

IN VIVO STUDY OF TERATOGENIC EFFECTS OF ANTIEPILEPTIC DRUGS USING ZEBRAFISH MODEL

Submitted in partial fulfilment of the requirements for the
award of Bachelor of Science degree in B.Sc.
Microbiology

By

ANUSIYA M (40300004)



DEPARTMENT OF BIOTECHNOLOGY

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)

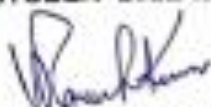
Accredited with Grade "A" by NAAC | 12B Status by UGC | Approved by AICTE
JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119

MAY 2023

DEPARTMENT OF BIOTECHNOLOGY

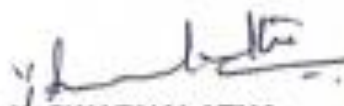
BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Anusya.M (40300004) who carried out the project entitled "IN VIVO STUDY OF TERATOGENIC EFFECTS OF ANTIEPILEPTIC DRUGS USING ZEBRAFISH MODEL" under our supervision from OCTOBER 2022 to MARCH 2023



Dr. V. RAMESHKUMAR

INTERNAL GUIDE



Dr. Y. SWARNALATHA

EXTERNAL GUIDE



Dr. V. RAMESH KUMAR

HEAD OF THE DEPARTMENT

Submitted for Viva voce Examination held on . 8/5/2023


Internal Examiner


External Examiner
8/5/2023

ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to the Board of Management of SATHYABAMA for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to **Dr. V. RAMESH KUMAR**, Head of the Department, and Department of **BIOTECHNOLOGY** for providing me with necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Guide, **Dr. Y. SWARNALATHA, PROFESSOR, DEPT OF BIOTECHNOLOGY, SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY** for her valuable guidance, suggestions and constant encouragement that paved the way for the successful completion of my project work.

I would also like to express my sincere gratitude to **Ms. SIDHIKA. K, Mr. NARENDRAN. C** and **Ms. VIJAYALAKSHMI. R** for their support and help for the successful completion of my project.

I am greatly beholden to my family and friends for their support, patience and forbearance during this venture.

I wish to express my thanks to all teaching and non-teaching staff members of the Department of **BIOTECHNOLOGY** who were helpful in many ways for the completion of the project.

ABSTRACT

Siddha is one of the ancient medical systems in India, considered as the mother medicine of ancient Tamilians /Dravidians in South India. It deals with the physical, psychological, social and spiritual well being of an individual. All medications used for treating various diseases or for restoring the balance of the body are made of natural herbs and plants. Siddha medicines are not just curative, but preventive in nature. Therefore, besides people suffering from chronic ailments, others too can use it to remain healthy. The roots of this system are intertwined with the culture of the ancient Tamil civilization. Epilepsy is the 4th most common neurological disorder. It is also called "seizure disorders. " Seizure is a disruption of the electrical communication between neurons. Epilepsy and seizures can develop in any person at any age. The present study was initiated with the aim of comparing anti-epileptic drugs from different system of medicine and its teratogenic effects using zebrafish model. The study was carried out to evaluate the teratogenic effects of antiepileptic drugs from two different systems of medicines a namely, Evaporate from (allopathy), and Ponnavarai (cassia auriculata) chooranam from (Siddha Sastric Medicine) using in- vivo zebrafish model. The present study focuses on the teratogenic effects of antiepileptic drugs available in two different system of medicine. The Developmental parameters were observed along with the mortality and hatchability values. The results will gave us a better understanding on the teratogenic and anticonvulsant effects of drugs available in alternate system of medicine.

KEYWORDS: Epilepsy, Zebrafish, Teratogenicity, Anti-epileptic drugs (AEDs), Siddha.

LIST OF CONTENT

CHAPTER NUMBER	CONTENT	PAGE NUMBER
	ABSTRACT	I
	LIST OF CONTENTS	II
	LIST OF ABBREVIATIONS	V
	LIST OF TABLES	VI
	LIST OF FIGURES	VII
CHAPTER 1	INTRODUCTION	1
	1.1 Teratogenicity	1
	1.2 Epilepsy	1
	1.2.1 Epilepsy in women	2
	1.2.2 seizures during pregnancy	3

	1.3 Zebrafish as a suitable model organism	3
	1.3.1 Zebrafish as a model organism	9
	1.3.2 Zebrafish maintenance	9
	1.3.3 Breeding circumstances	9
	1.3.4 Feeding	9
	1.4 Antiepileptic drug	10
	1.5 Siddha system of medicine	10
	1.5.1 Origin of siddha medicine	11
	1.5.2 Siddha sastric medicine	11
	1.5.3 Basic concepts of siddha medicine	11
	1.5.4 Formulations of siddha	12
	1.6 Commercially available (AED) in allopathy	12
	1.6.1 Pharmacological activity of valproate	13
	1.6.2 Indication and usage	13

	1.6.3 Contraindication	13
	1.7 ponnavarai chooranam	14
CHAPTER 2	LITERATURE SURVEY	16
CHAPTER 3	AIM AND SCOPE	26
	3.1 Objectives	26
CHAPTER 4	MATERIALS AND METHODS	27
	4.1 Maintenance	27
	4.2 Breeding procedure	27
	4.3 preparation of E3 medium	28
	4.4 Test compound preparation	28
	4.5 Treatment of embryos with drugs	28
	4.6 Larvae observation and evaluation of teratogenicity	29
	4.7 Mortality, Deformity and Hatchability	30
	4.7.1 Formulae	30
CHAPTER 5	RESULTS AND DISCUSSION	31

	5.1 Test compounds	31
	5.2 Embryo exposure	31
	5.3 Mortality rate	31
	5.4 Deformity rate	31
	5.5 Hatchability rate	31
	5.6 Result and Discussion	32
CHAPTER 6	SUMMARY AND CONCLUSION	37

ABBREVIATIONS

S.No.	Abbreviation	Full Form
1.	AED	Anti - epileptic drug
2.	PE	Pericardial edema
3.	YSE	Yolk sac edema
4.	TM	Tail Malformations
5.	HM	Head Malformations
6.	DB	Deformed Body
7.	LP	Less Pigmentation
8.	VAP	Valproic acid
9.	hpf	Hours post fertilization
10.	µg	Microgram
11.	ml	Milliliter
12.	CNS	Central Nervous System
13.	RM	Retinal malformations

LIST OF TABLES

TABLE NUMBER	CONTENT	PAGE NUMBER
5.1	PONNAVARAI CHOORANAM	32
5.2	VALPROATE	33
5.3	MORTALITY PERCENTAGE OF PONNAVARAI CHOORANAM	34
5.4	MORTALITY PERCENTAGE OF VALPROATE	35

LIST OF FIGURES

FIGURE NUMBER	CONTENT	PAGE NUMBER
1.1	Zebrafish	5
1.2	Lifecycle of Zebrafish	5
1.3	Developmental Stages Of Zebrafish	6
1.4	Ponnavarai Plant	15
1.5	Ponnavarai Chooranam	15
4.1	Maintenance of Zebrafish	27
4.2	Test Drug Concentrations	29

CHAPTER 1

INTRODUCTION

1.1 TERATOGENICITY

Teratogenesis is a process that causes birth defects or deformities in an embryo or fetus. It involves the investigation of the underlying causes and mechanisms of deformities or anomalies at birth. These might include ailments like intellectual disabilities that don't have any obvious structural defects. A teratogen is an agent (from outside the body) that causes malformations or birth defects. It is the study of non-genetic mechanisms causing birth abnormalities and deformities that are caused by environmental factors. Since the origins of deformity have been researched since ancient Greece (Vargesson, 2017). The term "teratology" is derived from Greek and essentially translates to "the study of monsters. "Teratogenic factors" are chemicals that can lead to congenital defects; they might be physical, chemical, hormonal, viral, or related to the mother's health (Haroun, H.S.W, et al, 2017). They are environmental elements that can interfere with normal fetal development and result in birth abnormalities if the mother is exposed to them. Teratogens are substances that have the potential to harm fetal or embryonic development. It may result in the termination of a pregnancy or congenital abnormalities, which are birth problems. The embryo or fetus is affected by the length of the exposure, the quantity of the teratogenic substance, and the developmental stage it is in at the time of the exposure. Various factors may cause embryo or fetus injury, which could result in a physical abnormality. It frequently causes developmental processes to deviate from the norm. Abnormalities include things like deformity, death, functional impairment, or even demise (Aihamdan,w, et al, 2020).

1.2 EPILEPSY

According to the ILAE (International League Against Epilepsy), "Epilepsy is a short-term event consisting of distinct signs and symptoms involving idiosyncratic nerve

activities in the CNS (Central nervous system) causing neurological disorders with abnormal behaviour and action."Genetic, structural/metabolic, and undiagnosed problems are some of the causes. Seizures are brought on by an excess of neuronal effusion in the central nervous system (Dahl-Hansen, E et al, 2019). Epilepsy has a complex etiology that is often diverse and frequently caused by a combination of risk factors, aberrant developmental patterns, and environmental influences (B.Mayri1 et al, 2019). The sickness is common and affects people of all ages. Genetics, brain infections, strokes, tumors, and extremely high or low temperatures are just a few of the causes. Sudden electrical activity in the brain is what makes it distinctive. It is believed to be the clinical symptom of an unexpected and excessive firing of a certain group of brain neurons. Excessive nerve-cell discharges in the brain that result in instance activity are what cause seizures. Frequently, they cause unconsciousness, an increase in muscle activity, or an odd sensation (Weckesser, A et al, 2013).

