PRECLINICAL SAFETY EVALUATION OF AADUTHEENDAPAALAI VER CHOORANAM

The dissertation Submitted by

Dr. L. SAKTHIMANIPRIYA ., M.D (s)

Under the Guidance of

Dr. R. MADHAVAN., M.D (s),

Associate Professor and Guide,

Head of the Department

Department of Nanju Maruthuvam,

National Institute of Siddha, Chennai - 47.

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Preclinical safety evaluation of AADUTHEENDAPAALAI VER CHOORANAM"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. R.Madhavan MD (S)**, Associate Professor, Head of the Department, Department of Nanju Maruthuvam, National Institute of Siddha, Tambaram sanatorium, Chennai - 47 and the dissertation has not formed the basis for the award of any degree, Diploma, Fellowship or another similar title

Date: DR. L.SAKTHIMANIPRIYA., MD(S).,

Signature of the candidate

Place:

BONAFIDE CERTIFICATE

Certified that I have gone through the thesis submitted by **Dr.L.SAKTHIMANIPRIYA, (Reg.No: 321716204)** a student of final year M.D (S), Branch VI – Department of Nanju Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai – 47 and the thesis work has been carried out by the individual only. This thesis does not represent or reproduce the thesis submitted and approved earlier.

Place:

Date:

Dr. R. Madhavan, MD(S).,
Name & Signature of the Guide
Associate Professor,
Department of Nanju Maruthuvam,
National Institute of Siddha, .
Chennai – 47

Dr. R. Madhavan., MD (S).,
Name & Signature of the HOD,
Associate Professor,
Department of Nanju Maruthuvam,
National Institute of Siddha,
Chennai – 47.

Dr. R. Meenakumari., MD (S).,
Name & Signature of the Director,
Professor,
National Institute of Siddha,
Chennai – 47.

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INTRODUCTION

1. INTRODUCTION

Siddha medicine is the oldest and the foremost medical systems in the world. The God Shiva transmitted the knowledge of medicine to Parvati, who in turn passed it on to Nandi, from whom it was given to the first practitioners of Siddha medicine the Siddhars. The word Siddha comes from the word Siddhi, which means an object to attain perfection or heavenly bliss. Siddhars were the ones who had attained "Siddhi". The main aim of Siddhars was to certify that the container of the soul for the attainment of happiness and to reach the God. They found diseases as one of the obstacle to reach God. So they bestowed to the world the Siddha medicine to treat diseases.

Siddha generally refers to aathma Siddha that is the eight supernatural powers. Those who attained or achieved the above- said powers are known as Siddhars. There were 18 important Siddhars in olden days and they developed this system of medicine. Hence it is called Siddha Medicine. The Siddha system of Medicine is Prevalent in South India, Sri Lanka, Malaysia, and Singapore, where the existence of Dravidian civilization was documented their experiences in Tamil language. This system owes its origin to the Dravidian culture which is of the Prehistoric Period [1].

According to Siddha medical science, the universe originally consisted by atoms which contributed to the five basic elements, viz., Nilam (earth), Neer (water), Thee (fire),Vali (wind) and Veli (space) which synchronize with the five senses of the human body, and they were the fundamentals of all the corporal things in the world. A close relationship is found existing between the external world and the internal system of human body. Siddhars maintain that the structure of the human body is a miniature of the world in itself. In other words, every substance visible or invisible, animate or inanimate is said to be formed of kinds of Panchaboothas, otherwise called the five elements which is said above. They might have been formed by one, two, three, four or

five elements noted above. This is the first principle or idea of a substance. Also *Sattamuni Gnanam* says that, Microcosm reflects macrocosm

அண்டத்திலுள்ளதே பிண்டம்
பிண்டத்திலுள்ளதே அண்டம்
அண்டமும் பிண்டமும் ஒன்றே
அறிந்துதான்பார்ககும்போதே

- சட்டமுனி ஞானம்.

(i.e.) The universe is a macrocosm made up of five primordial elements or boothas and the human being is a microcosm made up of the same five elements. The seers of ancient India propounded the Thiridhatu theory in accordance with which three vital elements namely the Vatha, Pitha and Kaba in their normal condition regulate all physiological activities and keep the body healthy. This Thiridhatu theory is also based on Panchaboothas. When these Thridhatus became abnormal or when their mutual harmony is disturbed they bring about ill health[2]. It is mentioned in *Thirukural* as follow,

மிகினும் குறையினும் நோய்செய்யும் நூலோர்
வளி முதலா எண்ணிய மூன்று.

- திருவள்ளுவர்

Siddha differs from western system of medicine in the method of approach to the medical problems which it views and interprets in terms of the three elemental theories and thus while considering the aetiology and treatment of disease, it gives more attention to the disorders of the body than to the extrinsic ones. A specific feature in Siddha care is the Kalpamurai also called as Kayakalpa where the human body can be fortified into rock like strength through serious of efforts with lifestyle, strengthening drugs and formulations.

