength
n-Hexane	Dark Yellow	Dark Green	Dark Red
Pet-ether	Yellowish	Dark Green	Dark Red
Methanol	Dark Green	Blue-Purple	Dark Purplish
Ethanol	Dark Green	Dark Purple	Purplish Black
Water	Yellow	Yellowish Green	Dark Yellow

In-vitro Antioxidant effects of *Ocimum basilicum* and *Ocimum sanctum*

Gutteridgde (1995) reported that various diseases are caused due to free radicals induced oxidative stress. *In-vitro* antioxidant activity of EEOBL and EEOSL Extracts by DPPH scavenging activity and FRAP Assay.

DPPH Free Radical-Scavenging Activity

The extract's ability to scavenge free radicals was determined using Krings & Berger's (2001) approach. As a standard and blank free radical scavenger, 50% methanol was utilised, while trolox served as a positive control. 600 µl of DPPH was added after varying the concentrations (30-300 µg/ml) using the stock of each sample (3 mg/ml). Following a 20-minute incubation period, the absorbance at 517 nm was determined for the reaction mixture.

Ferric Reducing / Antioxidant Power (FRAP) Assay

The extract's FRAP was calculated using Benzie & Strain's (1996) methodology. These quantities were then combined with 1 millilitre of FRAP reagent, and the mixture was incubated for 30 minutes in the dark. To keep track of the sample's capacity to convert ferric ions into ferrous ones, the absorbance of the reaction mixture was measured at 593 nm.

Table 11: Effect of EEOBL and EEOSL on FRAP Assay.

Conc (µg/ml)	% Inhibition	
	EEOSL	EEOBL
0.5	0.48±0.012	0.160±0.025
1.5	1.47±0.016	1.46±0.064
2.5	3.33±0.050	2.70±0.016
3.5	3.73±0.130	3.30±0.047
4.5	4.64±0.035	4.37±0.097
5.5	5.50±0.026	5.46±0.120

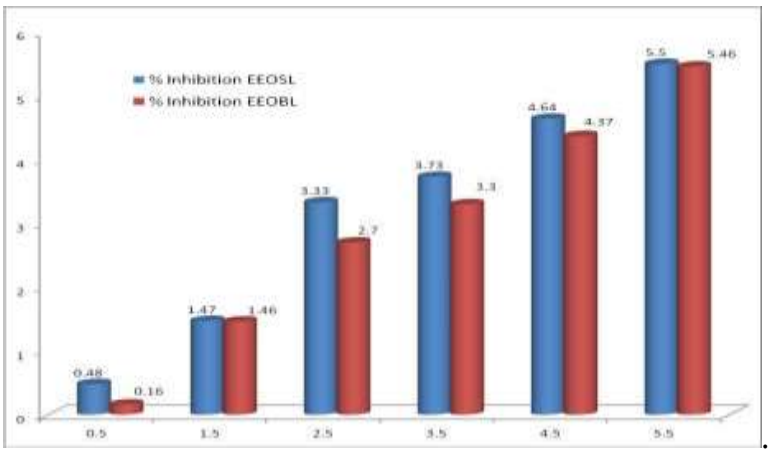

Fig. 10: Effect of EEOBL and EEOSL on FRAP Assay.

It was observed that reducing power of EEOBL and EEOSL extracts was increased with increasing dosage showed significant antioxidant activity. (Table 11; Fig. 10).

Safety & Toxicity Evaluation of EEOBL and EEOSL Extracts. Animal Groups

Group I: Control (1ml of Distilled water). Group II: Test drug (EEOBL 200 mg/kg). Group III: Test drugs (EEOBL 400 mg/kg). Group IV: Test drug (EEOSL 200 mg/kg). Group V: Test drugs (EEOSL 400 mg/kg).

EEOBL and EEOSL extracts were administered and physiological and behavioral changes were analysed.

Sub acute Toxicity Study

Table 12: Toxicity (mortality) of EEOBL and EEOSL extracts.