1.2.1 EPILEPSY IN WOMEN

Since epilepsy is a chronic condition, treatment typically involves a daily schedule that never ends. Epilepsy patients are frequently otherwise healthy when their condition is well-controlled, allowing them to fully engage in life, including childbirth (Harden, C.L et al, 2006). The frequent use of antiepileptic drugs (AEDs) during pregnancy among women with epilepsy raises concerns about the growing foetus' safety. Once birth anomalies are identified, treatment lowers the frequency of recurrent seizures as well as the related medical and neurological effects. Malformations of the urogenital system, the heart, and other organs are among them. Numerous specific concerns pertain to women with epilepsy, including the danger and treatment of pregnancy. Antiepileptic medications as well as frequent seizures while pregnant could be detrimental to the fetus. Currently, there are various registries of expectant mothers with epilepsy, and data from these studies are gradually becoming accessible (Gawel, K et al, 2020).

1.2.2 SEIZURES DURING PREGNANCY

The main arguments for providing daily antiepileptic medications (AED) to Pregnant women with epilepsy are the danger of seizures during pregnancy and any potential consequences they may have on the developing fetus experiencing your first seizure while expecting. The onset of the first seizure during pregnancy, however, may have several major impacts on the pregnant woman's ongoing care in a medical setting. Throughout the first trimester, metabolic changes, medications, and toxicology testing should all be evaluated. Since common physiological changes that occur during pregnancy during the second trimester may result in decreased blood pressure and vascular space dilating, various episodes are a big issue. In the third trimester, other diagnoses like posterior reversible encephalitic syndrome (PRES) and stroke are also possible. Vascular anomalies, infections, and any abrupt incident may result in mass lesions (Gawel, K et al, 2020). A first epileptic seizure could potentially affect the pregnancy depending on what caused it. Having seizures that affect a mother's awareness and focus can have an impact on the foetus' heart development and the newborn's weight. Although status epileptics during pregnancy are rare, antiepileptic drug-using women have occasionally reported fetal mortality and anomalies after status epileptics. Case studies indicate that the unborn heart may be affected, and about 2% of pregnant epileptic women experience seizures during birth. However, epilepsy does not necessitate a Caesarean delivery. With careful preparation throughout pregnancy, the likelihood of seizures developing can be reduced, but the baby may still be born with many anomalies and disorders related to the nervous system (Nowik et al, 2015).

1.3 ZEBRAFISH AS A SUITABLE MODEL

The benefits of zebrafish (*Danio rerio*) make them suitable model organisms for neurological research. Zebrafish are suitable for a range of research projects since they are small (approximately 2.5 cm long), have a short generation period (3–4 months), and may regularly generate hundreds of eggs. *Danio rerio*, a small tropical freshwater fish sometimes known as a zebrafish or brachydanio rerio, is a species that is exclusively found in the Ganges River and its tributaries in northern India.

Zebrafish are generally found near the seafloor because this reduces attacks from predators. Due to its simple breeding and maintenance requirements, high fertility, swift outward growth, transparent larval bodies, and numerous methods for successfully establishing genetically modified strains, it is a practical model animal for neuroscience because it has many advantages. Zebrafish have many advantages in biomedical research, particularly in the study of epilepsy, according to recent exceptional reviews. Being a "model", zebrafishes are still employed as a model organism for disease and translational research. Zebrafish and humans share numerous genes, developmental processes, and disease states because they are both vertebrates. Early embryogenesis can be seen in detail for development, pharmacological response, regeneration, and infection due to their optical transparency (Kimmel, C et al, 1995-203(3)). The majority of the adult zebra fish's physiology and anatomy are comparable to those of humans and other animals, and many of their disease processes closely resemble clinical sickness and its symptoms. Zebrafish can offer data when zebrafish embryos are easily accessible. Clinical research on human subjects using seizure models has substantially improved and enhanced our understanding of the mechanisms causing epilepsy (Rajkumar, S et al, 2016). Our understanding of epilepsy and the quest for viable treatments have already greatly improved. Zebrafish offer a range of opportunities for research into the mechanisms causing seizures in a variety of epilepsy patients, including people and other animals (Choonara I, 2018). Stages in the embryonic development of the zebrafish *Danio rerio*. The seven main stages of embryogenesis include the zygote, cleavage, blastula, gastrula, and stages for the development of the embryo of the zebrafish *Danio rerio* (Teame T, et al, 2019)

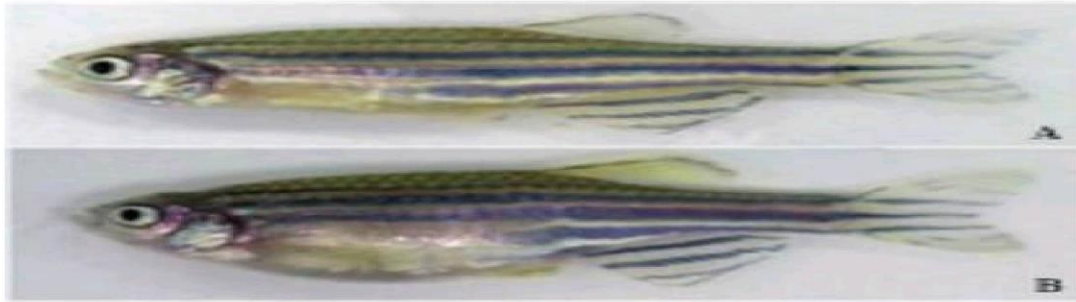


Fig 1.1 ZEBRAFISH (*danio rerio*) (Teame T, et al,2019)

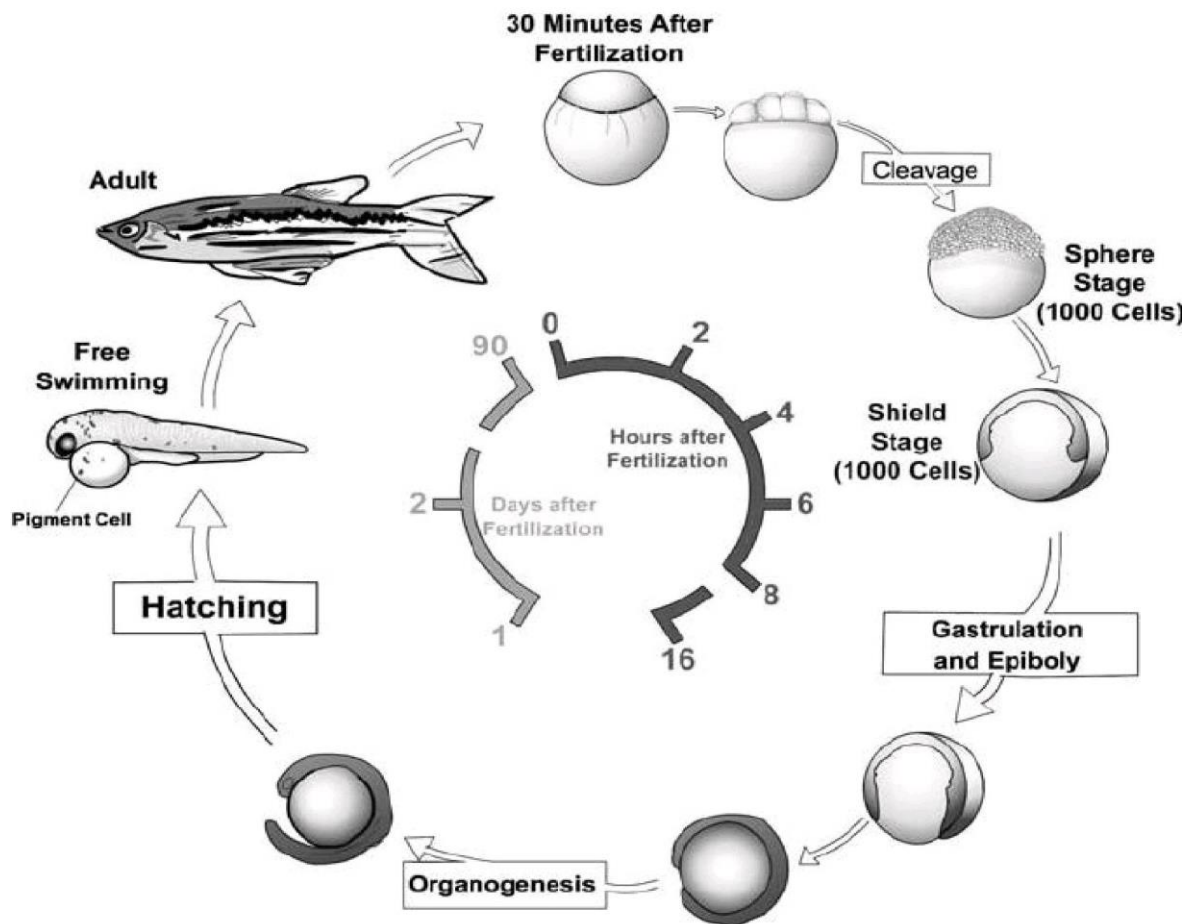


Fig: 1. 2 LIFE CYCLE OF ZEBRAFISH (Allison,D,Costa et al,2009)