The uniqueness of Siddha system of medicine is not only curing the ailments but also the mind to lead a peaceful life. In *Thirukurral,* it is stated as,

மனநலம் மன்னுயிர் காக்கும்

- திருவள்ளுவர்

The Siddha system of Medicine emphasizes on the patient, environment, age, sex, race, habits, mental frame work, habitat, diet, appetite, physical condition, physiological constitution of the diseases for its treatment which is individualistic in nature. Diagnosis of diseases are done through examination of pulse, urine, eyes, study of voice, colour of body, tongue and status of the digestion of individual patients. Siddha System has unique procedure treasure for the conversion of plants, metals and minerals as drugs [3].

Siddhars are precise in the treatment of poisons. They identified toxic substances and their antidote which is documented in Siddha literature. The Siddhars have astonishing knowledge in herbal, metal, mineral and animal products. Before going to any Siddha medical Preparation, the raw drugs, which are purified (detoxification) and then the purified drugs are including to the medicine.

The signs and symptoms of poison in humans, the way of diagnosis of poison and treatment are explained in Nanju Maruthuvam (Siddha Toxicology). It contains general antidote for poison and also explained the specific antidote for particular poison which is beneficial to the society [4].

In our Siddha system, they are two types of medicine which include 32 internal and 32 external medicines. Among 32 internal medicines, one of the simple forms of internal medicine in Siddha system is Chooranam. Chooranam is a fine dry powder of drugs. The term Chooranam may be applied to the powder of a single drug or a mixture of two or more drugs which are powdered separately prior to their being mixed to homogenity. The Chooranam should be very fine, amorphous and should be perfectly dry before the Chooranam is prepared the process of purification as to be done.

Purification of raw drugs is a process aimed at both purification as well as concentration of the raw drug. It usually involves process like cleaning, frying, soaking and grinding with herbal juices until impurities are removed. No medicinal preparation is

done without prior *suddhi* process. This process helps raw material or crude drugs to lose their undesirable or toxic effects and thereby giving better efficacy [5].

The shelf life of medicines indicates the potency of medicines. As per Siddha literature Agamarunthu paadal in Gunapadam thathu jeevam text,

“உயர்சூர ணம்பிட்டு வடகம்வெண்ணெய்நான்கி
னுயிர்மூன்று திங்களெண்ணெய்.....”

From the above quote the shelf life of chooranam (powder) is three months, but according to AYUSH guidelines the shelf life of chooranam is one year [6, 7].

Aadutheendapaalai is one among the medicinal plant mentioned in siddha literature. It is play an important role in diagnosing of snake poison. It is also used to treat the skin disease and virulent noxious bite.

Snake bite is a life threatening problem causing mortality from ancient period to till date. About 94,000 snake bite deaths are recorded globally and 15000 in India per year [8]. Even today treating the noxious cases is a dare to the medical profession, but treating such noxious cases through the Siddha system of medicine seems to be more abundant and cost effective, hence this meticulous formulation had been preferred in a broad vision.

I wished for working in the field of Siddha toxicology and was permitted to do the safety evaluation of ‘*Aadutheendapaalai Ver Chooranam*’ - a Siddha herbal preparation which is indicated for several illness and virulent noxious bite

AIM & OBJECIVES

2. AIM & OBJECTIVES

2.1 AIM:

To evaluate the safety profile (Acute and 28 days repeated dose oral toxicity study) of **"*Aadutheendapalai Ver Chooranam*"** – a herbal preparation in Wistar albino rats.