S. No.	EEOBL	EEOSL	Percent mortality (%)
1.	50	50	0
2.	100	100	0
3.	250	250	0
4.	500	500	0
5.	750	750	0
6.	1000	1000	0
7.	1250	1250	0
8.	1500	1500	0
9.	1750	1750	0
10.	2000	2000	0

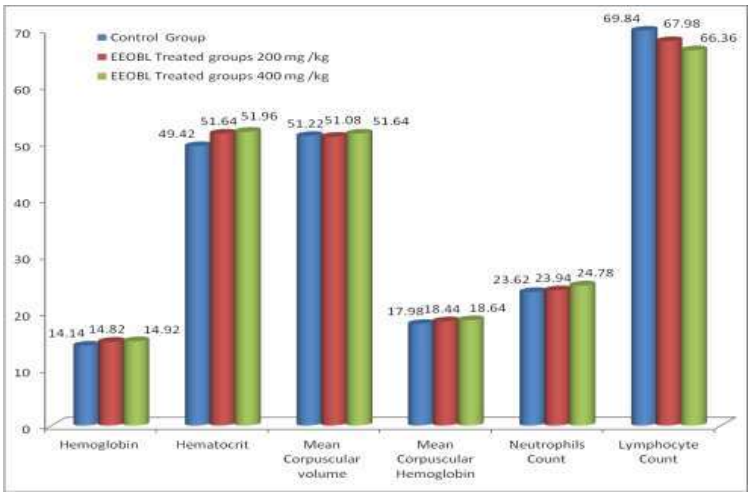

Fig. 11: EEOBL effects on blood analysis tools.

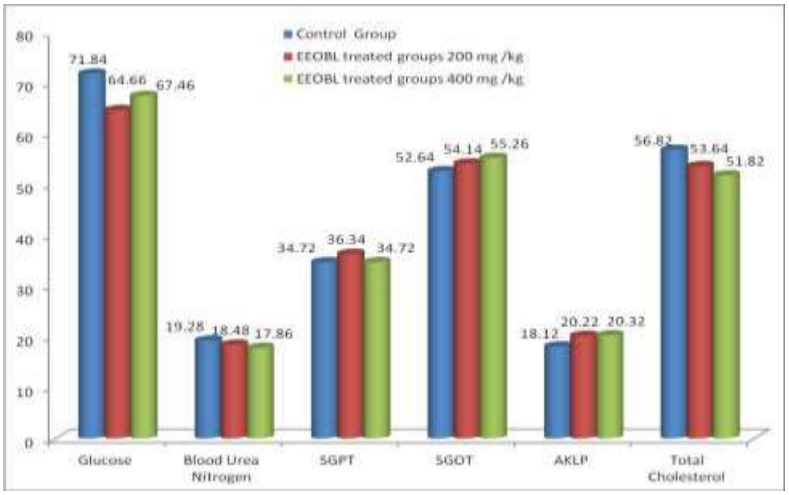

Fig. 12: EEOBL effect on LFTs and blood values.

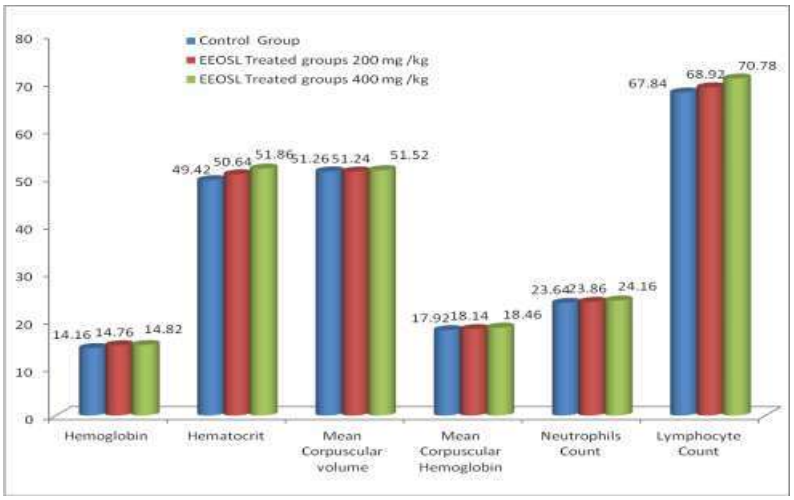

Fig. 13: EEOSL effects on different blood values.

