

ESTIMATION OF ZEBRA FISH GROWTH RATE USING WITHANIA SOMNIFERA INCORPORATED FISHFEED

Submitted in partial fulfilment of the requirements for the award of

Bachelor of Technology degree in Biotechnology

by

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**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited with Grade "A" by NAAC

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **MEGALA B (37230044)** and **SHOBANA S (37230082)** who carried out the project entitled "**ESTIMATION OF ZEBRA FISH GROWTH RATE USING WITHANIA SOMNIFERA INCORPORATED FISHFEED**" under our supervision from October 2020 to March 2021.

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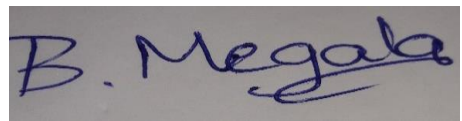
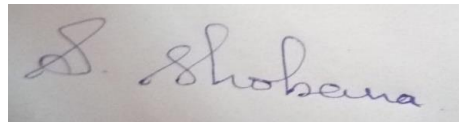
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DECLARATION

We **MEGALA B (37230044)** and **SHOBANA S (37230082)** hereby declare that the Project Report entitled “**ESTIMATION OF ZEBRA FISH GROWTH RATE USING WITHANIA SOMNIFERA INCORPORATED FISHFEED**” done by me under the guidance of **Dr. P. Prakash** at **Sathyabama Institute of Science and Technology, Chennai** is submitted in partial fulfilment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

DATE: 16.04.2021

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ABSTRACT

Withania somnifera (Ashwagandha) is widely mentioned in various traditional textbooks of Ayurveda, Siddha, Unani and Chinese medicine owing to its wide range of health benefits. It is used in treating a wide variety of illnesses including asthma, diabetes, hypertension, stress, arthritic diseases, cancer and also used to reduce levels of fat and sugar in the blood. *Lep Withania somnifera* *toguium rivurale* were collected from siddha medical and extracted using distilled water. Qualitative analysis of phytochemicals from the extracts showed the presence of carbohydrates, glycosides, phenols, terpenoids, saponins and proteins. Further the extract is given for GCMS analysis reported various significant compounds. Also In this present study, we bring out the growth promoting effects of *Withania somnifera* against Zebrafish growth rate was evaluated by measuring length and weight of Zebrafish at different time intervals with different concentration of *Withania somnifera*. Among them maximum growth rate was observed in 150 mg.

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LIST OF ABBREVIATIONS

mg	Milligram
cm	Centimeter
mm	Millimeter
L	Liter
ml	Milliliter
WS	<i>Withania Somnifera</i>
WFA	Withaferin A
GABA	gamma-Aminobutyric acid
ROS	Reactive Oxygen species
CRP	C-reactive protein
WBC	White cell Count
GC-MS	Gas Chromatography-Mass spectrometry
SPSS	Statistical Package for the Social Sciences
°C	degree celcius
%	Percentage
wt	Weight

1.INTRODUCTION

WITHANIA SOMNIFERA (Ashwagandha, Family Solanaceae) is commonly known as “Indian Winter cherry” or “Indian Ginseng”. It is one of the most important herb of Ayurveda used for millennia as a Rasayana for its wide ranging health benefits. Rasayana is described as a special herb or metallic preparation which promotes the longevity, prevents aging, provides positive health and mental faculties, increases memory, and impart resistance and immunity against diseases. These types of remedies are given to small children as tonics, and are also taken by the middle-aged and elderly to increase longevity. It is known as “Sattvic Kapha Rasayana” Herb (Changhadi, 1938). Most of the Rasayana herbs are adaptogen / anti-stress agents.

Ashwagandha is an ancient medicinal herb with multiple health benefits. It can reduce anxiety and stress, help fight depression, boost fertility and testosterone in men, and even boost brain function. Supplementing with ashwagandha may be an easy and effective way to improve your health and quality of life.

1.1 WHAT IS ASHWAGANDHA?

Ashwagandha is an herb that has been used in Ayurvedic medicine for thousands of years. In Sanskrit, the word "ashwa" means "horse," while "gandha" means "smell." The translation of Ashwagandha is roughly "the smell and strength of a horse," which alludes to its traditional use to support vitality and a healthy libido. Once ground, the herb smells like a horse and was thought to impart the power of one, too. Its species name, *somnifera*, means "sleep-inducing" in Latin. *Somnifera* indicates its traditional Ayurvedic use for supporting somnolence, or sleepiness. Ashwagandha's Hindi name is "asgandh," another nod to its potent odor that's reminiscent of horse sweat.

1.2 ORIGIN OF THE ASHWAGANDHA

The Ashwagandha plant (scientific name *Withania somnifera*) is a small evergreen shrub that is short and squat with velvety leaves and bell flowers that contain orange berries similar in appearance to small tomatoes.

Ashwagandha grows in India, the Middle East, and parts of Africa. It is widely grown in dry parts of subtropical regions. Rajasthan, Punjab, Haryana, Uttar Pradesh, Gujarat, Maharashtra, and Madhya Pradesh are major ashwagandha growing states in India.

VERNACULAR NAMES OF *WITHANIA SOMNIFERA*

- Arabic: Kaknaj-e-Hindi
- Bengali: Ashvaganda, Asvagandha
- English: Winter cherry
- Gujarati: Asan, Asana, Asoda, Asundha, Ghodaasoda
- Hindi: Asgandh, Punir
- Malayalam: Amukkiram, Pevetti
- Marathi: Askandha, Kanchuki, Tilli
- Odia: Asugandha
- Persian: Kaknaj-e-Hindi, Asgand Nagaori
- Sanskrit: Ashvagandha, Ashvakandika, Gandhapatri, Palashaparni
- Tamil: Amukkira, Asubam, Asuvagandi
- Telugu: Asvagandhi, Penneru, Pennerugadda, Dommadolu
- Urdu: Asgand, Asgand Nagori

(Anonymous, 2007, Chopra et al., 1980, Kirtikar et al., 1980).

1.3 HISTORY OF ASHWAGANDHA IN AYRURVEDA

Ayurveda, the traditional system of medicine practiced in India can be traced back to 6000BC (Charak Samhita, 1949). For most of these 6000 years Ashwagandha has been used as a Rasayana. The root of Ashwagandha is regarded as tonic, aphrodisiac, narcotic, diuretic, anthelmintic, astringent, thermogenic and stimulant. The root is often dried and ground, then given as a powder mixed with ghee, honey, and milk as Ashwagandha can often have a bitter taste. This warm beverage is often consumed before bedtime. Ashwagandha is included in our Golden Milk to help support a feeling of relaxation.

Ashwagandha is one of the most commonly used herbs for the "Vata" constitution, which is associated with air and space. Balanced Vata energy helps maintain supple skin and joints, a healthy body weight, vitality, healthy cognitive function, and a healthy nervous system.

Ashwagandha is also used traditionally as a tonic for memory, vitality, and hormonal function, which supports balanced Vata energy.

It is also used to support sleep quality, and the ground powdered root can be applied topically to help maintain healthy, comfortable joints.

It should be noted that Ashwagandha impacts the Ayurvedic mind-body type called Sadhaka Pitta, which has an effect on body and emotions.