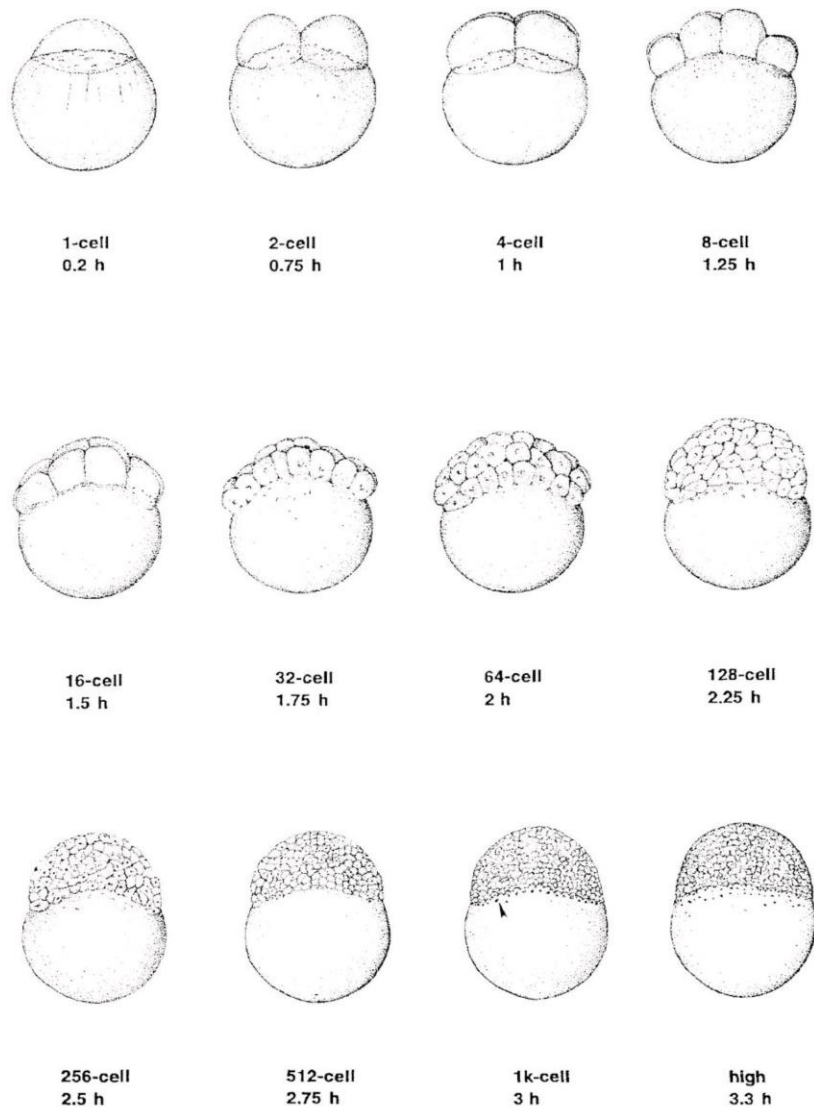
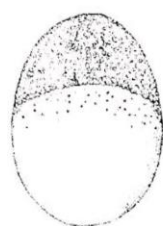


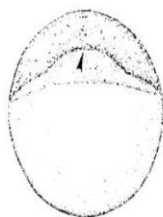
Fig. 1 (Legend to Fig. 1 appears on page 259).



oblong
3.7 h



sphere
4 h



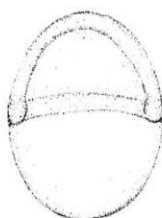
dome
4.3 h



30%-epiboly
4.7 h



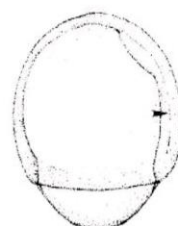
50%-epiboly
5.3 h



germ ring
5.7 h



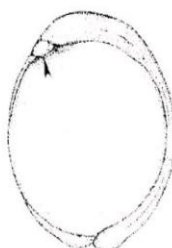
shield
6 h



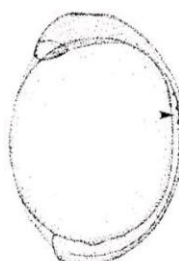
75%-epiboly
8 h



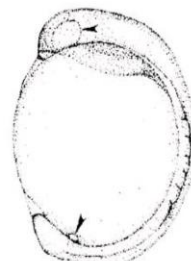
90%-epiboly
9 h



bud
10 h

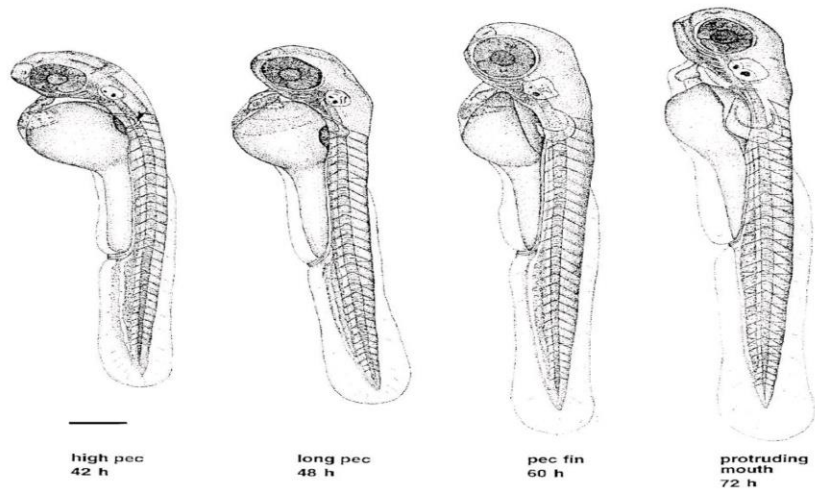


3-somite
11 h



6-somite
12 h

Fig. 1 (continued).



258

KIMMEL ET AL.

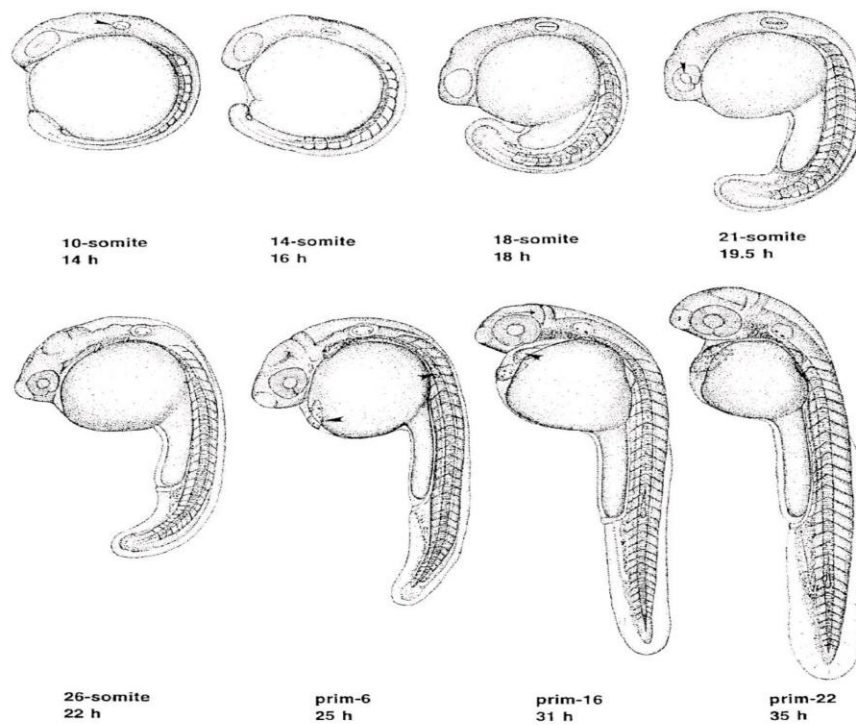


Fig. 1 (continued).

Fig1.3 DEVELOPMENTAL STAGES OF ZEBRAFISH(Kimmel,c et al, 1995)

1.3.1 ZEBRAFISH AS A MODEL ORGANISM:

Zebrafish can be maintained low in cost, making it possible to harvest hundreds to thousands of embryos every week for research. In vivo, the analysis must be possible with the zebrafish system (Ali, shaukat& et al, 2011). As a consequence of an experiment, parental care is low and has a reduced impact on epigenetic modifications.

1. Affordable,
2. Simple husbandry,
3. Low cost,
4. A rapid number of embryos were generated in less time,
5. External progress,
6. Optical clarity as its developed etc., (Longkumer,sentiyanger et al, 2022)

1.3.2 ZEBRAFISH MAINTENANCE

With a high-density rack, a semi-automatic recirculating water system, and all necessary, crucial water filtering and disinfection, the zebrafish habitat has been carefully built and tailored. The fish (n=7) were housed in 1-liter individual fish tanks from the zebrafish housing system. There, a submersible heater (300W) was employed to maintain a 28.2C temperature. To maintain photoperiod, a digital switch with various circuits was used. The pH of the housing system was kept at 7.3.

1.3.3 BREEDING CIRCUMSTANCES

For the breeding trials, both males and females of reproductive age were selected. A soft brush, petri dish, breeding chamber, egg separator, pebbles, and dropper are all part of the breeding setup (Chaudhary, Dr.Avdes& et al,2012).

1.3.4 FEEDING

Adult zebrafish were alternately given commercial diets from Perfect Companion three times each day. According to the statistics, the serving size was selected to

promote speedy consumption within 10 minutes. (Chaudhary, Dr.Avdesh& et al,2012).

1.4 ANTIEPILEPTIC DRUG

The drug is used to treat or prevent seizures or convulsions by regulating electrical activity in the brain. Anti-epileptic medications (AEDs) have become much more prevalent, mostly affecting children and elderly people, improving their quality of life and greatly reducing both morbidity and mortality. But they are very toxic and have a high level of toxicity. For many AEDs, behavioral problems and tiredness are the most frequent adverse drug reactions. Traditional medicines are used to control epileptic seizures. Drug toxicity is the basis for the treatment. This medication has a significant impact on seizure disorders (Aneja, S et al, 2013).

