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Formulation and Evaluation of Antiepileptic Parentral Preparation From Cynadon Dactylon

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ABSTRACT

Epilepsy is a challenging neurological disorder, prompting the investigation of novel therapeutic strategies. The purpose of this study was to develop and test an antiepileptic parentral formulation based on cynadon dactylon.A medicinal herb with excellent pharmacological characteristics.Various extraction processes were used to acquire the bioactive ingredient, which was then formulated into parenteral dose forms such as injection or infusion.The produced formulation was thoroughly evaluated for physicochemical properties, stability, and in vitro drug release..In vivo investigations were also done to evaluate the pharmacodynamic effectiveness and safety profile of the developed preparation.Preliminary data point to the potential of cynadon dactylon-based parentral preparation as an effective and safe antiepileptic drug.Further optimization and clinical research are required to establish their therapeutic usefulness in epilepsy care.

Objective: Clearly state the purpose of the study, emphasizing the development and evaluation of parentral formulation containing cynadon dactylon for antiepileptic activity.

Purpose:The purpose of formulating and evaluating an antiepileptic parenteral preparation from Cynodon dactylon is to explore its potential as a natural, effective, and safe alternative to conventional antiepileptic drugs, ensuring rapid delivery and addressing the need for novel therapies with fewer side effects and improved accessibility.

Result: Cynadon dactylon was extracted by maceration method. The extract identification test for cynadon dactylon extract was positive. This is noval method for to treat epileptic seizures. The antiepileptic parenteral preparation from Cynodon dactylon exhibited significant anticonvulsant activity and demonstrated good safety profiles in preliminary tests.

Conclusion: In conclusion, the antiepileptic parenteral preparation from Cynodon dactylon showed promising anticonvulsant efficacy and safety in initial evaluations, suggesting its potential as a natural alternative for epilepsy treatment.

Keywords: Cynadon dactylon, Epilepsy, Efficacy , Seizures.

INTRODUCTION

Seizures are transitory behavioral changes caused by the disorganized, synchronized, and rhythmic firing of groups of brain neurons. Epilepsy is a neurological illness characterized by the occurrence of seizures on a regular and unpredictable basis. The term epilepsy is derived from the Greek verb epilamvanein [to be seized], “to be taken hold of,” or “to be attacked,” implying that the individual experiencing a seizure is ‘possessed’ or at least out of control. Epilepsy is a broad term that refers to a number of different disorders. Seizures that occur repeatedly. Despite significant research, the pathogenesis remains poorly understood. Studies on several animal models have provided significant evidence for heterogenicity in the mechanism of epileptogenesis. Several biochemical hypotheses link epilepsy to decreased activity of the inhibitory GABAergic system and increased activity of excitatory amino acids (glutamate and aspartate systems).According to the most recent WHO report, there are approximately 50 million patients worldwide, with 85% of them living in developing countries. Every year, an estimated 2.4 million new cases are diagnosed worldwide, with at least half of these instances beginning in early childhood or adolescence. Epilepsy responds to therapy; around 70% to 80% of persons with epilepsy

can live normal lives if appropriately treated; yet, there remains fear, misunderstanding, discrimination, and social stigma. That has surrounded epilepsy for ages is still a major hindrance that has blocked therapy for epileptic sufferers.

Epilepsy increases a person's risk of premature death by approximately two or three times that of the general population. It is the most common serious brain disorder in the world, regardless of age, race, social class, nationality, or geography. Although numerous novel synthetic drugs have been approved for the treatment of seizures in recent years – high cost, unique adverse effect profiles and patients non- compliance led to cessation of therapy. The natural product althrough admininstration in the curved form. Many times demonstrate less side effects, improved efficacy and are cost effective. Attempts are being made to improve the products, such as using extraction techniques to remove non-useful components.Screening extracts for the presence or improvement of a desired pharmacological activity over the non-extracted form has become an important method in the search and development of natural medicines. According to Ayurvedic literature, the ethanol extract of Cynodon dactylon reduces the onset and severity of convulsions. The goal of this research is to learn more about Cynadon dactylon's aqueous extract and its anticonvulsant effects.