1.4 CHEMICAL COMPOSITION

The roots are reported to contain alkaloids, amino acids, steroids, volatile oil, starch, reducing sugars, glycosides. The biologically active chemical constituents of *Withania somnifera* (WS) include alkaloids (isopelletierine, anaferine,..etc.), steroidal lactones (withanolides, withaferins) and saponins (Mishra, et al.,2000). Anti stress agent in the ashwagandha are Sitoindosides and acylsterylglucosides. Active principles of Ashwagandha, for instance the sitoindosides VII-X and Withaferin-A, have been shown to have significant anti-stress activity against acute models of experimental stress (Bhattacharya et al., 1987). Many of its constituents support immunomodulatory actions (Ghosal et al., 1989). The aerial parts of *Withania somnifera* yielded 5-dehydroxy withanolide-R and withasomniferin-A (Atta-ur-Rahman et al., 1991).

1.5 CHARACTERISTICS OF THE PLANT

Ashwagandha is an annual to perennial, branched, under shrub to herb of about 30cm to 120cm height, minutely stellate and tomentose branches. Roots are fleshy, tapering, whitish brown. Leaves are ovate, and flowers are greenish. The mature fruits are orange berries.



Fig 1.5: *WITHANIA SOMNIFERA* (Ashwagandha)

MORPHOLOGY

WITHANIA SOMNIFERA is an evergreen, erect, branching, tomentose shrub, 30-150 cm in height. Leaves are simple, ovate, glabrous, and up to 10 cm long. Flowers are greenish or lurid yellow, small about 1 cm long; few flowers (usually about 5) born together in axillary, umbellate cymes (short axillary clusters). Fruits are globose berries, 6 mm in diameter, orange red when mature, enclosed in the inflated and membranous persistent calyx. Seeds are yellow, reniform and 2.5 mm in diameter (Anonymous, 2007). The drug Asgand consists of the dried mature roots of the plant which has the following morphological properties:

MACROSCOPIC

The stout fleshy roots when dry are cylindrical, gradually tapering down, straight, unbranched, 10-17.5 cm long and 6-12 mm in diameter. The main roots bear fiber-like secondary roots. The outer surface of the roots is brownish white and interior is creamy white when broken. They have a short and uneven fracture, a strong odour and mucilaginous bitter and acrid taste (Anonymous, 1982).

MICROSCOPIC

The young root has a single layered epidermis followed by a parenchymatous cortex of 4-5 layers of cells, the endodermis being conspicuous by the presence of casparian stripes. The cork cambium arises in the outermost layer of the cortex. The endodermis persists even after the secondary growth has taken place (Shah et al., 1993).

1.6 HEALTH BENEFITS OF ASHWAGANDHA

CAN REDUCE THE BLOOD SUGAR LEVEL

In several studies, ashwagandha has been shown to lower blood sugar levels. In the studies it found increased in insulin secretion and improved insulin sensitivity in muscle cells. Both in healthy people and those with diabetes in studies it suggested that it can reduce the blood sugar levels. (Gorelick J et al., 2015 and Ashwinikumar A Raut et al., 2012)

MIGHT HAVE ANTICANCER PROPERTIES

Ashwagandha has anticancer activities. Studies have found that withaferin- a compound in ashwagandha – helps induce apoptosis (Apoptosis is a form of programmed cell death, or “cellular suicide”). It also delay or prevent the growth of new cancer cell in human body in several ways. In the studies it believed that withaferin in ashwagandha to promote the formation of Reactive Oxygen Species (ROS)

(Reactive oxygen species are free radicals. A type of unstable molecule that contains oxygen and that easily reacts with other molecules in a cell. A build-up of reactive oxygen species in cells may cause damage to DNA, RNA, and proteins, and may cause cell death). Thus this formation of the Reactive Oxygen Species inside the cancer cells disrupt their function. And also it may cause cancer cells to become less resistant to apoptosis.(Avani R Vyas et al.,2013)

Animal studies gives idea that it may treat several types of cancer which includes breast, lung, colon, brain, and ovarian cancer. (Kamel F Khazal et al.,2014 and Senthilnathan P et al.,2006)

CAN REDUCE THE CORTISOL LEVELS

Cortisol is a stress hormone released by the adrenal glands. It's important for helping your body deal with stressful situations, as your brain triggers its release in response to many different kinds of stress. Under stressful conditions, cortisol provides the body with glucose by tapping into protein stores via gluconeogenesis in the liver. This energy can help an individual fight or flee a stressor. However, elevated cortisol over the long term consistently produces glucose, leading to increased blood sugar levels. (K Chandrasekhar et al.,2012)

Studies on ashwagandha shown that it may help to reduce cortisol level in human body. Studies in chronical stressed adults, those who supplemented with ashwagandha had significantly greater reduction in cortisol, compared with the control group. Those taking the highest dose experienced a 30% reduction on average.

MAY HELP REDUCE STRESS AND ANXIETY

In studies ashwagandha is best known for its ability to reduce stress. It helps in reducing the stress by blocking the stress pathway in the brain by regulating chemical

signal in the nervous system. Several human studies have shown that it can reduce the symptoms in people with stress and anxiety disorder. (Manuel Candelario et al.,2015)

MAY REDUCE SYMPTOMS OF DEPRESSION

A few studies suggest ashwagandha may help reduce depression. Taking high concentration of ashwagandha per day results in 79% reduction in severe depression. (C Andrade et al.,2000)

CAN BOOST TESTOSTERONE AND INCREASE FERTILITY IN MEN

In several studies it results that ashwagandha supplements may have very high effects on testosterone levels and reproductive health in men. In research study in infertile men the group treated with ashwagandha result shows increased sperm count and motility. In this treatment the result led to a significant increase in testosterone levels. (Gupta A et al.,2013 and Wankhede S et al.,2015)

In the treatment with ashwagandha reports in increased antioxidant levels in their blood.

MAY INCREASE MUSCLE MASS AND STRENGTH

Many research has shown that the ashwagandha may improve body composition and increase strength. Taking safe and effective dosage of ashwagandha for 30 days results in gaining of muscle strength. Also it results that ashwagandha had significantly increase of gain in muscle strength and size. It also reduce in body fat percentage. (Ashwinikumar A Raut et al.,2012 and Wankhede S et al.,2015)

MAY REDUCE INFLAMMATION

Several animal studies have shown that ashwagandha helps decrease inflammation. Studies in humans have found that it increases the activity of natural killer cells, which are immune cells that fight infection and help you stay healthy. It has also been shown to decrease markers of inflammation, such as C-reactive protein (CRP)(C-reactive protein is a substance produced by the liver in response to inflammation.) This marker is linked to an increased risk of heart disease. Withaferin A and 3-b-hydroxy-2,3-dihydrowithanolide F isolated from *WITHANIA SOMNIFERA* show promising antibacterial, antitumoral, immunomodulating and anti-inflammatory properties (Zahra Samadi Noshahr et al.,2015)

MAY IMPROVE BRAIN FUNCTION

Studies suggest that ashwagandha can alleviate memory and brain function problem caused by any injury or disease. Research has shown that it promotes antioxidant activity that protects nerve cells from harmful free radicals. In animal studies treated with ashwagandha had nearly a complete reversal of spatial memory impairment. This has been likely caused by a reduction in oxidative stress. Ashwagandha has traditionally been used to boost memory in Ayurvedic medicine. (Baitharu I et al.,2013)

INVOLVES IN NEURODEGENERATIVE DISEASES

Neurodegenerative diseases such as Parkinson's disease, Huntington's disease, and Creutzfeldt– Jakob disease, Alzheimer's disease are studied with ashwagandha shows result as slows, stops, reverses or removes atrophy and synaptic loss. In this ashwagandha can be used to treat these neurodegenerative diseases at any stage of disease. Ashwagandha has been described as a nervine tonic in Ayurveda and thus it is most common ingredient of Ayurvedic tonic. (Bhattacharya et al., 1995)

2.REVIEW OF LITERATURE

Withania Somnifera had been in Ayurveda, the traditional system of medicine practiced in India can be traced back to 6000 BC (Charak Samhita, 1949). For most of these 6000 years Ashwagandha has been used as a Rasayana. The root of Ashwagandha is regarded as tonic, aphrodisiac, narcotic, diuretic, anthelmintic, astringent, thermogenic and stimulant. According to Charaka Samhita, Susruta Samhita and other ancient texts, Ashwagandha is known as Balya (increases strength), Brusya (sexual performance enhancer), vajikari (spermatogenic), Kamarupini (libidoenhancing), Pustida (nourishing). Ashwagandha is named such as its root smells like horse ("Ashwa") urine and believe to provide power like horse when consumed.