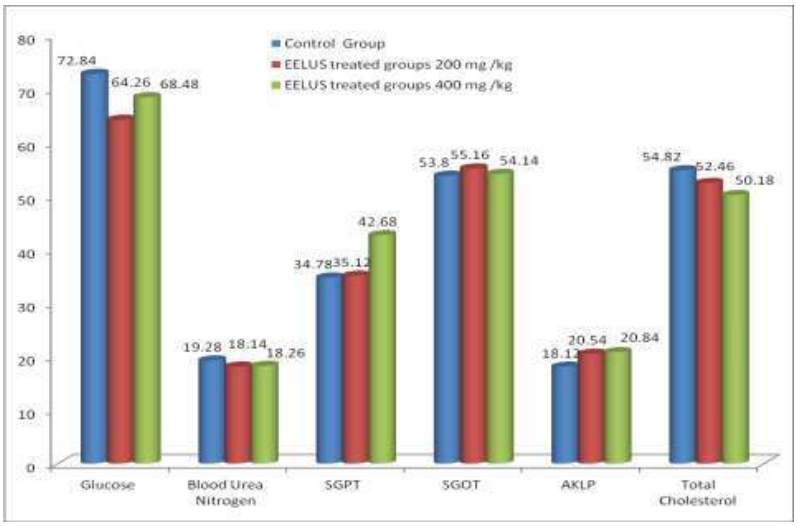

Fig. 14: EEOSL effects on blood quality.

***In-vivo* anti-epileptic activity**

Animal study was approved with Form B: IEC/IAEC/2023/10 dated 02-11-2023. MES (Maximal Electroshock Seizures) Induced Seizures Model was used (Loscher and Schmidt, 1988).

Grouping of Animals

Table 13: Animal groups.

Group	Treatment / Dose	Animals
I	Control / Normal saline (25 ml/kg)	6
II	Electroshock Group (Toxic Control Group)	6
III	EEOBL-A (100 mg/kg) + Electroshock	6
IV	EEOBL-B (200 mg/kg) + Electroshock	6
V	EEOSL-A (100 mg/kg) + Electroshock	6
VI	EEOSL-B (200 mg/kg) + Electroshock	6
VII	Combination of EEOBL-A Extract (100mg/kg) + EEOSL-A Extract (100mg/kg) + Electroshock	6
VIII	Standard "Phenytoin sodium" (25 mg/kg) + Electroshock	6
IX	Standard "Saraswat Syrup" (Baidyanath Formulation) + Electroshock	6

Treatment schedule

Group I (Normal Control): Aniamls were given water *ad libitum.*

Group II (Toxic Control group): In the present investigation, seizures were induced in wistar albino rats by maximal electroshock method (MES).

Group-III & IV (Electroshock + EEOBL Extract A/B): EEOBL-A (100 mg/kg) and EEOBL-B (200 mg/kg) respectively for 07 days and then seizures were induced by MES method.

Group-V & VI (EEOSL (A/B + Electroshock): Albino rats of Group V and VI were given pre-treatment with EEOSL-A (100 mg/kg) and EEOSL-B (200 mg/kg) respectively and then seizures were induced by MES method.

Group-VII (Combination of EEOBL-A and EEOSL-A Extract + Electroshock): Group VII animals was given treatment with combination of EEOBL-A extract (100 mg/kg) and EEOSL-A extract (100 mg/kg) followed by induction of seizures by MES.

Group-VIII (Standard drug - Phenytoin + Electroshock): Phenytoin sodium (Standard) was used (25 mg/kg). Group VIII animals were given treatment with standard drug Phenytoin followed by induction of seizures by MES.

Group-IX (Baidyanath Ayurvedic Formulation Saraswat Syrup (Standard) + Electroshock): Saraswat Syrup was used as the standard antiepileptic drug (5 ml/kg). Animals were given treatment with Saraswat Syrup for followed by induction of seizures by MES.

Table 14: Effects of EEOBL and EEOSL on MES-induced seizures in rats.