Cynadon dactylon:

Cynadon dactylon, commonly known as Durva and Arugampallu, is widely used in Indian home traditions and worshipping ceremonies. This grass holds significant therapeutic value for treating many ailments. Natural resources have always been valued and cherished in Indian culture and tradition, regardless of their importance. Mother Nature has supplied us with an abundance of such materials. Indian civilisations have used these resources since their inception. Durva has been used for medical reasons in the form of lep or kadha since the Vedic era, and it has always been associated with deity worship.

It is found at elevations of up to 3000 meters throughout India. It is found on sandy soils in warmer climate zones around the world. It grows in climates between 30 degrees south and 30 degrees north latitude. It can also be found raging from acidic to alkaline conditions near the shoreline, mainly in aseptic regions and controlled areas with rainfall between 670 and 1750mm. However, it cannot thrive in shaded areas since it requires moisture in the soil and full sunlight to flourish properly. During the winter, the grass goes dormant and turns brown. This herb's entire structure can be utilized for a variety of medical applications.

Durva is a glabrous, prostrate, creeping, stoloniferous perennial herb with rhizomes. It has three morphological divisions. Durva grass may grow up to 7.5 cm per day in damp conditions, making cultivation more easier than with other grasses. Dry environments impede growth to some extent. Spriggling of th grass can produce growth after 4-8 weeks and can easily cover the region of 30-45 cm apart in ground.

The grass reproduces mostly by seed, which germinate prolifically following a fire. New shoots and leaves sprout swiftly, receiving food from subsurface reserves.Root suckers aid in sprouting when the roots are damaged. Thus, the species is recognized for its rapid growth. Pollination occures by wind in essential as they shows self- incompatibility which produces inbreeding depression. The chromosomal expression of distinct varities follows a pattern of 2X=4X=36.

Durva grass in epilepsy:

Durva grass has been linked to epilepsy. Epilepsy is a disorder characterized by recurring, spontaneous seizures, with brain malfunction being the most common cause. Epidemology shows that it is one of the most frequent neurological disorders, as the incidance revealed roughly 50 new cases per year/ 100,000 population. Approximately 1% of the population suffers from epilepsy (stafstrom and carmant, 2015). Incidence and prevalence rates can be used to calculate the frequency of epilepsy in a population. The age-adjusted incidence of epilepsy varies by geographical region, ranging from 16/100,000 person years to 111/100,000 person years.

Durva grass has the potential to treat epileptic seizures, as evidenced by significant results in various studies conducted over the years. Evaluation of aerial sections of durva grass on CNS activities is indicated due to nature's function in suppressing generalized tonic-clonic seizures. The rise in the serotonin and GABA level of brain was responsible for analgesic and anticonvulsant activities was indicated the role. A study on brain toxicity in mouse fetuses was conducted to demonstrate the protective potential of durva grass extract, which resulted in a reduction in macro and microscopic neuropathological conditions in models. This was suggested due to its antioxidant property, which neutralized the effect in various parts of the growing brain, thereby helping to prevent teratological changes in brain cells and tissues.

Parentral formulation:
Parental products are sterile preparations that contain one or more active ingredients and are meant to be administered to the body via injection, infusion, or implantation. They are packaged in either single or multi-dose packages. The parentral route of drug administration offers various advantages, including quick beginning of action, consistent pharmacokinetics , and suitable for individuals with limited gastrointestinal function. The parentral route for administering antiepileptic formulations can be very beneficial in emergency settings or for patients who require immediate seizure control.

Advantages of parentral preparation
1. Parentral products bypass pre-systematic or first-pass metabolism, resulting in a fast commencement of action.
2. Drugs that cannot be administered orally can be administered via this technique.
3. Patients who are vomiting or unconscious cannot take the medicine orally. In such circumstances, the medicine can be administered using this technique.
4. Modifications to the formulations can prolong the drug's activity.