Withania Somnifera (L) is an important drug commonly known as asgand which has been used either single or in combination with other drugs in unani as well as Ayurvedic system of medicine for centuries. It has been described by Dioscorides (78 AD) in his book "Kitab-ul-Hashaish". . Asgand consists of the roots of *Withania somnifera* which has various therapeutic actions such as anti-inflammatory (Muhallil-e-Warm), sedative (Musakkin), alterative (Muaddil) and aphrodisiac (Muqawwi-e-Bah). (Qamar Uddin et al.,2012).

Withania Somnifera (Linn), is an erect, evergreen, perennial shrub which is a widely used medicinal plant considered as aphrodisiac and rejuvenating, anti-inflammatory and anti tumouragent (Naidu PS, et al., 2003). It is best known classically for its rejuvenating properties, and hence called "Indian Ginseng" (Singh and Kumar, 1998). Roots of *Withania somnifera* (Ashwagandha) reportedly exhibit antioxidant, immune modulatory and haematopoietic properties (Mishra LC, et al., 2000).

The active pharmacological components of *Withania somnifera* constituents are withanolides (Steroidal lactones with ergostane skeleton) and alkaloids (Elsakka M, et al., 1990). It also possesses antistress, immune modulatory, anti-oxidant and antibacterial activity. (Lingaiah and Nagaraja Rao, 2013)

Ashwagandha (roots of *Withania somnifera* DUNAL) is one of the most valuable herbal drugs used in Indian traditional medicine (Ayurveda) as a rasayana drug that is capable of imparting long life, youthful vigor, and good intellectual powers

Ashwagandha is clinically used for the treatment of general debility, consumption, nervous exhaustion, insomnia, loss of memory, and so on. These traditional uses imply that Ashwagandha may possibly be useful at improving neurodegenerative diseases. Indeed, this herbal drug has been reported to exert various pharmacological effects such as anti-inflammatory, anti-tumor, anti-oxidant, immunomodulatory, and anti-neuropsychiatric disease effects.

Withania Somnifera is commonly used in emaciation of children (when given with milk, it is the best tonic for children), debility from old age, rheumatism, vitiated conditions of vata, leucoderma, constipation, insomnia, nervous breakdown, goiter etc. The paste formed when roots are crushed with water is applied to reduce the inflammation at the joints (Bhandari, 1970). It is also locally applied in carbuncles, ulcers and painful swellings (Kritikar KR and Basu, BD 1980). The root in combination with other drugs is prescribed for snake venom as well as in scorpion-sting. It also helps in leucorrhoea, boils, pimples, flatulent colic, worms and piles. The Nagori Ashwagandha is the supreme among all Ashwagandha varieties. Maximum benefit appears when fresh Ashwagandha powder is used.

Studies have indicated a potential role of ashwagandha as a nootropic, promoting cognitive function and enhancing memory (Dhuley J.N, 2001), presumably due to its cholinomimetic activity (Schliebs et al., 1997). Results have been promising for neurological disorders such traumatic brain injury, brain tumor, and neurodegenerative pathologies (Kuboyama T et al., 2014 and Singh et al., 2008). Efficacy of ashwagandha extracts and isolated constituents has been reported in experimental models of neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD), which are associated with the disruption of neural networks and premature death of neurons. Clinical studies with ashwagandha have shown promise in bipolar disorder, enhancing cognitive and psychomotor performance, and ameliorating cognitive dysfunction (Pingali U et al., 2014).

Withania consists of withanolides (Kirson I et.al., 1971) as their major constituents which is 28 carbon containing steroidal lactone in which C-26 and C-22 are normally oxidized and form a 6-membered of lactone ring. Now due to several pharmacological investigations it is proved that Withania extract contains several important contents (Mirjalili M et.al., 2009).

WITHANIA SOMNIFERA has been considered the major source of drug in the treatment of diabetes mellitus in Indian medicinal system and the other ancient system in the world, long times diabetes mellitus was treated orally with their extract, these plant products are more considered to be less toxic and are free from side effects.

Identified neuroprotective phytoconstituents of Ashwagandha are sitoindosides VII–X, withaferin A, withanosides IV, withanols, withanolide A, withanolide B, anaferine, beta-sitosterol, withanolide D with key pharmacological effects in brain disorders mainly anxiety, Alzheimer's, Parkinson's, Schizophrenia, Huntington's disease, dyslexia, depression, autism, addiction, amyotrophic lateral sclerosis, attention deficit hyperactivity disorder and bipolar disorders. The literature survey does not highlight any toxic effects of Ashwagandha. Further, multiple available marketed products and patents recognized its beneficial role in various brain disorders; however, very few data is available on mechanistic pathway and clinical studies of Ashwagandha for various brain disorders is scarce and not promising. (sumaira Saleem et al.,2020 and Sultan Zahiruddin et al.,2020)

The folk healers used the plant to treat several diseases such as fever, cancer, asthma, diabetes, ulcer, hepatitis, eyesores, arthritis, heart problems, and hemorrhoids. The plant is famous for the anti-cancerous activity, low back pain treatment, and muscle strengthening, which may be attributed to the withanolide alkaloids. *W. somnifera* is also rich in numerous valued secondary metabolites such as steroids, alkaloids, flavonoids, phenolics, saponins, and glycosides. A wide range of preclinical trials such as cardioprotective, anticancer, antioxidant, antibacterial, antifungal, anti-inflammatory, hepatoprotective, anti-depressant, and hypoglycemic have been attributed to various parts of the plant. Different parts of the plant have also been evaluated for the clinical trials such as male infertility, obsessive-compulsive disorder, antianxiety, bone and muscle strengthening potential, hypolipidemic, and antidiabetic. This review focuses on folk medicinal uses, phytochemistry, pharmacological, and nutraceutical potential of the versatile plant. (Morgan A. Pratte,BS et al.,2014)

Withaferin A (WFA) is a natural compound produced by *Withania somanifera*, which has been used as a traditional medicinal herb for centuries. Over the past few decades, investigators have begun to delve into the wealth of natural compounds to find new therapeutic approaches for a host of diseases. WFA, a steroid Lactone, is an example of a natural compound that has been investigated as a potential therapy for several disorders including certain type of cancers, metabolic disorders, inflammatory conditions, and neurodegenerative disorders. (Behl T et al., 2020)

The research and studies of Ashwagandha's activities in the inhibition and reduction of tumour growth have shown encouraging evidence that this remarkable herb may prove to be extremely effective in the treatment of tumor type diseases including cancer (Singh and Gilca, 2010). It also improves the white cell count (WBC) and function, which are depleted in the chemotherapeutic treatment of cancer. Ashwagandha in the treatment of fibroid tumors of the uterus showed reduction of uterine bleeding tendencies and disappearance of fibroids after long treatment. (Abbas SS et al., 2005).