1.5 SIDDHA SYSTEM OF MEDICINE

One of the oldest traditional medical systems still in use, the Siddha system, had its beginnings in South India around 5000 BC. It has Dravidian roots and has literary works in Tamil throughout. This system was influenced by local tradition, which has roots in the ancient Dravidian civilization. Siddha were holy people who made important contributions to medicine; the word "Siddha" refers to accomplishments. It was said that 18 Siddha contributed to the creation of this therapeutic method. The most well-known of the eighteen is Agasthiyar, and several of his writings are still used often as standard texts on surgery and medicine by Siddha medical professionals. Tamil-speaking areas of India are the primary locations for Siddha practice, and Siddha literature is published in this language. The Siddha system is mainly intended for therapeutic purposes. In all of the literature, only Tamil is used as a classical language. (South Indian language), which is spoken by people in Sri Lanka to some extent as well as the Indian states of Tamil Nadu and Kerala, plays a part in Siddha (Thangapandiammal, S et al, 2019).

1.5.1 ORIGIN OF SIDDHA MEDICINE

One of the country's oldest medical systems is Siddha medicine, an antiquated medicinal practice with roots in southern India. The Siddha system's foundation consists of alchemy, mysticism, ancient spiritual practices, Siddha philosophy, and all of these things. The Siddha system of medicine has been practiced in the historic Tamil region and is the most significant of all other medical systems around the globe. The Dravidian culture of the formerly submerged territory of Kumarikandam, which is the Lemurian continent located in India, is very old, according to ancient archaeological findings. The uniqueness of the Siddha System is illustrated by its continued, more than 5000-year contribution to humanity in the struggle against (Thangapandiammal, S et al, 2019).

1.5.2 SIDDHA SASTRIC MEDICINE

The Sastric Chooranam of the Siddha medical system follows a strictly scientific method. Native American communities all over the world are well aware of the significance of multiple plants in treating human health issues, which is relevant to the drug release of the numerous herbs used in the formulation. By looking up its botanical name and Siddha name in the components and indications of Siddha Sastric remedies from the old system. By preventing sickness, the Siddha system of medicine can help us live a healthy, natural lifestyle (soundharya, R et al, 2019).

1.5.3 BASIC CONCEPTS OF SIDDHA MEDICINE

According to Siddha's philosophy, the universe is made up of the five basic elements of earth, water, fire, and air in the same way as the human body. A fundamental Siddha principle is that the Pancha Bhootham (five elements), Arusuvai (six tastes), and Uyirhathu (three senses of humor) are interrelated. There are 96 basic laws (thathuas), among which Mannu (earth), Neer (water), Thee (fire), Vaayu (air), and Aakaayam (space) are the five components that make up Bootham. The five senses that Pori possesses are its eyes, ears, nose, tongue, and skin. The five senses that Pulan possesses are sight, hearing, smell, taste, and touch. The five motor organs of

Kanmenthiriyam are the hands, legs, mouths, rectums, and sex organs (Sofia, H.Nalini 2007)

1.5.4 FORMULATION OF SIDDHA MEDICINE

Natural ingredients make up Siddha medicines. The Siddha system employs formulations and therapeutic methods that are uniquely created for each patient and ought to be modified by their body type, surroundings, and conditions (Saraf, Shailendra, et al, 2011).

1.6 AED (ANTIEPILEPTIC DRUGS)COMMERCIALY AVAILABLE IN ALLOPATHY:

These medications should only be prescribed by pediatricians who are well-versed in the pharmacokinetics, indications, dosage, side effects, and possible drug interactions of the medicine. There are several of them already available on the market, including lamotrigine, topiramate, levetiracetam, valproate, zonisamide, vigabatrin, lacosamide, carbamazepine, rufinamide, stiripentol, retigabine, brivaracetam, ganaxolone, and perampanel, among others(Aneja, S et al,2013).

VALPROATE

Epilepsy and Bipolar illness are both treated with sodium valproate. It is occasionally used to stop migraines. Only those with a prescription can purchase this medication. You can take it as pills, capsules, or a liquid. Being a simple branched-chain fatty acid with no structural ties to any other Antiepileptic medication, valproic acid (N-dipropyl acetic acid) or valproate (VPA) is a unique medication. Four decades ago, when other chemicals were being examined for antiepileptic drugs. VPA has been shown to have antiepileptic effects in a variety of animal models in the absence of seizures. VPA has a wide antiepileptic spectrum, making it a medication. Valproate can particularly alter gene expression due to its more recently discovered histone deacetylase (HDAC) inhibitory characteristics. Anti-epileptic drugs (AEDs) like valproic acid are frequently prescribed to treat seizures. The outcome of this Sodium valproate reduces the brain's excessive electrical activity, which prevents epilepsy. This is accomplished by raising the activity of a GABA neurotransmitter in the brain. In the brain, GABA is

believed to be produced more often and not be broken down as a result of sodium valproate. Through its ability to stop sodium from entering nerve cells, sodium valproate may also be able to stabilize electrical nerve activity. Valproate plays a major role as an antiepileptic drug that was prescribed (Rajkumar, S et al, 2016)

1.6.1 PHARMACOLOGICAL ACTIVITY OF VALPROATE

VPA is virtually completely bioavailable. All commonly used VPA formulations conventional tablets, enteric-coated tablets, sustained-release tablets, capsules, and oral and intravenous solutions, have a 96–100% efficacy range. However, the formulation affects how quickly something is absorbed. VPA is quickly absorbed. Patients with chronic hepatitis, kidney illness, aging, pregnancy, and patients with renal disease have lower protein-bound fractions than healthy individuals in the presence of other medications that are protein-bound, such as acetylsalicylic acid or phenylbutazone over the past few decades; several clinical trials have shown that VPA is efficient. Many forms of seizures are treated. Different neurological or psychiatric illnesses may benefit from the use of VPA which involves GABAergic, glutamatergic, and serotonergic neurotransmission (Ayano, G. 2016).

1.6.2 INDICATION AND USAGE

(Valproic acid) is prescribed for the treatment of simple and complicated absence seizures, as well as adjuvant therapy in patients who have various seizure types, including absence seizures (Tolou-Ghamari, Z et al, 2015).

1.6.3 CONTRAINDICATION

Dark urine and yellowing of the skin or whites of the eyes, however, these symptoms may be less noticeable in those with brown or black skin, which are potential indicators of liver disease. Long-lasting, intense stomach pain, vomiting, or nausea may be symptoms of acute pancreatitis (an inflamed pancreas) (Johannessen, C.U., et al, 2003).

1.7 PONNAVARAI (CASSIA AURICULATA) CHOORANAM

Bogar - 7000 alias Bogar Saptha Kaandam

Gunapaadam (Mooligai vaguppu) (Material medical - Plant kingdom).The churna is freely flowing and keeps its effectiveness for a year when kept in sealed containers. Powder formulations used in the Allopathic medical system are comparable to churna formulations. To treat Malakattu (constipation), Kaalkaivalippu (epilepsy), Valippugal (convulsions), Kaaichchal (fever), Irumal (cough/bronchitis), vitiated conditions of the vata and pitta, and skin conditions like Sori (itching), Sirangu (scabies), Thole vedippu/Saruma vedippu (skin fissures), In addition to Araikkaduvan, other terms for rashes include Mega pudaigal (venereal rashes), Thole thadippu/Thole kandukandaka thadiththal (rashes), Neenga thinavu (severe itching), Kanakkadi/Poochchikadi/vandu kadi (wasp sting/insect bite), and others(Nambirajan, Get al,2018). *Cassia auriculata* L. is one such plant that has been utilized in traditional medicine. The plant, which is extensively found in India and Sri Lanka, is a member of the Fabaceae family. Avarai panchaga chooranam, also known as Kalpa herbal tea, is a type of herbal tea that is made from five plant parts: roots, leaves, flowers, bark, and unripe fruits. It is a plant that primarily spreads on the ground. The leaves are tiny, with a white lower surface and a green top surface (Sudharshini, K et al,2019). The flowers are quite tiny and reddish. Fruits have five glands for color. The roots of plants are very useful in medicine. The leaves and seeds of this plant are used to make the medicine punarnava(Bhowmik, D et al, 2012).



Fig: 1.4 PONNAVARAI CHOORANAM



Fig: 1.5 PONNAVARAI PLANT

CHAPTER 2

LITERATURE SURVEY

Vargesson reviews the history and mechanisms of thalidomide-induced teratogenesis, a well-known example of a teratogenic agent that caused significant birth defects in thousands of babies in the 1950s and 1960s. The author discusses the teratogenic effects, including its impact on development and the role of oxidative stress and inflammation. Overall, Vargesson's article provides a comprehensive and up-to-date review of thalidomide-induced teratogenesis, highlighting the importance of this tragic event in the history of medicine and the ongoing efforts to understand and prevent birth defects(Vargesson, 2017).

Heshmat SW Haroun published "Teratogenicity and Teratogenic Factors" in the MOJ Anatomy and Physiology Journal on MedCrave in 2017. In this article, the author provides a detailed overview of teratogenicity and teratogenic factors, including definitions, historical background, and examples of teratogenic agents. The author also discusses the various factors that can influence the severity and type of teratogenic effects, Overall, the article by Haroun provides a comprehensive review of teratogenicity and teratogenic factors, highlighting the need for continued research in this area to prevent and mitigate the impact of birth defects(Haroun, H.S.W, et al, 2017).

Aihamdan,w Entitled "Knowledge regarding teratogens among women of childbearing age at a large tertiary care center in Saudi Arabia" was published in the Journal of Family Medicine and Primary Care. The study aimed to assess the knowledge of women of childbearing age in Saudi Arabia regarding teratogens, which are agents that can cause birth defects. The authors surveyed 300 women attending a tertiary care center in Saudi Arabia and analyzed their responses to questions related to teratogenic agents and their effects on fetal development. The results of the

study indicated that overall, the women had a low level of knowledge about teratogenic agents and their potential effects on fetal development (Aihamdan,w, et al, 2020).