Groups	Flexion (sec)	Extension (sec)	Convulsion (sec)	Recovery time (min)	% pro-tection
Group I	5.22 ± 0.32	8.86 ± 0.38	25.68 ± 4.12	4.14 ± 0.56	--
Group II	3.48 ± 0.32*	8.18 ± 0.52	24.16 ± 3.68	3.22 ± 0.46	--
Group III	3.12± 0.42**	7.28 ± 0.36	17.12±3.26*	2.84 ± 0.20	60.28
Group IV	2.96±0.24**	7.12 ± 0.28*	16.98 ± 3.16	2.68±0.16*	68.44
Group V	3.28 ± 0.14*	8.12 ± 0.52	22.34 ± 3.72	3.18 ± 0.16	52.65
Group VI	3.22 ± 0.12*	7.96 ± 0.42	20.28 ± 3.62	2.98 ± 0.12	56.16
Group VII	2.88± 0.18***	6.96 ± 0.42*	16.86 ± 3.12	2.54 ± 0.18*	73.28
Group VIII	1.33 ±0.05***	1.66±0.55***	14.83±1.28*	1.13±0.04***	81.20
Group IX	2.80± 0.20***	6.75 ± 0.48*	16.60 ± 3.16	2.44 ± 0.16*	76.44

Nate: values: Mean ± SE (*P < 0.05,; ***P < 0.001).

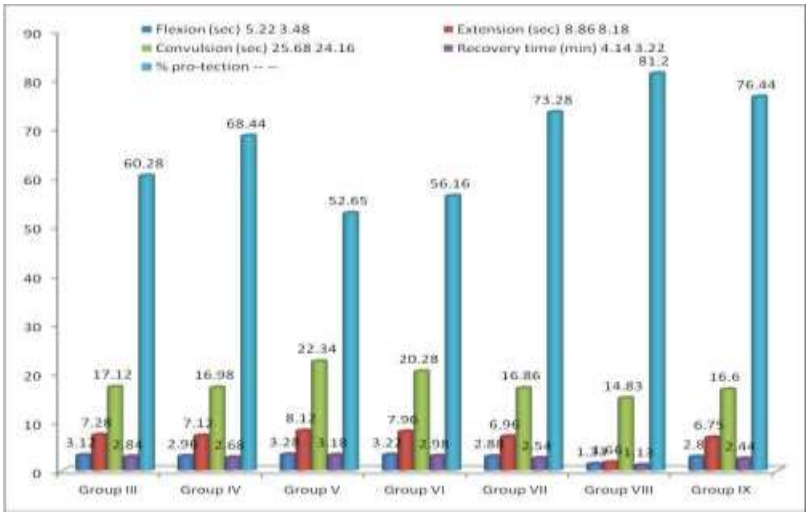

Fig. 15: Effect on MES-induced seizures in rats.

RESULTS AND DISCUSSIONS

During October and November of 2023, fresh leaves were evaluated pharmacognostically to determine whether secondary plant metabolites (SPMs) were present, phytochemical screening was also done. *Ocimum basilicum* and *Ocimum sanctum* leaves underwent pharmacognostic analysis to verify the purity and authenticity of the plant material that was obtained. Authenticated specimens (IEC/Pharm/Herb/2023/2311 & IEC/Pharm/Herb /2023/ 2312) samples were deposited in Herbarium Bank.

Various extracts non-polar to polar and solvent extracts made from dried, coarsely ground sweet basil leaves. The leaves of Shyama / Krishna basil were also extracted in different ways, such as *O. sanctum* petroleum ether extract (OSPEE), *O. sanctum* chloroform extract (OSCE), *O. sanctum* methanol extract (OSME), *O. sanctum* ethanol extract (OSEE), and *O. sanctum* aqueous extract (OSAE), which are non-polar to polar and solvent extracts. The

leaves were shade dried and coarsely ground separately. Chemical groups (PPMs and SPMs) such as phenolics, flavonoids, alkaloids, proteins, tannins, steroids, glycosides, terpenes, amino acids, carbohydrates, and reducing sugars were found in the qualitative analysis of *Ocimum basilicum* and *Ocimum sanctum* leaves extracts.

In estimation of Total Phenolic Content in EEOBL and EEOSL extracts appearance of blue colour on addition of few drops of alcoholic ferric-chloride in extract of leaves of *O. basilicum* and *O. sanctum* indicated phenolic compounds which were analysed spectrophotometrically. TPC in *O. sanctum* was 11.44±6.36 µg GAE/mg DW extract whereas phenolic content in the leaves extract of *O. basilicum* was 10.84±6.48 µg GAE/mg DW extract. In estimation of TFC in EEOBL and EEOSL extracts absorbance of standard and sample extracts by spectroscopic / colorimetric (pink colour of TFC content) analysis shown that high concentration of TFC was observed in EEOSL extract (8.92 mg of Quercetin Equivalent/100 mg of Crude extract) in comparison of EEOBL extract (8.54 mg of Quercetin Equivalent/100 mg of Crude extract). In Physico-chemical analysis of *O. basilicum* and *O. sanctum* Linn ash values indicated that drug were free from contamination. High water soluble and ethanol soluble extractive values indicated water soluble salts (Table 5-6).