Behavioral experiments have lent support to the GABA-mimetic activity of Ashwagandha root extract. GABAergic neurodegeneration due to neuroleptic-induced excitotoxicity and oxidative stress is one of the etiopathological mechanisms in the pathophysiology of tardive dyskinesia (Gunne L.M et al., 1993) and GABA agonists are shown to be effective in ameliorating the symptoms of tardive dyskinesia. The beneficial effect of Ashwagandha root extract might be due to its GABA mimetic activity. Ashwagandha, its constituents and the metabolites of its constituents promote the growth of nerves after taking it for 7 days.

Ashwagandha also exhibited an antidepressant effect, comparable with that induced by imipramine, in two standard tests, the forced swim-induced 'behavioral despair' and 'learned helplessness' tests. The investigations support the use of Ashwagandha as a mood stabilizer in clinical conditions of anxiety and depression. (Abdel-Magied EM et al., 2001).

3. AIM AND SCOPE

3.1 AIM

To estimate the Phytochemical and Antioxidant from WITHANIA SOMNIFERA.

To prepare a fish feed with WITHANIA SOMNIFERA.

To estimate the growth rate incorporated with WITHANIA SOMNIFERA in fish feed.

3.2 OBJECTIVE

To evaluate the phytochemical compounds.

To evaluate the metabolites by GCMS.

To estimate the growth rate incorporated with WITHANIA SOMNIFERA.

To quantify the safe concentration of WITHANIA SOMNIFERA intake.

To analyse the growth statistically by SPSS.

4.MATERIALS AND METHODS

4.1 MATERIALS

1. Rectangle boxes
2. Weighting balance
3. Measuring cup
4. White cloth
5. Measuring tape
6. Rubber tube
7. Animal model

4.2 METHODOLOGY

4.2.1 SAMPLE COLLECTION

The herb sample ashwagandha was collected from the Ayurvedic medicinal shop, Kanchipuram, Tamil Nadu as a powder form.

4.2.2 PHYTOCHEMICAL ANALYSIS

Detection of alkaloids

Add few drops of Mayer's reagent to 1 ml of the extract. Formation of yellow coloured precipitate indicates the presence of alkaloids.

Detection of carbohydrates

Add few drops of Benedict's reagent to the extract and heat it for 5 minutes. Appearance of orange red colour indicates the presence of reducing sugars.

Detection of glycosides

To 1 ml of the plant extract add 2 ml of ferric chloride and immerse the contents in boiling water bath for about 5 minutes. Cool the mixture and extracted with equal volume of benzene and treat it with equal amount of Ammonia solution. Formation of rose pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Detection of saponins

Take 1ml of the plant extract and dilute it with distilled water to 5 ml and shake the contents continuously for five minutes. Formation of foam indicates the presence of saponins.

Detection of phytosterols

To 1ml of the extract add 1ml of chloroform and few drops of conc. Sulphuric acid. Shake the contents and allow it to stand for few minutes. Appearance of golden yellow colour indicates the presence of triterpenes.

Detection of phenols

Add 3-4 drops of ferric chloride solution to 1ml of the extract. Observe for the formation bluish black colour which then indicates the presence of phenols.

Detection of tannins

To 2ml of the extract add few drops of ferric chloride solution appearance of blue colour indicate the presence of blue colour hydrolysable tannins and green colour indicates the presence of condensed tannins.

Detection of flavonoids

To 2ml of the extract add 2ml of sodium hydroxide solution. Intense yellow colour formation indicates the presence of flavonoids.

Detection of proteins and amino acids

To 1ml of the extract add few drops of concentrated Nitric acid. Formation of yellow colour indicates the presence of proteins.

4.2.3 GC-MS ANALYSIS

5 gm of sample with 50 ml methanol allowed overnight under shaking condition then removed the solids. The GC-MS analysis of the extract was carried out using Triple quadrupole mass spectrometer with Fused silica 30mm of length of capillary

column, diameter and film thickness is 0.25mm. where the condition was set as described previously.

4.2.4 TREATMENT WITH *WITHAMNIA SOMNIFERA*

4.2.4.1 SEPARATION OF FISHES

Collected Zebra fish are separated into 7 parts of each containing 6 fishes each for the treatment with *WITHANIA SOMNIFERA*. 7 parts are labelled from control, T1 to T6.



Fig 4.2.5 Separation of fishes

4.2.4.2 WEIGHING THE *WITHANIA SOMNIFERA*

WITHANIA SOMNIFERA is measured using weighting balance machine. In that powdered herb is measured as 50mg, 100mg, 150mg, 200mg, 250mg, 300mg.

4.2.4.3 FEEDING *WITHANIA SOMNIFERA*

Zebrafish is treated with *WITHANIA SOMNIFERA* by mixing it with one litre of fresh water. From 50mg to 300mg of the powdered herb is mixed with one litre of fresh water each separately and the mixed water is filled in the fishbowls from T1 to T6.

4.2.4.4 CHANGING THE WATER

Two days once the old water had been changed with the fresh water. In that time of changing the water the powdered form of *WITHANIA SOMNIFERA* is mixed with fresh water.

5.RESULT AND DISCUSSION

5.1 PHYTOCHEMICAL ANALYSIS

Through the phytochemical screening of chemical constituents related to biological activity of the plant root extract are alkaloids, flavonoids, carbohydrates, tannins and terpenoids. These indicate that *WITHANIA SOMNIFERA* root extract contain a significant amount of this phytochemicals which confirms its antioxidant property. In the medicinal effects described in the ayurvedic and siddha tannins, phenolic acids, flavonoids and alkaloids are the important ingredients to prevent against oxidative stress and reduce the activity of cholinesterase and xanthine oxidase and diminish the mucus secretion in the airway glands. *withania somnifera* act as a phytomedicine in joint disorders.

Table 5.1.1: Phytochemical Analysis of *WITHANIA SOMNIFERA*

PHYTOCHEMICALS	ROOT EXTRACT
PHENOLICS	+
FLAVONOIDS	+
CARBOHYDRATES	+
ALKALOIDS	+
TANNINS	+
TERPENOIDS	+
TRITERPENOIDS	—
ANTHRAQUINONES	—
SAPONINS	—
GLYCOSIDES	—

“+” Represents presence of the chemical substance and “-“Represents absence of the chemical substance.

The WS sample is collected and quantified for pure form. Zebra fish is selected as the animal model for the work. 15 days age zebrafish is collected and separated into 7 parts. The powder is measured separately (from 50mg to 300mg) increased with 50mg each and mixed with the fresh water (that water is filled in the fish bowls). The old water is changed two days once.