Essential requirements for formulation:
In the preparation of parentral product, the following substance are added to make a stable preparations.
1. Vehicals
2. Additives
a. Solubilising agent
b. Stabilisers
c. Buffering agents
d. Antibacterial agents
e. Chelating agents
f. Suspending agent

Vehicles
There are two types of vehicles, which are typically employed for the production of injections.
1. Aqueous vehicles- water is utilized as vehicle for majority of injections since water is tolerated well by the body and is safest to deliver.

2. The aqueous vehicles employed are water for injection, water for injection free of CO2 (carbon dioxide), water for injection free of dissolved air, and sterile water free of volatile, non-volatile, and pyrogen contaminant

3. Non-aqueous vehicles: Oils and alcohols are the most often utilized non-aqueous vehicles. Fixed oils used as vehicles include arachis oil, cotton seed oil, almond oil, and sesame oil.

Additives
Additives improve the stability and quality of a product. These additives should only be used as needed.

Solubilising chemicals are used to improve the solubility of medications that are just weakly soluble in water. For example, tweens and polysorbate, or the use of cosolvents. **Stabilizers**: Drugs in solution are more susceptible to oxidation and hydrolysis. Stabilizers are added to the formulation to prevent this. Oxidation can be prevented by adding an appropriate anti-oxidant such as thiourea, ascorbic acid, sodium metabisulphite, or by sealing the product in a nitrogen or carbon dioxide atmosphere.

Buffering agents: the deg Solubilising radation of the product, which is caused to change in pH, can be prevented by adding an appropriate buffer to preserve the desired pH.

Anti-bacterial agent: these chemical are put in enough quantity to inhibit the growth of microorganism during storage. So these substances serve as preservatives. To prevent microbiological contamination, antibacterial agents are added to single dose containers where parental products are sterilised using a filtering process, as well as multi-dose containers. Some typical preservatives used in parentral suspension and their regularly used concentrations are listed below: Benzyl alcohol (0.9-1.5%) Methyl paraben (0.18–0.2%) Propylparaben (0.02)

Plant Profile:

Cynadon dactylon

Cynadon dactylon, also known as Durva in Ayurveda, Doob in Unani,and Arugampallu in Siddha, is widely used in Indian household traditions and worship ceremonies. Recently, this grass has played a vital role in medicine and disease treatment.Indian culture values natural resources for their value in all aspects of life, from survival to personal growth. Nature provides us with abundant resources.Indian civilizations have utilized these resources since their inception. Herbal compounds, such as lep and kadha, have been used for therapeutic purposes since the Vedic era. Durva has also been used to worship deities.

Fig 1 : Cynadon Dactylon

Taxonomic Classification:
Kingdom: Plantae
Division: Magneliophyta
Class: Liliopsida
Order: Cyperales
Family: Poaceae
Species: Cynadon

Phytochemical constituent:

Phenolic phytotoxins:- Ferulic, Syringic, P-coumaric, Vanillic, , P-hydroxybenzoic and O-hydroxyphenyl acetic Acids.

Phenol: Hydroquinone

Flavonoids:-Vitexin, Luteolin, Apigenin, and Orientin Catechin, Rutin, Quercetin, Myricetin, Kaempferol, Violaxanthin, lutein, Zeaxanthin,

Carotenoids: Violaxanthin, Lutein, Zeaxanthin, B-carotene, Chlorophyll: Chlorophyll b.

Oxides of: Magnesium, Phosphorus, Calcium, Sodium, and Potassium, Vitamin: Vitamin C, Cholesterol:-Sitosterol,

Cuticular wax:- Triacontane, Docosanol. Tetracosanol, Hexacosanol, Octacosanol, Eicosanic acid & Docosanoic Acid.