The fluorescence analysis showed color change in day light to UV light indicated that *Ocimum basilicum* and *Ocimum sanctum* Linn leaves powders contains high concentration of phenolic, tannins, flavonoids and terpenes as SPMs, nature of solvent and nature of chemical reaction involved in analysis (Table 7-8). Further, antioxidant activity of the EEOBL and EEOSL Extracts were evaluated. In DPPH assay, percentage inhibition in EEOSL was in range of 12-62% while in EEOBL extract it was 7-47%. In FRAP assay of EEOBL and EEOSL extracts, observed values were expressed in terms of µg ferrous sulphate equivalents (FSE) /mg extract. It was observed that reducing power of EEOBL and EEOSL extracts was increased with increasing dosage showed significant antioxidant activity. No mortality was seen in rats up to 2000 mg/kg/b.wt in trials on acute toxicity. Atherosclerosis, diminished engine function, and other behavioural changes were noted. Significant changes in haematological markers, disturbances in renal function, and mildly aberrant behaviour were also noted. Still, there were no appreciable changes in the liver enzymes SGPT, SGOT, T-Prot, and AKLP. According to Tables 12 and Fig. 11-14, the EEOBL and EEOSL extracts produced a very large safety margin, so they should be categorised as Non-Toxic Constituent. Finally, in pharmacological investigation for *In-vivo* anti-epileptic activity of EEOBL and

EEOSL extracts individually / separately and in combination (EEOBL + EEOSL). EEOBL-B extract (Group IV) was found to be effective (P<0.05) in epileptic seizures like standard drug Phenytoin / Saraswat Syrup. Pre-treatment with EEOSL-A / EEOSL-B (Group V & VI) extracts induced dose dependent anticonvulsant effects. EEOSL-B (Group VI) extract was found to be more effective than EEOSL-A but less effective than standard drug Phenytoin / Saraswat Syrup (Table 13-14). Finally, pre-treatment with combination of EEOBL-B and EEOSL-B (Group VII) extracts very significantly decreased flexion phase of MES-induced seizure and very significantly decreased the duration of convulsion (***P<0.001). Further, both EEOBL and EEOSL very significantly decreased the convulsion phase. EEOSL produced better anti-epileptic effects then EEOBL (Table 14; Fig. 15).

CONCLUSIONS

The EEOBL and EEOSL extracts induced a very wide safety margin (no mortality upto 2000 mg). Finally, in *in-vivo* anti-epileptic activity, EEOSL-B extract was found to be more effective than EEOSL-B but less effective than standard drug Phenytoin / Saraswat Syrup. Finally, combination of EEOBL-B and EEOSL-B very significantly decreased convulsion. Much more elaborated pharmacological research is proposed.

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REFERENCES

1. Abhay, K. P., Pooja, S., Nijendra, N. T. *Asian Paci. J. of Trop. Biomed*, 2014; 4(9): 682-694.
2. Achaliya, G.S., Wadodkar, S.G., Dorle, A.K. *Indian J. Pharmacol*, 2005; 37: 33-36.
3. Arenal, A., Martin, L., Castillo, N.M., Gonzalez, R. *Asian Pacific J. ofTropical Biomed*, 2012; 5(8): 654-657.
4. ArunG.ChandraK.K. *Modern Ayurveda*. CRC Press, 2012; 317-336.
5. Balandrin, N.F., Kinghorn, A.D., Farnsworth, N.R. *In Human Medicinal Agents from Plants*. Kinghorn, A. D., Balandrin, M. F., Eds, 1993; 2-12.
6. Bast, F., Rani, P., Meena, D. *The Scientific World of Journal*, 2014; 6-9.
7. Benedec, D., Oprean, R., Tamas, M. *Farmacia*, 2009; 57: 625–629.
8. BenzieI. F and StrainJ.J. *Anal. Biochem*, 1996; 239: 70–76.