5.1.1 GCMS ANALYSIS

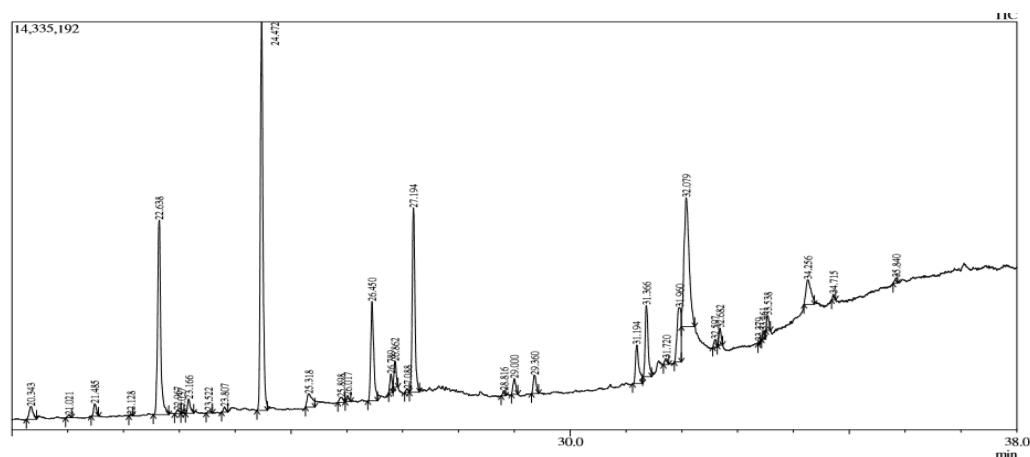
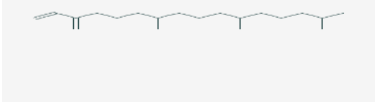
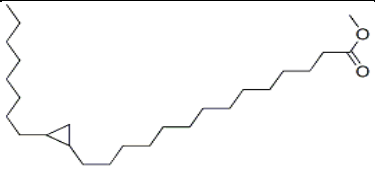
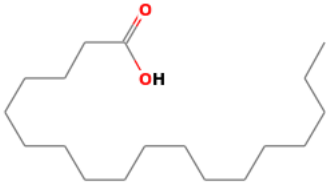
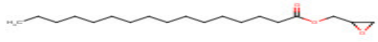
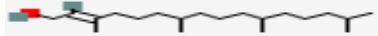


Fig 5.1: Analysis of *withania somnifera* by GCMS

Table 5.1.2: Result of GCMS analysis

S.no	Compounds	Structure	Bioactivity
1	Neophytadiene		Anti-inflammatory, analgesic and antipyretic activities
2	Cyclopropanetetradecanoic acid		Anti-microbial activity
3	Hexadecanoic acid, methyl ester		Antibacterial and antifungal activities

4	Octadecanoic acid		Antimicrobial
5	Glycidyl palmitate		Preparation of isophosphatidic acid which inhibits apoptosis, Ojochenemi
6	2-methyloctacosane		Antifungal activity
7	Cyclohexanol		Anti-microbial and cytotoxic activities
8	Oxirane		Anti-bacterial activity
9	3,7,11,15-Tetramethyl-2-hexadecen-1-ol		Antimicrobial, anticancer, anti-inflammatory, anti-diuretic activities

Withania somnifera. Analysis carried out by GC-MS of the methanolic extracts of whole plant revealed presence of compounds at molecular level viz. Oleic Acid, Phytol, n-hexadecanoic Acid, 9-Octadecenoic acid(Z)-, methyl ester, Hexadecanoic acid, methyl ester, 2-Methoxy-4-vinylphenol, Azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl), 17-Octadecynoic acid, O- Neophytadiene,. All these bioactive compounds are responsible for the antibacterial property and other therapeutic uses of the plant, eg.

alkaloids have pharmacological effects and are used as medications. Also some phytochemicals have antioxidant and anticancer activity.

5.2 INITIAL MEASUREMENT IS CALCULATED

At the 1st day length and weight of the fish is calculated.

Table 5.2: Initial measurements of the zebrafish

SI.NO	AMOUNT OF <i>WITHANIA</i> <i>SOMNIFERA</i> (mg)	AMOUNT OF WATER (litre)	INITIAL LENGTH (cm)	INITIAL WEIGHT (mg)
CONTROL	0	1	1.6	30
T1	50	1	1.6	30
T2	100	1	1.6	30
T3	150	1	1.6	30
T4	200	1	1.6	30
T5	250	1	1.6	30
T6	300	1	1.6	30

The Initial length of the zebra fish is calculated as 1.6cm and weight is 30mg. these fishes are given *WITHANIA SOMNIFERA* along with their feed for treatment.

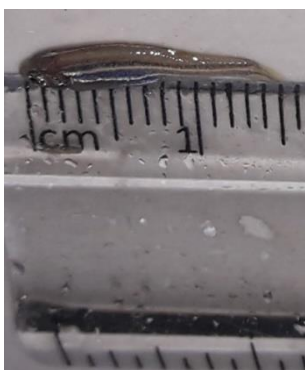


Fig 5.2.1 Initial length of the zebrafish



Fig 5.2.2 Initial weight of the zebrafish

5.3 10th DAY RESULTS

At the 10th day the height and weight of the zebrafish is calculated.

Table 5.3: 10th day measurements of the zebrafish

SI.NO	AMOUNT OF <i>WITHANIA</i> <i>SOMNIFERA</i> (mg)	AMOUNT OF WATER (litre)	10 th DAY LENGTH (cm)	10 th DAY WEIGHT (mg)
CONTROL	0	1	1.9	54
T1	50	1	1.8	68
T2	100	1	1.75	46
T3	150	1	1.72	42
T4	200	1	1.65	38.33
T5	250	1	1.63	36
T6	300	1	1.61	34

At the 10th day length and weight of the zebrafish is calculated. In the 10th day results control set has an average length as 1.9cm and weight as 54mg. And comparing with other set of treated zebrafish test set 1 given with 50mg of the *WITHANIA SOMNIFERA* that has been two days once along with the fresh water gives the high value of length as 1.8cm and weight as 68mg.

Other than this from test 2 to test 6 gives the result in the decreasing range least with 300mg treated set.