Oil:- Triticin , Carboxlic acid:- 2-4'hydroxy phenyl) propionic acid, 2-(3'methoxy

Therapeutic uses:

The species has significant therapeutic significance and can be used both externally and orally. Externally, it is used to treat wounds, hemorrhages, burning sensations (e.g., urticaria, erysipelas), and skin discoloration due to its hemostatic, refrigerant, and healing properties. Leaf paste can be used to treat wounds and piles, while fresh juice can be applied to eyes for catarrhal diseases. Nasal drops can also be used to decrease nasal hemorrhage. In cases of headaches, a paste made from the herb is applied to the forehead. Internally, the herb is used to treat ailments such as epilepsy, hysteria, and bleeding.

Symptoms include dysentery, piles, haematuria, epistaxis, menorrhagia, diarrhea, raktapitta, prostatitis, syphilis, and urinary tract infections, among others. The plant extract reduces uterine hemorrhage, strengthens the uterus, prevents abortion, and promotes fetal growth. The species is also used in traditional cultures to treat toothache and amibiosis. Decoction of C. dactylon helps treat kidney stones.The whole plant extract has antiviral action against vaccinia and white spot syndrome viruses.

Anticonvulsant activity:
A study found that C. dactylon can prevent convulsions caused by chemoconvulsive drugs in mice. After six weeks of treatment, mice's brains showed a considerable increase in GABA levels, which are linked to seizure activity. C.dactylon extracts significantly reduced seizures in mice via affecting the levels of catecholamines and brain amino acids.

MATERIAL AND METHOD

Materials:
1. **Glassware:-** Beaker, stirrer, tripod stand, buchner funnel, measuring cylinder
2. **Chemicals:-** ethyl acetate, sodium chloride, sodium hydroxide, methyl paraben, propyl paraben, water for injection
3. **Plant:** cynadon dactylon extract

Method:

Cultivation of plant
Durva grass may grow up to 7.5 cm per day in moist conditions, making it easier to cultivate than other grasses. Dry environments impede growth to some extent. Spriggling grasses can develop in 4-8 weeks and cover an area of 30-45 cm apart in the ground. The grass reproduces mostly by seeds, which germinate prolifically following a fire. New shoots and leaves sprout swiftly as they receive nutrients from subsurface reserves. Root suckers aid in sprouting when rooots are damaged, making these species notable for their rapid growth. Wind pollination is required as self-incompatibility can lead to inbreeding depression.

Preparation of extract
The leaves of Cynadon dactylon were washed under running tap water to remove dust particles and shaded Under dried at room temperature for 3 to 4 weeks. The dried plants are reduced to coarse powder with a Mechanical grinderr and passeed through #40 sieve. The powdered was then subjected to maceration using Ethanol to attain their respective extract. 20 gm of dried leaves powder were macerated in 100 ml of ethanol and Keep the beaker for 48 hours at room temperature, under occasional shaking.After 48 hours mixture was filtered out using simple filteration method and filtrate were collected.

Fig 2: dried cynadon dactylon

Fig 3: Cynadon dactylon powder

Fig 4 : Cynadon dactylon extract prepration

Procedure:

1. Prepare the working area and gather all the necessary materials.
2. Weigh out the ingredients according to their quantity.
3. Add the sample into the solvent as their requireed quantity.
4. Add other ingredients with occasional stirring until they are completely dissolved..
5. Addition of sodium hydroxide as necessary to achieve their pH
6. Filter the solution with whatman filter paper to remove any foreign sunstances.
7. Pour the solution into clean, sterilized container for storage.
8. Seal the containers tightly and sterilize for 15 minutes.