9. Bhutada, P., Mundhada, Y., Bansod, K., Dixit, P., Umathe, S. *Epilepsy & Behavior*, 2010; 18: 207–210.
10. Browne, T.R. *Am J Hosp Pharm*, 1978; 35: 1048–1056.
11. Bunrathep, S., Palauvej, C., Ruangrungsi, N. *J. of Health Res*, 2007; 21: 201–206.
12. Chauhan, K. A. *A review of medicinal plants showing anticonvulsant activity*.University of Rajasthan, Jaipur, 2018; 17-23.
13. Chen, Z., Brodie, M.J., Kwan, P. *Curr. Opin. Neurol*, 2020; 33: 185–90.
14. Chiang, L.C., Cheng, P.W., Chiang, W. *Clin Exp Pharmacol Physiol*, 2005; 32(10): 811-816.
15. Daowan, L, Broetz-Oesterhelt, H., Werner, E.G., Muller, V.W. *Fitoterapia*, 2013; 91: 100-106.
16. Das, M. K. and Kumar, B. *Int. J. Res. in Ayur. and Pharm*, 2012; 3(5): 645-647.
17. De-Almeida, S., Edvaldo, R., Vieira, D.P. *J biomedicine & biotech*, 2009; 1-6.
18. DeSmetK., HanselChandlerF. *Adverse Drugs*. Heidelberg, 1997; 145-162.
19. DebjitB., ChiranPDMargretC. *Arch. Appl. Sci. Res*, 2009; 1(2): 32-56.
20. Devinsky, O., JetteNSchefferI.E. *Nat. Rev. Prim*, 2018; 4: 24-27.
21. FisherSAcevedo, CBogaczA., *Epilepsia*, 2014; 55: 475–482.
22. Fisher, R.S., Cross, J.H., D'Souza, C. *Epilepsia*, 2017; 58: 531–542.
23. Forsgren, L., Beghi, E., Sillanpaa, M. *Eur. J. Neurol*, 2005; 12: 245–253.
24. Galanopoulou, A. S. *Curr. Neuropharmacol*, 2008; 6: 1-20.
25. Giselezapata-sudo et al. *J of Ethenopharmacology*, 2010; 130: 9-12.
26. Gutteridgde, J.M. *Free Radic. Res. Comm*, 1995; 19: 141-158.
27. Harborne, J. B. *Phytochemical Methods.* Springer Publ, 1973; 1-288.
28. Heidari, M.R., Glogowski, J., Oun, A. *Pak. J. Biol. Sci*, 2006; 9: 772-776.
29. Hema, B., Anwar, F., Bhupendra, S. *Int. J. Appl. Res. in Natl. Prod*, 2009; 2: 1-4.
30. Ilhan, K., Nazife, Y., Mehlika, B. *Afri. J. Trad. Compl. & Altern. Med*, 2008; 5(4): 363–369.
31. Jamal, J., Khalighi, A., Vivanco, J. *J. Agric. Food Chem*, 2002; 50(21): 5878–5883.
32. JayaprakashaK., SinghPSakariah, K. *Food Chem*, 2001; 73: 285-290.
33. JeongC. H.Choi, GNKimJH. *Food Chem*, 2010; 118: 278–28234.
34. Jerome, E. J. *Physiol Behav*, 2018; 176(5): 139–48.
35. John, A., Ojewole, O. *Brain Research Bulletin*, 2008; 75: 126-32.
36. Kamtekar, R., Pathak, N., Sriram, S. *J. of Appl. Pharmaceu. Sci*, 2009; 4(09): 61-65.
37. Kathirvel, P. and Ravi, S. *Nat. Prod. Res*, 2012; 26: 1112–1118.