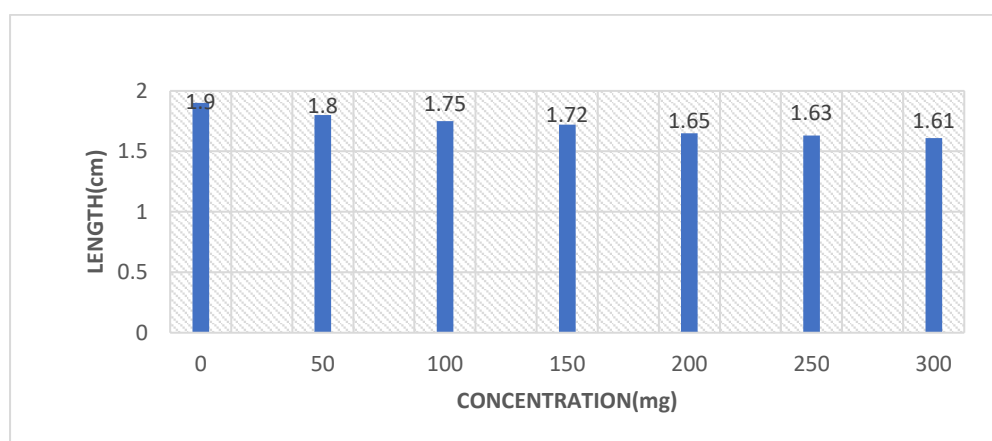


Fig 5.3.1: Graphical representation of the 10th day length

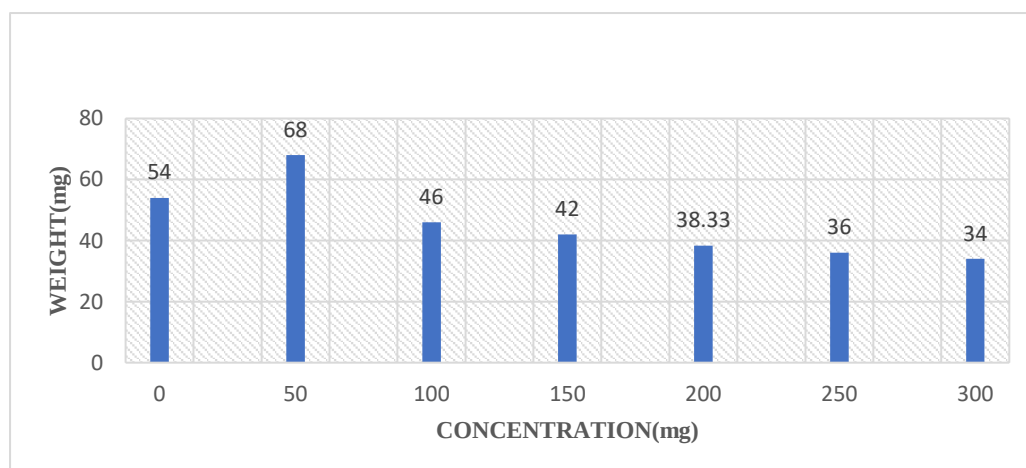


Fig 5.3.2: Graphical representation of the 10th day weight

Comparing with all the sets the 50mg treated set has a high weight of 68mg. At this stage it is concluded that treating the zebrafish with 50mg for 10 days given a good result with increase in the weight. Also found that giving less concentration for minimum period gives better result.

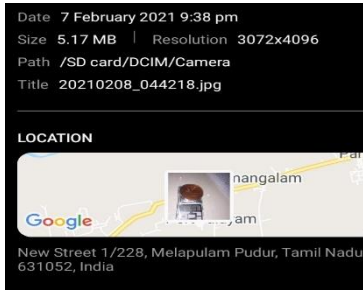


Fig 5.3.3: 10th day control set fish weight

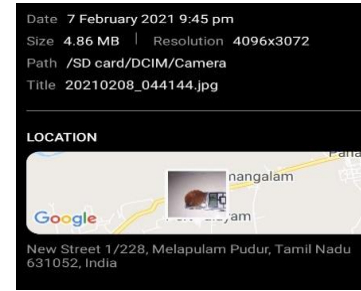


Fig 5.3.4: 10th day 50mg treated fish weight



Fig 5.3.5: 10th day control set fish length

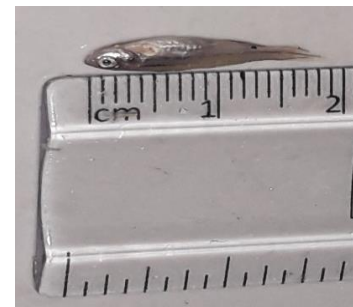


Fig 5.3.6: 10th day 50mg treated fish length

5.4 20th DAY RESULTS

At the 20th day the height and weight of the zebrafish is calculated.

Table 5.4: 20th day measurements of the Zebrafish

SI.NO	AMOUNT OF <i>WITHANIA</i> <i>SOMNIFERA</i> (mg)	AMOUNT OF WATER (litre)	20 th DAY LENGTH (cm)	20 th DAY WEIGHT (mg)
CONTROL	0	1	2.05	75
T1	50	1	2.08	93.33
T2	100	1	1.92	85
T3	150	1	1.93	56.66
T4	200	1	1.95	66.66
T5	250	1	1.98	85
T6	300	1	2.1	96.66

At the 20th day the length and weight of the zebrafish is calculated. In the 20th day results comparing with all the set 300mg treated set has a high average length and weight that is highest length is 2.1 and weight is 96.66. The length and weight of 300mg treated zebrafish at this stage shows a drastic change.

From the 10th day result to 20th day result of test set 6 with high concentration of 300mg *WITHANIA SOMNIFERA* treated gives a good result only after some period of time. This shows that when it is given in high concentration it reacted slowly and gives result in growth after certain period.

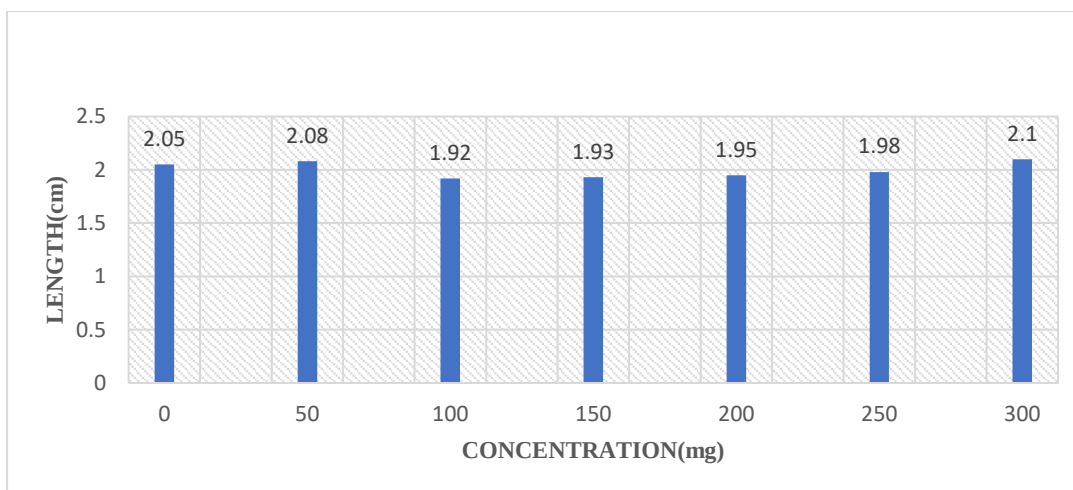


Fig 5.4.1: Graphical representation of the 20th day length

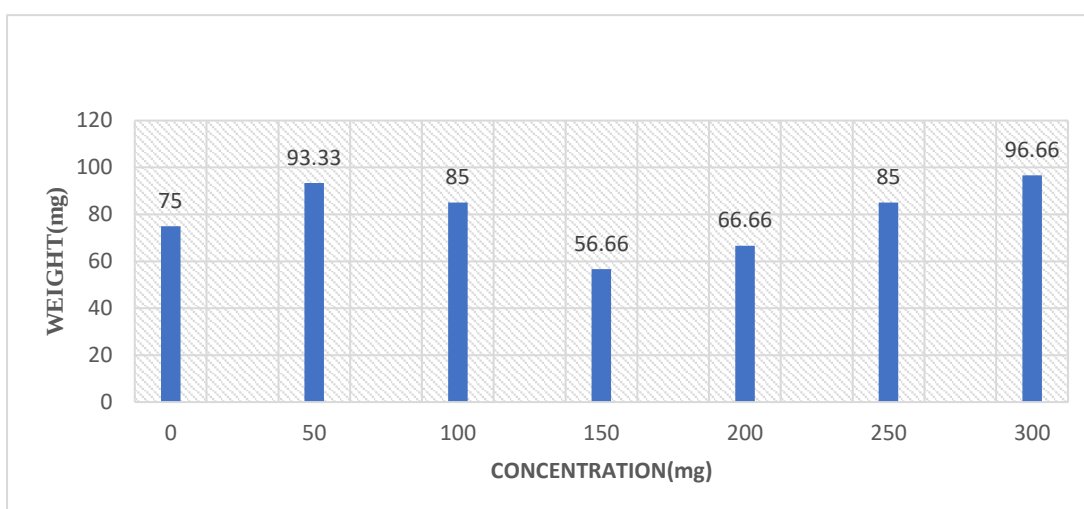


Fig 5.4.2: Graphical representation of the 20th day weight

Out of that result it is get to know that the fishes treated with low concentration (that is 50 mg) gives a good result in their weight gain at a short time elsewhere the fishes treated with high concentration (that is 300mg) gives a good result in their weight gain after some period of time.