Dahl-Hansen, E gave a review on "Epilepsy at Different Ages—Etiologies in a Norwegian Population" which presents a study conducted in Norway to investigate the etiologies of epilepsy at different ages. The study aimed to determine if the etiologies of epilepsy varied by age group and if the results were consistent with those reported in other populations. The authors conducted a retrospective study of patients diagnosed with epilepsy at a tertiary center in Norway between 2007 and 2016. They categorized patients into four age groups: neonates (0–28 days), infants (29–2 years), children (3–18 years), and adults (19 years and older). The article will be of interest to healthcare providers and researchers working in the field of epilepsy(Dahl-Hansen, E et al, 2019).

B. Mayuri, D. Sudheer Kumar, and P. Kishore published"A Review on Epilepsy" in the Journal of Medical Science and Clinical Research (JMSCR) in March 2019. The authors provide a comprehensive review of epilepsy, covering topics such as epidemiology, pathophysiology, classification, diagnosis, and treatment. Overall, "A Review on Epilepsy" provides a useful and comprehensive overview of epilepsy and is a valuable resource for healthcare professionals and researchers working in the field(B.Mayri1 et al, 2019).

C. Cynthia L. Harden and Lakshmi Nadiminti's" The review on "Treatment considerations during pregnancy for women with epilepsy" was published in the journal Comprehensive Epilepsy Women's Health. The authors of the article are. The article is on the treatment considerations that are relevant for women with epilepsy during pregnancy with an increased risk of maternal mortality, miscarriage, and preterm labor. They also discuss the potential risks associated with antiepileptic drug (AED) use during pregnancy, including an increased risk of birth defects and developmental delays. The importance of a multidisciplinary approach to the management of epilepsy during pregnancy, involving obstetricians, neurologists, and other healthcare providers as needed in the management of seizures and AED use

and emphasize the importance of careful monitoring and individualized treatment plans to optimize both maternal and fetal outcomes (Harden, C.L et al, 2006).

Annalisa Weckesser and Elaine Denny's review on "Women living with epilepsy, experiences of pregnancy, and reproductive health: A review of the literature" was published in the journal *Seizure*. The authors are from Birmingham City University, Centre for Health and Social Care Research, Faculty of Health in Birmingham, UK. The article is a review of the literature on the experiences of women with epilepsy during pregnancy and reproductive health, particularly about pregnancy and contraception. The article provides an overview of the physiological changes that occur during pregnancy and how these changes can affect the management of epilepsy. The authors also discuss the potential risks associated with antiepileptic drug (AED) use during pregnancy, including an increased risk of birth defects and developmental delays (Weckesser, A et al, 2013).

Gawel,k review of "Seizing the Moment: Zebrafish Epilepsy Models" provides an overview of the use of zebrafish as a model organism for studying epilepsy. The authors highlight the advantages of using zebrafish, including their transparent embryos that allow for easy observation of brain development and their genetic similarity to humans. The article also discusses the various types of epilepsy models that have been developed using zebrafish, including chemical induction, genetic mutations, and electrical stimulation. The authors describe how these models have been used to study the underlying mechanisms of epilepsy as well as test potential therapies(Gawel, K et al, 2020).

Kimmel,c gave a review on The zebrafish (*Dania rerio*) has become an important animal model for research in various fields of science, including veterinary medicine. This is because zebrafish share a high degree of genetic and physiological similarity with humans, making them a valuable tool for studying disease mechanisms, drug discovery, and toxicology. In veterinary medicine, zebrafish are commonly used to study various aspects of fish health, including infectious diseases, environmental toxicology, and aquaculture. Overall, the use of zebrafish as an animal model(Kimmel, C et al, 1995-203(3)).

Choonara's review of the zebrafish can be divided into several stages", each marked by specific morphological and molecular events. Here is a brief overview of the different stages. Throughout the stages, various molecular and genetic events drive the development of the zebrafish can use, including the activation and repression of specific genes, cell signaling pathways, and epigenetic modifications. The zebrafish is a popular model organism for studying vertebrate development due to its transparency, ease of genetic manipulation, and rapid development(Choonara I, 2018).

Teame T review on the embryonic development of the zebrafish can be divided into several stages, each marked by specific morphological and molecular events. Here is a brief overview of the different stages: Fertilization: Fertilization occurs when the sperm enters the egg. Gastrulating: During gastrulating, cells from the blastula migrate inward to form the three germ layers: the ectoderm, mesoderm, and endoderm. This process establishes the basic body plan of the embryo. Segmentation: At around 10 hours post-fertilization, the embryo becomes segmented, with somites forming along its length. The somites will give rise to the musculature and skeleton. Pharyngula: At this stage, the embryo resembles a tadpole with a distinct head and tail. The eyes and brain begin to form, and the heart starts beating. Then hatching occurs and it will take 3 months to mature as an adult fish(Teame T, et al,2019).

Ali Shaukat review on the High fecundity and rapid development of transparent embryos Ease of genetic manipulation Genetic and molecular similarities between humans lower cost and space requirements compared to larger animal models. Disadvantages; Limited ability to study complex behaviors Differences in physiology and anatomy compared to humans Limited translatability to humans Ethical concerns associated with the use of animals in research Lack of well-established disease models in zebrafish(Ali, shaukat& et al, 2011).

Longkumer,sentiyanger gave an article on title Zebrafish are prolific breeders, producing hundreds of eggs per mating, allowing for the efficient production of large numbers of experimental animals, but zebrafish are ideal for studying developmental

processes and the effects of drugs and other treatments. Disadvantages: Lack of immune system complexity: Zebrafish have a simpler immune system than mammals, which limits their usefulness for studying aspects of the human immune system. Limited behavioral studies: While zebrafish can be used to study basic behavioral processes, they are not well-suited for studying complex behaviors or cognitive processes. Physiological differences There are important differences between zebrafish and humans that must be taken into account when interpreting results from zebrafish studies(Longkumer,sentiyanger, et al, 2022).

Chaudhary and Dr. Avdesh's review on Zebrafish (*Danio rerio*) is a popular animal model used in biomedical research. They are relatively small, easy to breed and maintain, have a short lifespan, and have a high fecundity rate, making them ideal for genetic and developmental studies. Guidelines for the maintenance and breeding of zebrafish under laboratory conditions include housing and maintenance, feeding, breeding, and health monitoring. Zebrafish should be housed in a variety of tanks with a volume of at least 2-3 liters per fish, with a recirculating water system, a light cycle of 14 hours of light and 10 hours of darkness per day, and a spawning substrate such as marbles or a mesh net. Zebrafish should be monitored regularly for signs of disease, including abnormal behavior, fin rot, skin discoloration, or changes in appetite (Chaudhary, Dr.Avdesh& et al,2012).

Rajkumar, S review on Regular Care and Maintenance of a Zebrafish (*Danio rerio*) Laboratory provides a detailed guide on the regular care and maintenance of zebrafish in a laboratory setting. It outlines essential equipment, water quality, feeding requirements, handling and transporting, and standard operating procedures for breeding and maintaining zebrafish colonies. It is a useful resource for researchers and laboratory technicians involved in zebrafish research(Rajkumar, S et al, 2016).

Nowik gave a review of the article: Zebrafish have become a valuable tool in epilepsy research over the past few decades, providing a unique model system for studying the underlying mechanisms of seizures and the development of new treatments. Recent studies have used zebrafish to investigate the molecular and cellular mechanisms of epilepsy, as well as to test the efficacy of antiepileptic drugs

(AEDs). Zebrafish have a high degree of genetic similarity to humans, making them a valuable model system for investigating the genetic basis of epilepsy. In the future, zebrafish are likely to continue to play a prominent role in epilepsy research, particularly in the development of new treatments. Optogenetics, a technique that allows researchers to control the activity of specific neurons using light, is also being used to identify specific neuronal populations involved in seizure generation and to develop targeted therapies that modulate their activity(Nowik et al,2015).

Imti Choonara on the issue of anti-epileptic drug toxicity in children is affiliated with the Academic Division of Child Health at the University of Nottingham and the Derbyshire Children's Hospital. The article highlights the potential risks of AEDs in children, such as liver toxicity, bone marrow suppression, and skin reactions. It also emphasizes the importance of monitoring AED therapy in children, including regular blood tests and close observation. Healthcare professionals should be aware of the potential risks and take appropriate measures to minimize the risk of toxicity. Overall, the article serves as a reminder of the potential dangers of AED therapy in children and the importance of careful monitoring and management(Aneja, S et al, 2013).

Sofia, H review on the Basic Principle in Siddha Pharmaceutical Science: An Overview" provides a comprehensive overview of the fundamental principles of Siddha Pharmaceutical Science is an important aspect of the Siddha system of medicine about plant-based medicines, the concept of "Pancha Bootha," and the importance of drug preparation. It also discusses the role of in modern times, highlighting its potential for the development of new drugs and treatments. Further research is needed to advance the understanding and application of Siddha pharmaceutical science (Sofia, H. Nalini 2007).

Saraf, Shailendra gave a review of the article the Siddha system of medicine is an ancient medical system that originated in India over 5,000 years ago. It is based on the concept of balance between the five elements (earth, water, fire, air, and ether) and the use of natural remedies and physical therapies. It has been documented in various ancient texts, including the Agastya Samhita, Charaka Samhita, and Susruta Samhita. Despite its ancient origins, it continues to be practiced today and is

recognized as one of India's traditional systems of medicine (Saraf, Shailendra, et al, 2011).

Thangapandiammal and H. Nalinisofia the article "Origin and Antiquity of the Siddha System of Medicine – A Review" explores the history and evolution of the Siddha system of medicine, which emphasizes the holistic treatment of disease by balancing the three senses of humor (Vatha, Pitha, Kapha) in the body. It also discusses its integration into the Indian healthcare system and its recognition as a distinct medical system by the Indian government. The article provides a comprehensive overview of the history and evolution of the Siddha system of medicine, highlighting its importance in the development of Indian medicine and its continued relevance in modern times(Thangapandiammal, S et al, 2019).