38. Khandeval, K.R. *Pharmacog. Prac*, 2008; 19: 61-67.
39. Kokate, C. K. *Pract. Pharmacog.* Vallabh, 2005; 3: 107-111.
40. Krings, U. and Berger, R.G. *Food Chemistry*, 2001; 72: 223-229.
41. Kruger, H., Wetzel, S.B., Zeiger, B. *J. Medicinal Plants*, 2002; 9: 335–344.
42. Kupiec, T. and Raj, V. *J. Anal. Toxicol*, 2005; 29: 755-758.43.
43. Lee, S.A., Yoo, H.J., Lee, B.I. *Seizure*, 2005; 14: 157–163.
44. Lee, S.J., Umano, K., Lee, K.G. *Food Chemistry*, 2005; 91: 131–137.
45. Loscher, W. and Schmidt, D. *Epilepsy Res*, 1988; 2: 145-181.
46. Manigauha, A., Shibamoto, T., Mishra P.K. *Int. J.Pharm. Res*, 2009; 1: 1119-1121.
47. Moghimi, A, Bande, S.S., Kashi, A. *J. Bio. Sci*, 2008; 8: 426-430.48.
48. Najim, I.M. *Lancet Neurol*, 2018; 17(4): 295–297.
49. Nassiri, A.S. Bhatti, I.A., Marjan, L. *BMC Compl. & Alter. Med*, 2007; 7: 26-29.
50. Ngugi, A.K., Bottomley, C., Newton, C.R., *Epilepsia*, 2010; 51: 883–890.
51. Nisar, M., Estevam, C.S., Alves, P.B. *J. of Ethanopharmacol*, 2008; 116: 490-494.
52. Oliveira, J.S., Porto, L.A., Niculau, E.S. *Med. & Arom. Plants*, 2009; 8(3): 195–202.
53. Olufunmilayo, A., Omoniyi, K, Olayemi, A. *J. of Ethnopharm*, 2007; 113: 111–114.
54. Ozcan, M., ChalchatC., Ryvlin, P. *Czech J. Food Sci*, 2002; 20: 223–228.
55. Pal, D., et al. *Indian journal of experimental biology*, 2009; 47: 743-747.
56. Patel, P. and Moshé, S.L. *Epilepsia Open*, 2020; 5(1): 22–35.57.
57. Paton, A. A. *Africa. Kew Bull*, 1992; 47(3): 403-405.
58. Pemminati et al. *Indian J. Pharmacol*, 2007; 39: 87-94.
59. Politeo, O., Jukic, M., Milos, M. *Food Chem*, 2007; 101(1): 379-385.
60. Putievsky, E. and Galambosi, B. *Medicinal and Aromatic Plants-Industrial Profiles*. Amsterdam, 2005; 10: 37–63.
60. Quintans, J., Macdonald, L., Lovelyn, J. *Phytomedicine*, 2008; 15: 619-24.
61. Rao, V. S., Karanth, B., Sudhakar K. *J of Ethanopharmacol*, 2005; 102: 351-356.
62. Rezvani, M.E., Roohbakhsh, A., Megha, R. *J. Ethnopharmacol*, 2010; 127: 313-8.
63. Scheffer, I.E., Berkovic, S., Connolly, M.B. Epilepsia, 2017; 58: 512-521.
64. Shirishkumar, D., Capovilla, G., Sharma, S. *Pharmacological evaluation of anticonvulsant activity*, 2009; 1: 131-137.
65. Singh, E., Sharma, S., Dwivedi, J. *Scholars Res. Lib*, 2012; 2(1): 39-48.
66. Singh, A. and Trevick, S. *Neurologic Clinics*, 2016; 34: 837-847.
67. Sofowora, A. J. *Alter. Complem. Med*, 1993; 2(3): 365-372
68. Stephen, L. J. and Brodie, M. J. *Neurol Clin*, 2009; 27: 967–992.

69. Sundaramurthi, P., Dhandapani, S., Ponnusamy, S., Subbaiyan, M. *Hitek J.Bio. Sci. & Bioengg*, 2012; 1(1): 7-11.
70. Tateo, F. *J. of Essential Oil Research*, 1989; 1: 137–138.
71. Tiwari, S., Sharma, V., Mujawar, M., Mishra, Y.K. *Sensors* (Switzerland), 2019; 19(7): 232-234.
72. Trease, G.E. and Evans, W.C. Pharmacog, 1985; 17: 149-153.
73. Vyawahare, N.S., Khandelwal, A.R., Nikam, A.P. *J. of Herbal Medicine and Toxicology, 2007;* 1: 9-14.
74. Xu, Y., Nguyen, D., Mohamed, A. *Seizure*, 2016; 41: 167–74.
75. Zhizhen, Z., Tramacere, L., Franco, V. *Phytomedicine*, 2009; 16: 485-493.