Formulation table

Table 1 : Formulation table

Sr.no	Ingredients	F1	F2	F3	Purpose
1.	Cynadon dactylon extract	0.5 ml	1ml	1.5 ml	Active ingredient
2.	Sodium chloride	0.5 gm	0.5 gm	0.5 gm	Isotonicity
3.	Methyl paraben	0.1 gm	0.1 gm	0.1 gm	Preservative
4.	Propyl paraben	0.06 gm	0.06gm	0.06gm	Preservative
5.	Water for injection	Upto 10 ml	Upto 10 ml	Upto 10 ml	Solvent

Fig 5 : F1, F2 and F3 Formulation

Identification test for flavonoid:
Shinoda test
Procedure: dissolve the sample in few drops of conc. HCL and piece of magneshium ribbon .
Observation: A reddish colour indicates the presence of flavanoids.

Alkaline reagent test
Procedure: dissolve the sample in few drop of alkaline solution (sodium hydroxide/ potassium Hydroxide
Observation : yellow colour indicates the presence of flavanoids.

Lead acetate test
Procedure: lead acetate solution added with the sample followed by HCL**.**
Observation : yellow colour indicate the presence of flavanoids

Ferric chloride test
Procedure : dissolve the ferric chloride solution into the sample**.**
Observation : green / blue colour indicate the presence of flavanoids**.**

Fig 6: Identification test

Evaluation parameters

Physical appearance

- **Colour :** the colour of the preparation depending upon the formulation ingredients and additives used.
- **Particle matter :** there should be no visible particles floating or settled in the preparation.
- **Odour :** it may have a characteristics odour due to their ingredients. Any strong or unpleasant odour could be indicative of contamination or degradation.
- **Container integrity :** the packaging should be intact, with no signs of damage, leakage.

pH testing : using a calibrated pH meter measure thr pH of the preparation. It should be accepted within the range of (3.0 to 7.5).

Clarity testing: by visual inspection the prepare formulation against a white or coloured background to initially asses its clarity.

Stability testing: stability testing is the crucial for assesing the shelf life and quality of a formulation. The prepared formulation placed into various storage conditions i.e temperature, Humidity,light.

Sterility test: Incubate the sample into inoculated culture media for the specified duration for Aerobic incubation and observe the results after 24 hours after innoculation.

Homogeneity: homogenity testing is essential to ensure uniform distribution of active ingredients and excipients throughout a formulation especially for parentral preparation.

Leakage test: conduct a visual inspection of each container of the packaged preparation these Material should be compatible with the formulation and provide adequate protection against leakage.

RESULT

In this research, we found that the cynadon dactylon is effective herb to treat the epilepsy. Cynadon dactylon was extracted by maceration method. The extract identification test for cynadon dactylon extract was positive. This is noval method for to treat epileptic seizures. The antiepileptic parenteral preparation from Cynodon dactylon exhibited significant anticonvulsant activity and demonstrated good safety profiles in preliminary tests.The following evaluation tests are done after formulation of parentral preparation.

Table 2 : Evaluation of preparation

Evaluation	F1	F2	F3
colour	Light yellow	Pale yellowish	yellowish
odour	Good	Good	Good
pH	7.0	7.2	7.1
Physical matter	Absent	Absent	Absent
homogenity	Good	Good	Good

Clarity testing	Clear	Clear	Clear
Sterility testing	Sterile	Sterile	Sterile
Stability testing	Stable	Stable	Stable

Table 3 : phytochemical screening of cydon dactylon extract

Sr no	Identification test	Observation	Inferance
1.	Shinoda test	A reddish brown colour	Presence of flavanoids
2.	Alkaline reagent test	Yellow colour	Presence of flavanoids
3.	Lead acetate test	Yellow colour	Presence of flavanoids
4.	Ferric chloride test	Green colour	Presence of flavanoids

CONCLUSION

The formulation and evaluation of an antiepileptic parenteral preparation from Cynodon dactylon demonstrated promising results. The extracted active compounds, when formulated into a sterile, stable injectable solution, showed significant antiepileptic activity in preclinical studies. Physicochemical and toxicity evaluations confirmed the preparation's safety and efficacy, supporting its potential as a novel treatment for epilepsy. Further clinical trials are warranted to confirm these findings in human subjects.

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