Soundharya, R review on Siddha Sastric drugs are traditional Indian medicinal preparations based on the principles of the Siddha system of medicine. Studies have shown that they have therapeutic properties and can be used to treat various diseases. However, they should be used under the guidance of a qualified practitioner to ensure their safety and effectiveness(soundharya, R et al, 2019).

Sharma,suvasini review the newer generation of anti-epileptic drugs (AEDs) developed in recent years, including Levetiracetam, Topiramate, Zonisamide, Lamotrigine, Oxcarbazepine, Eslicarbazepine Acetate, Lacosamide, Rufinamide, Perampanel, and Brivaracetam. It highlights the importance of selecting the appropriate AED based on the type of epilepsy and the patient's individual needs and discusses the potential benefits and drawbacks of each drug(Aneja, S et al,2013).

Ayano, G. reviews the article on the title of the Clinical and biochemical properties of sodium valproate, including its Mechanism of Action, pharmacokinetics, clinical uses, adverse effects, and drug interactions. It discusses the use of sodium valproate in the treatment of epilepsy, bipolar disorder, and other neurological conditions and highlights its potential hepatotoxicity and teratogenicity. It concludes that sodium valproate is a valuable therapeutic agent for the management of epilepsy

and bipolar disorder, but caution is needed regarding its potential side effects and drug interactions (Ayano, G. 2016).

THEERDHALA, SRIKRISHNA's article on "FORMULATION AND EVALUATION OF SODIUM VALPROATE SUSTAINED RELEASED TABLETS" is available. This article was written by a team of authors from the Faculty of Pharmacy at the University of Lahore in Pakistan. It describes the development and evaluation of sustained-release tablets of sodium valproate, a drug used to treat epilepsy and bipolar disorder. The authors used different polymers and excipients to prepare the tablets and evaluated their release profiles and other properties. The article provides detailed information on the methods and results of the study and could be useful for researchers and pharmaceutical companies interested in developing sustained-release formulations of sodium valproate (THEERDHALA, SRIKRISHNA 2022).

Tolou-Ghamari, Z review on Valproate has been used for decades to treat epilepsy and bipolar disorder, but its use has been associated with serious adverse effects, including birth defects, liver failure, and pancreatitis. Healthcare providers should carefully weigh the risks and benefits of valproate and consider alternative medications when appropriate. Research is ongoing to develop more effective and safer treatments. Valproate has been used for decades to treat epilepsy and bipolar disorder, but its use has been associated with serious adverse effects, including birth defects, liver failure, and pancreatitis. Healthcare providers should carefully weigh the risks and benefits of valproate and consider alternative medications when appropriate. Research is ongoing to develop more effective and safer treatments (Tolou-Ghamari, Z et al, 2015).

Johannessen's article "Valproate: Past, Present, and Future" provides a comprehensive review of the history, pharmacology, and clinical uses of valproate, a medication used in the treatment of epilepsy, bipolar disorder, and migraine headaches. The authors begin by discussing the early history of valproate, including its discovery in the 1960s and its initial use in Europe for the treatment of epilepsy. They then go on to describe the pharmacology of valproate, including its mechanisms of action and pharmacokinetics. Finally, It is the discussion of the future of valproate,

including its potential use in the treatment of other neurological and psychiatric disorders, as well as the development of new formulations and delivery systems. Valproate is a medication commonly used in the treatment of bipolar disorder. It works by increasing the amount of a neurotransmitter called gamma-aminobutyric acid (GABA) in the brain, which can help to stabilize mood. Pharmacokinetics: Valproate is rapidly and almost completely absorbed after oral administration. Pharmacodynamics: Valproate works by increasing the amount of GABA in the brain. GABA is an inhibitory neurotransmitter that can help to calm overactive neurons, which can contribute to manic episodes in bipolar disorder. Therapeutic Effects: Valproate is effective in the treatment of epilepsy, migraine headaches, and some other neurological disorders with its indications (Johannessen, C.U., et al, 2003).

Bhowmik, D. The study you are referring to investigated the ant-diabetic activity of the bud and flower of *Azadirachta indica* (Neem) (Cassia auriculata L.) and found the treatment of anti-diabetic activity with the extract. These are both important factors in the development and management of diabetes mechanisms of action and to confirm the safety and efficacy of *Azadirachta indica* in humans. It is important to note that while the study provides promising results, it was conducted on animal models and the findings may not necessarily translate to humans. Additionally, the use of herbal supplements (Bhowmik, D et al, 2012).

Nambirajan review on the traditional Indian herb *Punarnava* and its medicinal importance. The article provides a detailed overview of the plant, its distribution, and its traditional uses in Ayurveda medicine. According to the article, *Punarnava* has been traditionally used for the treatment of a wide range of ailments, including jaundice, asthma, cough, and heart disease. The plant is rich in phytochemicals such as alkaloids, flavonoids, and steroids, which are responsible for its various medicinal properties. The authors conclude that *Punarnava* is a valuable medicinal plant with a wide range of therapeutic applications. However, they also note that further studies are needed to fully understand its pharmacological properties (Nambirajan, Et al, 2018).

Sudharshini, K.'s article on Chooranams are traditional herbal formulations used in Ayurvedic medicine, which is one of the oldest medical systems in the world. Several studies have been conducted to investigate the antimicrobial activity of chooranams against drug-resistant pathogens. Among the selected chooranams, Ponnavarai, Gunma Uppu, and Parangipattai, the following observations have been reported: Poonakali: Ponnavarai is a herbal preparation used for the treatment of fever, cold, and cough. A study conducted by reported that Ponnavarai exhibited (Sudharshini, K., 2019)

CHAPTER - 3

AIM AND SCOPE

To observe the teratogenic effects of anti-epileptic drugs using the zebrafish model.

3.1 OBJECTIVES

1. To analyze the effects of chosen Antiepileptic drugs on Zebrafish.
2. To examine the fetal effects of various AEDs (valproate), (ponnavarai chooranam) Using the zebrafish model.
3. To determine the anomalies and to compare them with control.

CHAPTER 4

MATERIALS AND METHODOLOGY

4.1 MAINTENANCE

The adult wild-type zebrafish (*Danio rerio*) is easily obtainable, readily maintainable, and under appropriate conditions yields a larger number of non-adherent, fully transparent eggs. Adult wild-type zebrafish for breeding stock were purchased and they were maintained in typically relevant dechlorinated water. Aeration and filtration were provided to the aquaria. Fishes were fed with dry flake food 2 times daily. The excess feces were removed. Fishes were acclimatized to laboratory conditions for 15 days prior to breeding.



Fig 4.1 MAINTAINANCE OF ZEBRAFISH

4.2 BREEDING PROCEDURE

15 days after acclimatization to laboratory conditions, fish were grouped based on gender and kept in fully aerated glass tanks filled with tap water. The culture conditions are $25 \pm 10^\circ\text{C}$ for a 14 hr day/10 hr night. The fish spawning was conducted in a spawning tray to obtain eggs for the experiments. Male and female fish were

taken at a ratio of 1:2 in a larger spawning tray covered by mesh were placed over a tray comprising of water. In the afternoon, the day before spawning, 10 females and five males were placed in the spawning setup. The embryos were recovered the very next morning.

4.3 PREPARATION OF E3 MEDIUM

To prepare a 60X stock, dissolve the ingredients in H₂O, to a final volume of 2 L. Adjust the pH to 7.2 with NaOH. Autoclave. To prepare 1X medium, dilute 16.5 ml of the 60X stock to 1 L with distilled water.

COMPOSITION

34.8g of NaCl (Sodium chloride), 1.6g of KCl (Potassium chloride)

5.8g of CaCl₂ (Calcium chloride), 9.78g of MgCl₂ (Magnesium chloride)

(Optimization of pH 7.2)

4.4 TEST COMPOUND PREPARATION

The Siddha formulation selected for the study is ponnavarai chooranam. the allopathy drug used was valproate. The concentrations of both drugs were optimized and five different concentrations of each drug were prepared. The concentrations were 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, and 50µg/ml E3medium and Ethanol were used as control.

4.5 TREATMENT OF EMBRYOS WITH DRUGS

Eggs were separated into 3 groups

Group 1: E3 medium and Ethanol were considered as positive and negative control respectively.

Group 2: Embryos treated with valproate at various concentrations namely 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml

Group 3: Embryos treated with Ponnavarai chooranams at various concentrations namely 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml

For the observation time, the duration of 24 hours, 48 hours, 72 hours, and 92 hours post fertilization.