Apart from that two concentration, control set give a stable growth elsewhere others gives very slow growth.

From this it can be concluded that the low concentration gets absorbed quickly and respond in their growth in short time on the other side high concentration gets absorbed slowly and respond in their growth after certain time.



Fig 5.4.3: 20th day control set fish weight



Fig 5.4.4: 20th day 50mg treated fish weight

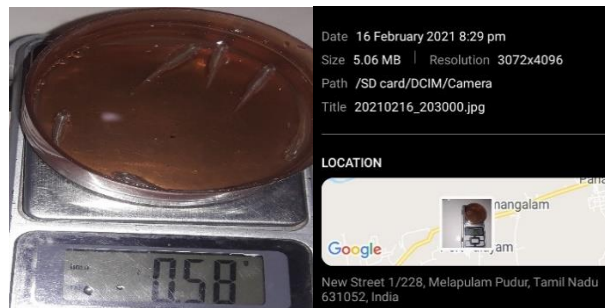


Fig 5.4.5: 20th day 300mg treated fish weight



Fig 5.4.6: 20th day control set fish length

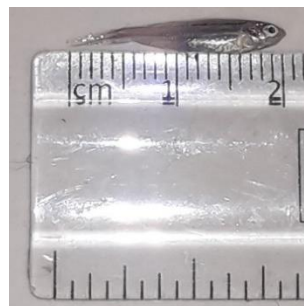


Fig 5.4.7: 20th day 50mg treated fish length



Fig 5.4.8: 20th day 300mg treated fish length

5.5 30th DAY RESULTS

At the 30th day the height and weight of the zebrafish is calculated.

Table 5.5: 30th day measurement of Zebra fish

SI.NO	AMOUNT OF <i>WITHANIA</i> <i>SOMNIFERA</i> (mg)	AMOUNT OF WATER (litre)	30 th DAY LENGTH (cm)	30 th DAY WEIGHT (mg)
CONTROL	0	1	2.25	116.6
T1	50	1	2.3	114
T2	100	1	1.95	101.6
T3	150	1	1.96	98
T4	200	1	1.98	85
T5	250	1	2.08	96
T6	300	1	2.35	115

At the 30th day the length and weight had been calculated. In the 30th day result control set had a highest weight of 116.6mg and high concentration set 300mg had a highest length of 2.35cm. Only a minute changes occurred between the control set and 300mg concentration treated set.

From the 20th day result to 30th day result no huge changes occurs in the high concentration of 300mg and low concentration of 50mg. Only a tiny change occurs in the increase in weight of the low and high concentration of *WITHNIA SOMNIFERA*

treated set fishes. Comparing the low and high concentration treated fishes the fish treated with 50mg had lower length and weight than 300mg concentration treated fish.

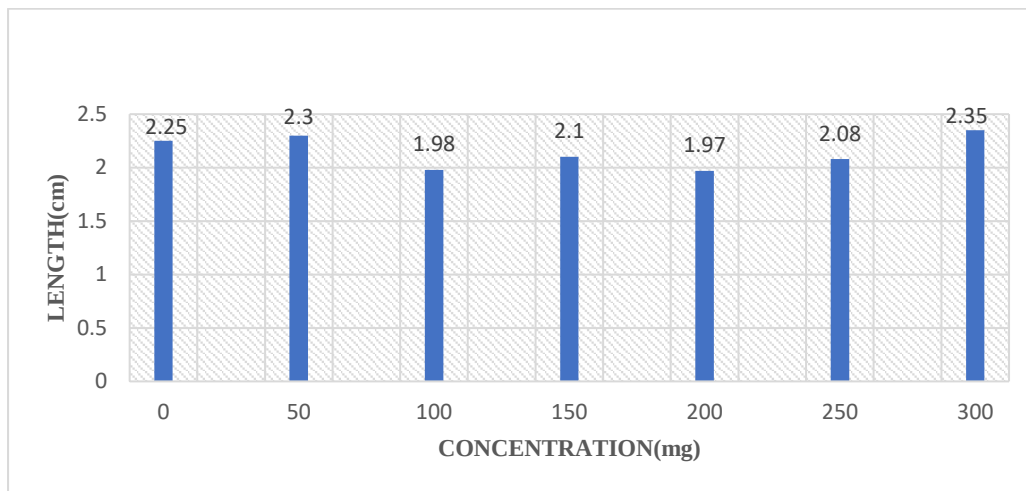


Fig 5.5.1: Graphical representation of the 30th day length

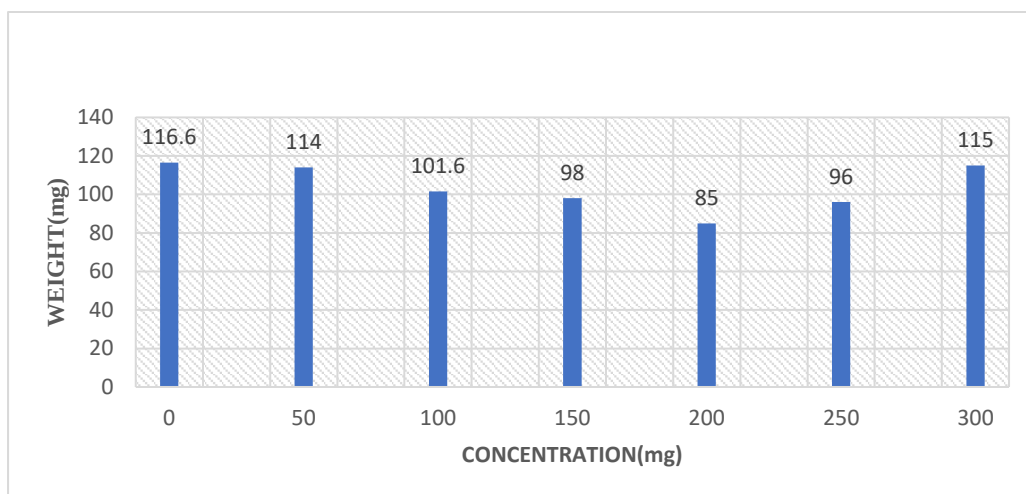


Fig 5.5.2: Graphical representation of the 30th day weight

From this result it is get to know that after 30 days of growth of zebrafish treated with 300mg and 50mg of *WITHANIA SOMNIFERA* had only a minute changes can be seen in the weight and length of the zebrafish. In this control set fishes had a stable growth both in their length and weight while treated set of fishes doesn't had a stable growth in their weight.