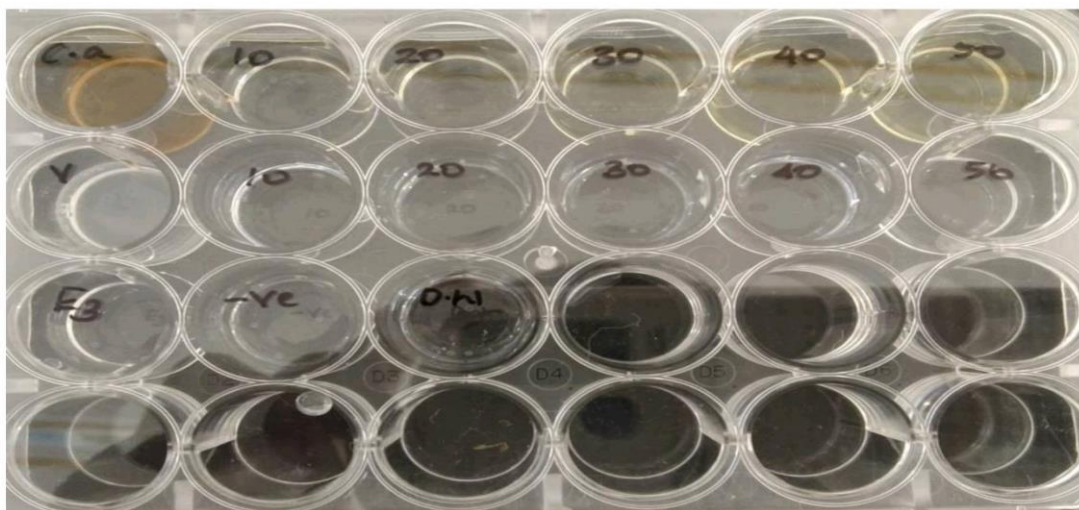


Fig: 4.2 TEST DRUG CONCENTRATIONS

4.6 LARVAE OBSERVATION AND EVALUATION OF OF TERATOGENICITY

Survival and morphological defects were assessed every day for 24 hours post fertilization to 48 hours, 72 hours, and 92 hours post fertilization using a stereomicroscope

coupled with a good megapixel phone camera. Analyzing effects after post-fertilization was performed to track the fate of the treated embryos and to give information about the different checkpoints. To assess teratogenicity surviving larvae were observed for morphological defects and the number of larvae possessing at least one morphological defect was reported.

4.7 MORTALITY, DEFORMITY, AND HATCHABILITY

Hatchability, deformity, and mortality rates were recorded at 24, 48, 72, and 96 hours post-fertilization, and death was defined by the coagulation of embryos and no visual heartbeat.

4.7.1 FORMULAE

The formula for mortality percentage

$$\text{Mortality\%} = (\text{No of dead fishes} / \text{total no of fishes}) \times 100$$

The formula for deformity percentage

$$\text{Deformity \%} = (\text{No of deformed fishes} / \text{total no of live fishes}) \times 100$$

The formula for hatchability percentage

$$\text{Hatchability \%} = (\text{No of hatched fishes} / \text{no of live fishes}) \times 100$$

CHAPTER 5

RESULTS AND DISCUSSION

5.1 TEST COMPOUNDS

Five different concentrations (10µg/mL, 20µg/mL, 30µg/mL, 40µg/mL, and 50µg/mL) of Valproate and the Siddha formulation Ponnavarai chooranam were prepared using an E3 medium. E3 medium and Ethanol were considered as the control.

5.2 EMBRYO EXPOSURE

The recovered embryos were exposed to the test compounds, live embryos were transparent and the dead ones were opaque. The embryos exposed to all five groups of drug solutions were observed under the microscope and the images were captured.

5.3 MORTALITY RATE

The mortality percentage of the embryos that were subjected to the concentrations was calculated and represented graphically.

5.4 DEFORMITY RATE

The embryos that were observed to possess morphological deformities or structural malformations were calculated and represented.

5.5 HATCHABILITY RATE

The embryos usually start hatching 48 hours after fertilization. The hatchability% of the embryos exposed to the solutions was calculated and was represented.

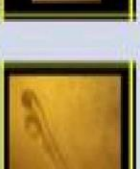
S.NO	PONNAVARAI CHLOORANAM	24 Hrs	48 Hrs	72 Hrs	96 Hrs
1	10				
2	20				
3	30				
4	40				
5	50				
6	E3 Medium (POSITIVE CONTROL)				

TABLE 5.1: IMAGES OF EMBRYOS EXPOSED TO PONNAVARAI

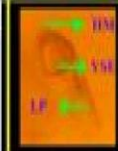
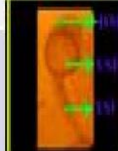
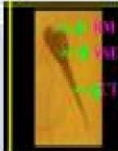
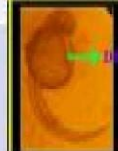
S.no	VALPORAG E	24 hrs.	48 hrs.	72 hrs.	96 hrs.
1	10				
2	20				
3	30				
4	40				
5	50				
6	Ethanol (NEGATIVE CONTROL)				

TABLE 5. 2: IMAGES OF EMBRYOS EXPOSED TO VALPROATE

Sno	Concentration (ug/ml)	Mortality (%)for 24hpf	Mortality (%) for 48hpf	Mortality (%) for 72hpf	Mortality (%) for 96hpf
1	10	10	30	40	40
2	20	10	30	50	50
3	30	15	30	50	60
4	40	20	40	50	60
5	50	20	40	60	70
6	E3	0	0	0	15
7	Ethanol	60	70	85	100

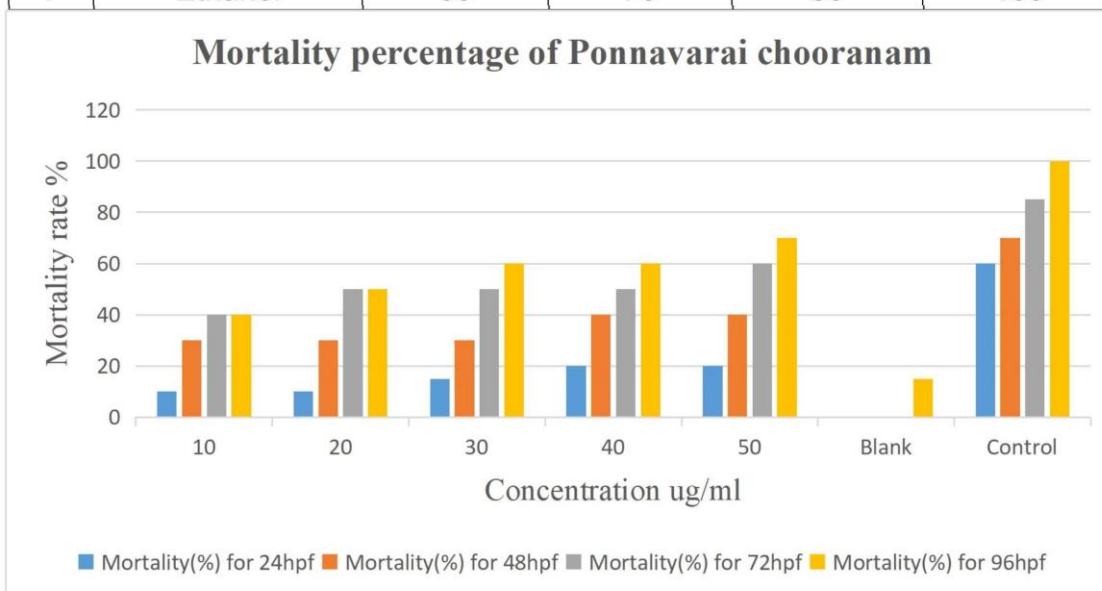


TABLE 5.3: MORTALITY PERCENTAGE OF PONNAVARAI CHOORANAM AT VARIOUS CONCENTRATIONS 24 HOURS POST FERTILIZATION (HPF).

Sno	Concentration (ug/ml)	Mortality (%)for 24hpf	Mortality (%) for 48hpf	Mortality (%) for 72hpf	Mortality (%) for 96hpf
2	20	30	50	65	75
3	30	45	50	65	75
4	40	50	55	70	80
5	50	50	60	70	90
6	E3	0	0	0	15
7	Ethanol	60	70	85	100

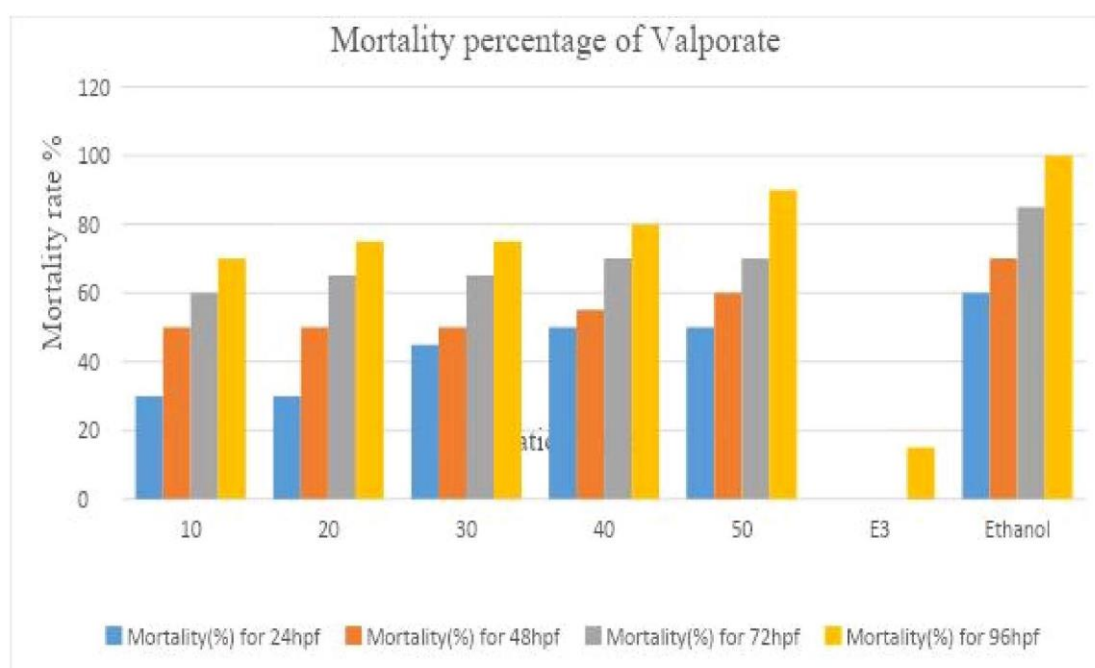


TABLE 5.4: MORTALITY PERCENTAGE OF VALPROATE AT VARIOUS CONCENTRATIONS AT VARIOUS HOURS POST FERTILIZATION (HPF).

DISCUSSION

The present study was initiated with the aim of evaluating a gestational supplement named Ponnavarai chooranam from the Siddha system of medicine as prophylactic for teratogenic effects using the zebrafish model. Valproate is an anti-epileptic medication that is associated with an increased risk of multiple birth defects in humans and is considered a teratogen, hence valproate has been employed so as to induce malformations to the embryos. Ponnavarai chooranam is a Siddha medication that is prescribed against many antiepileptic disorders. The study was carried out so as to understand the effects of Ponnavarai chooranam on embryos. To know whether the Siddha formulation really helps in developing offspring without any morphological malformations our groups of test compounds of five different concentrations were considered namely valproate and Ponnavarai chooranam combination of both the drugs where the Siddha formulation was added after 24,48,72,96 hours post fertilization and combination of both the drugs were observed no malformations observed in embryos exposed to ponnavarai chooranam. But in valproate allopathy drug at 48 hours the embryos did not hatch due to malformations. At 72 hours post-fertilization, there were retinal and tail malformations observed. At 96 hours the fish with less pigmentation was observed. In the positive control E3 medium, there is malformation the hatched fish were healthy. Due to high toxicity, embryos were found dead in ethanol. The mortality% and the deformity% were lower in the test compounds involving the Siddha formulation Ponnavarai chooranam compared to the valproate compositions.

CHAPTER 6

SUMMARY AND CONCLUSION

The present study was initiated with the aim of evaluating a gestational supplement named Ponnavarai choornam from the Siddha system of medicine as prophylactic teratogenic effects using the zebrafish model. Valproate has been used in the study so as to induce malformations in the embryos as Valproate is a teratogen. The study was carried out in order to find out whether the Siddha formulation really helps in overcoming the malformations induced by Valproate. The results of the study were found to be compromising. The results showed that the Siddha formulation was effective against the malformations possessed by the embryos upon exposure to the teratogen. Embryos that received ponnavarai choornam after 8 and 24 hours post-fertilization were found to have recovered from the structural malformations initially caused due to Valproate exposure. Mortality% and deformity% were lower in embryos exposed to the test solutions involving Ponnavarai choornam.

REFERENCES

1. Al-Hamdan, W., Moukaddem, A., AlOtaibi, N., Aledrees, A., Alhatem, N., Alshehri, N., & Aladham, M. Knowledge regarding teratogens among women of childbearing age at a large tertiary care center in Saudi Arabia. *Journal of Family Medicine and Primary Care*, 9(6), 3088. (2020).
2. Allison D'Costa, & Shepherd, Iain. Zebrafish Development and Genetics: Introducing Undergraduates to Developmental Biology and Genetics in a Large Introductory Laboratory Class. *Zebrafish*. 6. 169-77. 10.1089/Zeb.2008.0562 (2009).
3. Ali, Shaukat & Champagne, Danielle & Spaink, Herman & Richardson, Michael. Zebrafish Embryos and Larvae: A New Generation of Disease Models and Drug Screens. 93. 115-33. 10.1002/bdrc.20206(2011).
4. Aneja, S., & Sharma, S. Newer anti-epileptic drugs. *Indian Pediatrics*, 50, 1033-1040 (2013).
5. Ayano, G. Bipolar disorders and valproate: Pharmacokinetics, pharmacodynamics, and therapeutic effects and indications of valproate: Review of articles. *Bipolar Disorder*, 2(109), 2472-1077 (2016).
6. B. Mayuri¹, D. Sudheer Kumar², P. Kishore¹. A Review on Epilepsy *Journal of medical science and clinical research*(volume7)10.18535 (2019).
7. Bhowmik, D., Kumar, K. S., Srivastava, S., Paswan, S., & Dutta, A. S. Traditional Indian herb Punarnava and its medicinal importance. *Journal of Pharmacology and Phytochemistry*, 1(1), 52-57 (2012).
8. Chaudhary, Dr. Avdesh & Chen, Mengqi & Martin-Iverson, Mathew & Mondal, Alinda & Ong, Daniel & Rainey-Smith, Stephanie & Taddei, Kevin & Lardelli, Michael & Groth, David & Verdile, Giuseppe & Martins, Ralph. Regular Care and Maintenance of a Zebrafish (Danio rerio) *Journal of visualized experiments: JoVE*. 69. 10.3791/4196 (2012).
9. Choonara I. Anti-Epileptic Drug Toxicity in Children. *Children (Basel, Switzerland)*, 5(5), 57. [10.3390/children5050057](https://doi.org/10.3390/children5050057) (2018).

10. Dahl-Hansen, E., Koht, J., & Syvertsen, M. Epilepsy at different ages—Etiologies in the Norwegian population. *Epilepsia Open*, 4(1), 176-181 (2019).
11. Gawel, K., Langlois, M., Martins, T., van der Ent, W., Tiraboschi, E., Jacmin, M., ... & Esguerra, C. V. Seizing the moment: Zebrafish epilepsy models. *Neuroscience & Biobehavioral Reviews*, 116, 1-20 (2020).
12. Harden, C. L., & Nadiminti, L. Treatment considerations during pregnancy for women with epilepsy. *Women's Health*, 2(3), 415-423 (2006).
13. Haroun, H. S. W (2017). Teratogenicity and teratogenic factors. *MOJ Anat&Physiol*, 3(1), 00082 (2017).
14. Johannessen, C. U., & Johannessen, S. I. Valproate: past, present, and future. *CNS drug reviews*, 9(2), 199-216 (2003).
15. Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B., & Schilling, T. F. Stages of embryonic development of the zebrafish. *Developmental Dynamics: an official publication of the American Association of Anatomists*, (1995). 203(3), 253–310. 10.1002/aja.1002030302.
16. Kundap, U. P., Kumari, Y., Othman, I., & Shaikh, M. F. (2017). Zebrafish as a Model for Epilepsy-Induced Cognitive Dysfunction: A Pharmacological, Biochemical and Behavioral Approach. *Frontiers in pharmacology*, 8, 515. 10.3389/fphar.2017.00515 (2017).
17. Longkumer, Sentiyanger & Jamir, Ajungla & Pankaj, Pranay. (2022). Maintenance and Breeding of Zebrafish under Laboratory Conditions for Animal Research. *Agricultural Science Digest - A Research Journal*. 10.18805/ag.D-5599 (2022).
18. Nambirajan, G., Karunanidhi, K., Ganesan, A., Rajendran, R., Kandasamy, R., Elangovan, A., & Thilagar, S. Evaluation of antidiabetic activity of bud and flower of *Azadirachta indica* (Cassia auriculata L.). *Biomedicine & Pharmacotherapy*, 108, 1495-1506. [10.1016/j.biopha.2018.10.011](https://doi.org/10.1016/j.biopha.2018.10.011) (2018).
19. Nowik, Natalia & Podlasz, Piotr & Przyborowska, Anna & Kasica, Natalia & Sienkiewicz, Waldemar & Kaleczyc, Jerzy. Zebrafish: An animal model for

- research in veterinary medicine. *Polish Journal of veterinary sciences*. 18. 10.1515/pjvs-2015-0086 (2015).
20. Rajkumar, S., & Lekha, G. S. Basic Principles in Siddha Pharmaceutical Science-An Overview. *IOSR Journal of Pharmacy and Biological Sciences*, 11(6), 43-48 (2016).
21. Saraf, Shailendra & Shukla, Shiv Shankar. Fundamental aspect and basic concept of Siddha medicines. *Systematic Reviews in Pharmacy*. 2.48.10.4103/0975-8453.83439 (2011).
22. Sofia, H. Nalini. A study on RATHTHA MOOLAM (Dissertation subject) DOCTOR OF MEDICINE (SIDDHA BRANCH-1-Maruthuvam) (2007).
23. Soundharya, R., Aruna, V., & Amruthavalli, G. V. Absolute science of Siddha sastric drugs-An experimental proof. *Int J Complement Alt Med*, 12(2), 75-77 (2019).
24. Sudharshini, K., Anto, S., Saravanan, D., & Selvaraj, R. Antimicrobial Activity of Selected Chooranams (Poonaikali, Ponnavaarai, Gunma Uppu, Parangipattai) against Drug-Resistant Pathogens. *Int J Pharm Sci Rev Res*, 46(1), 139-145 (2017).
25. Teame T, Zhang Z, Ran C, Zhang H, Yang Y, Ding Q, Xie M, Gao C, Ye Y, Duan M, Zhou Z. The use of zebrafish (*Danio rerio*) as biomedical models. *Animal*; 9(3):68-77. doi: 10.1093/af/vfz020 Jun (2019)
26. Thangapandiammal, S & Sofia, H. Nalini. ORIGIN AND ANTIQUITY OF SIDDHA SYSTEM OF MEDICINE 338534466 (2019).
27. THEERDHALA, SRIKRISHNA. FORMULATION AND EVALUATION OF SODIUM VALPROATE SUSTAINED RELEASE TABLETS. 6. 2446-2452 net/publications (2020)
28. Tolou-Ghamari, Z., & Palizban, A. A. Review of sodium valproate clinical and biochemical properties. *Zahedan Journal of Research in Medical Sciences*, 17(8). (2015).
29. Vargesson, N. Medical Sciences, University of Aberdeen, Aberdeen, UK Lucas Fraga, School of Medicine, Medical Sciences and Nutrition, Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK December 2017

30. Weckesser, A., & Denny, E. Women living with epilepsy, experiences of pregnancy and reproductive health: a review of the literature. *Seizure*, 22(2), 91-98 (2013).
31. Wilkins, Benjamin & Pack, Michael. Zebrafish Models of Human Liver Development and Disease. *Comprehensive Physiology*. 3. 1213-30.10.1002/cphy.c120021.(2013)