Fig 5.5.3: 30th day control set fish weight



Fig 5.5.4: 30th day 50mg treated fish weight

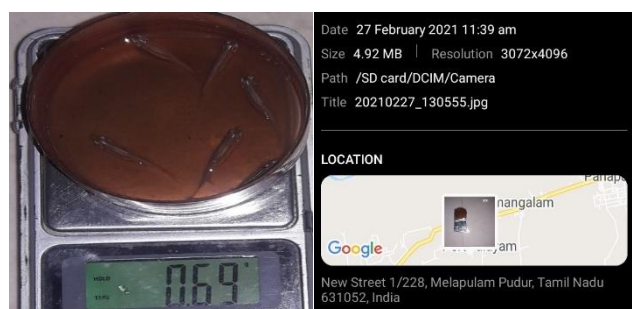


Fig 5.5.5: 30th day 300mg treated fish weight



Fig 5.5.6: 30th day control set fish length

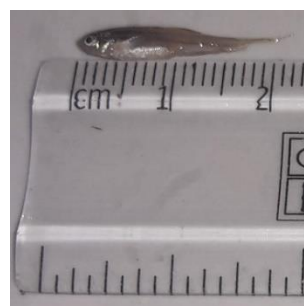


Fig 5.5.7: 30th day 50mg treated fish length

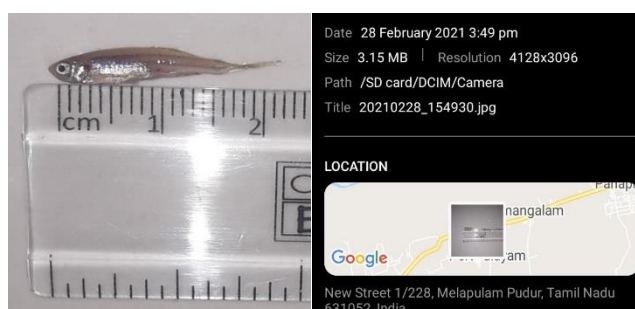


Fig 5.5.8: 30th day 300mg treated fish length

5.6 70th DAY RESULTS

At the 70th day the height and weight of the zebrafish is calculated.

Table 5.6.1: 70th day measurement of Zebra fish

SI.NO	AMOUNT OF <i>WITHANIA</i> <i>SOMNIFERA</i> (mg)	AMOUNT OF WATER (litre)	70 th DAY LENGTH (cm)	70 th DAY WEIGHT (mg)
CONTROL	0	1	2.45	150
T1	50	1	2.5	170
T2	100	1	2.53	172.5
T3	150	1	2.65	198
T4	200	1	2.60	177
T5	250	1	2.68	192
T6	300	1	2.7	195

After 30 days the supply of ashwagandha along with fish feed had been stopped and the length and weight calculated. In that result 150mg treated fishes had highest weight and 300 mg treated fishes had highest length.

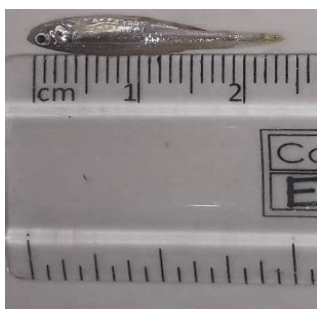


Fig 5.6.1:70th day control set fish length



Fig 5.6.2:70th day 300mg treated fish length

Table 5.6.2: Growth of *zebra fish* fed with and without Ashwagandha on 70th day

S.NO	C		50		100		150		200		250		300	
	L	W	L	W	L	W	L	W	L	W	L	W	L	W
1	2.35	140	2.55	160	2.53	171	2.75	188	2.4	175	2.58	190	2.65	205
2	2.55	155	2.3	175	2.6	167	2.44	192	2.58	170	2.45	185	2.72	194
3	2.25	140	2.7	180	2.7	173	2.6	203	2.45	185	2.7	189	2.7	190
4	2.3	145	2.4	168	2.47	180	2.48	194	2.3	174	2.62	196	2.8	188
5	2.5	165	2.5	172	2.57	170	2.74	210	2.62	187	2.75	180	2.95	195
6	2.4	160	2.38	168	2.3	168	2.54	200	2.7	176	2.9	198	2.44	203
7	2.7	145	2.6	176	2.43	166	2.8	198	2.9	175	2.6	205	2.7	185
8	2.55	150	2.72	167	2.38	174	2.5	195	2.55	165	2.78	195	2.46	200
9	2.5	160	2.35	170	2.57	179	2.75	194	2.7	180	2.74	182	2.98	198
10	2.4	140	2.5	164	2.75	177	2.9	206	2.8	183	2.68	200	2.6	192
AVE %	2.45	150	2.5	170	2.53	172.5	2.65	198	2.6	177	2.68	192	2.7	195

L = Length

W = Weight

Ave% = Average percentage

5.7 ZEBRA FISH TREATED WITH WITHANIA SOMNIFERA

WEIGHT GAIN (%) = FINAL WEIGHT – INITIAL WEIGHT

For control = 150 - 30

= 120

For 50mg = 170 – 30

=140

For 150mg= 198 - 30

= 168

For 300mg= 195 – 30

= 165

LENGTH GAIN (%) = FINAL LENGTH – INITIAL LENGTH

For control = 2.45 – 1.6

= 0.85

For 50mg = 2.5 – 1.6

= 0.9

For 150mg= 2.65 – 1.6

= 1.05

For 300mg= 2.72 – 1.6

= 1.12

AVERAGE DAILY WEIGHT GAIN = $\frac{\text{FINAL WEIGHT} - \text{INITIAL WEIGHT}}{\text{DAYS OF CULTURE}}$

For control = 120/70

= 1.71

For 50mg = 140/70

= 2

For 150mg= 168/70

= 2.4

For 300mg= 165/70

= 2.35

$$\text{AVERAGE DAILY LENGTH GAIN} = \frac{\text{FINAL LENGTH} - \text{INITIAL LENGTH}}{\text{DAYS OF CULTURE}}$$

$$\text{For control} = 0.85/70$$

$$= 0.0121$$

$$\text{For 50mg} = 0.9/70$$

$$= 0.0128$$

$$\text{For 150mg} = 1.05/70$$

$$= 0.015$$

$$\text{For 300mg} = 1.12/70$$

$$= 0.016$$

$$\text{SPECIFIC GROWTH RATE} = \frac{\ln \text{FINAL WEIGHT} - \ln \text{INITIAL WEIGHT}}{\text{DAYS OF CULTURE}}$$

$$\text{For control} = \ln(150) - \ln(30) / 70$$

$$= 1.6094 / 70$$

$$= 0.0229$$

$$\text{For 50mg} = \ln(170) - \ln(30) / 70$$

$$= 1.7346 / 70$$

$$= 0.0247$$

$$\text{For 150mg} = \ln(198) - \ln(30) / 70$$

$$= 1.8870 / 70$$

$$= 0.0269$$

$$\text{For 300mg} = \ln(195) - \ln(30) / 70$$

$$= 1.8718 / 70$$

$$= 0.0267$$

$$\text{FEED CONSERVATION RATE} = \frac{\text{TOTAL FEED (g)}}{\text{WEIGHT GAIN (g)}}$$

$$\text{For control} = 0.21 / 120$$

$$= 0.0017$$

$$\text{For 50mg} = 0.960 / 140$$

$$= 0.0068$$

$$\text{For 150mg} = 2.460 / 168$$

$$= 0.0146$$

$$\text{For 300mg} = 4.710 / 165$$

$$= 0.0285$$

5.8 CONCLUSION

To estimate the growth rate zebrafishes had been treated with Withania Somnifera for a short period of 30th days and maintained for other 40 days to conclude its growth. In the result, after 40 days of maintenance, there is a good growth rate in the 150mg treated fishes than other treated and untreated fishes. From these results it is be known that 150mg treated fishes had highest growth (Length 2.65 cm, weight 198mg) which is also a stable growth from beginning. As it has stable growth in fishes it is concluded that intake of moderate dose of Withamnia Somnifera gives better results in growth and it is a safe dose without any negative symptoms to the health. Comparing with high and low doses intake of moderate dose will give good results in the growth rate.

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