2.2 OBJECTIVES:

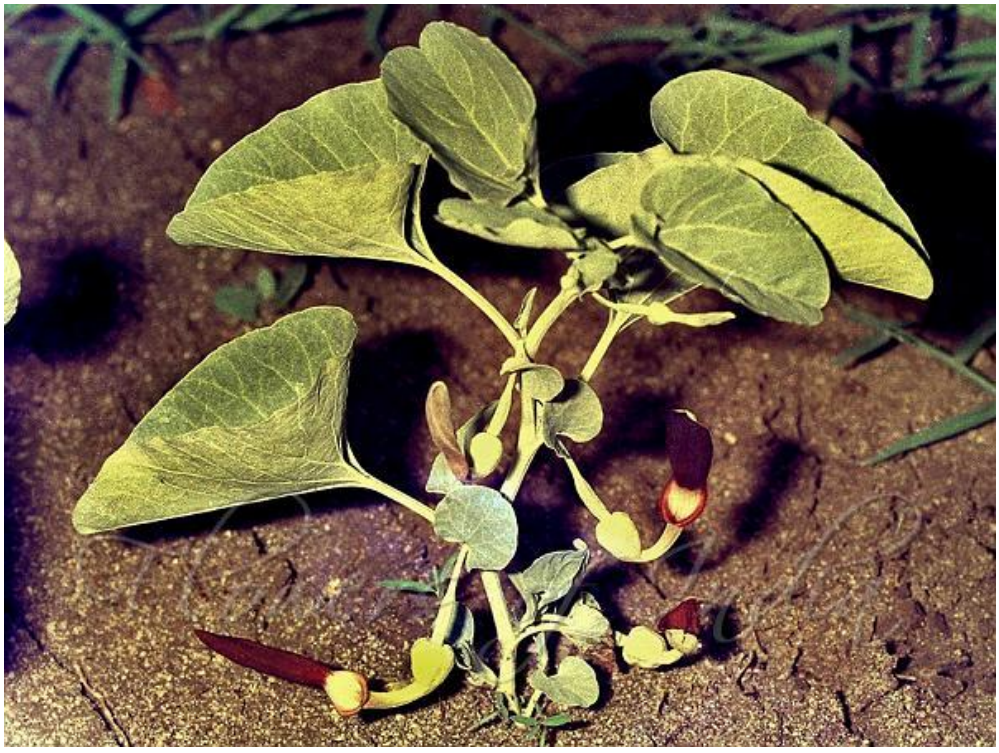
- To collect the raw drug
- To authenticate ***Aadutheendapalai ver*** by Botanist.
- To analyze the Physicochemical analysis, Biochemical analysis, Phytocompound analysis using TLC & HPTLC, Pesticide residual, Sterility and Aflatoxin of ***Aadutheendapalai ver Chooranam.***
- To find out the safe dose of the ***Aadutheendapalai ver Chooranam*** through toxicity Studies on Rodents as per OECD guidelines 423 & 407.

LITERATURE REVIEW

3. REVIEW OF LITRATUTE

In the present work literature review was carried out by using various resources like Siddha literature, modern literatures, internet resources like PubMed, Science direct, Google scholar and Scopus journals etc. The review was documented in the following aspects.

Figure 1: ஆடுதீண்டாப்பாளை



3.1.1 SIDDHA ASPECT

ஆடுதீண்டாப்பாளை

வேறு பெயர்:

ஆடுதீன்னாப்பாளை

ஆடுதொடாப்பாளை

பங்கம்பாளை

இது பூண்டு இனத்தைச் சேர்ந்தது. இது தென்னிந்தியாவில் திருவாங்கூரிலும் கீழக்கரையைச் சேர்ந்த நாடுகளிலும் சிங்களத்திலுள்ள மணற்பாங்கு அல்லது நன்செய் நிலங்களிலும் ஏராளமாகக் கிடைக்கும்.

ப.உ:இலை, விதை, வேர், சமூலம்

சுவை: கைப்பு (குமட்டல்)

தன்மை: வெப்பம்

பிரிவு: கார்ப்பு

செய்கை:

புழுக்கொல்லி

சூதகமுண்டாக்கி

வெப்பமுண்டாக்கி

உரமாக்கி

நீர்மலம்போக்கி

உடற்தேற்றி

முறைவெப்பகற்றி

பண்பு:

குடற்புழு, சிலந்தி, பூச்சி நஞ்சுகள், கரும்படை, கரப்பான் வாதநோய்-80, பன்றி மாமிசத்தோல் போன்ற படை ஆகிய இவைகள் ஆடுதீண்டாப்பாளையால் நீங்கும். பலமும் விந்தும் உண்டாகும்.

கிரந்திகரப் பன்வெக்கை கேசநலி மாந்தை

யரந்தை வினையை யறுக்கும்- துறந்து

பிரியொனா நோய்களையும் பின்முன் பாராமல்

மறியுணா மூலியடை வாய்.

(தே. வெண்பா)

இது கிரந்தி, கரப்பான், மயிர்கொட்டடீப் போதல், கணச்சூடு ஆகியவற்றைப் போக்கும்

ஆடுதொடாப் பாளைக்ககக் கிருமி வன்சிலந்தி

நீடுகருங் குட்டம் நிறைகரப்பான்-ஆடிடச்செய்

எண்பது வாய்வும்இகல்குட்ட முத்தீரும்

திண்பெறுநற் றாதுவுமாஞ்செப்பு.

(அ.கு)

மேற்கண்ட நோய்களுக்கு கால் பலம் நிறையுள்ள இலையைக் கால்படி வெந்நீரில் இரண்டு மணிநேரம் ஊறவைத்து வடிகட்டி 15 மி.லி. முதல் 30 மி.லி. வீதம் கொடுக்கலாம்.

உலர்ந்த இலையை மேற்கண்டபடி ஊறல்குடிநீர்செய்து கொடுக்க நுண்புழுக்கள் சாகும்.

விதை

விதைகளைப் பொடித்து 4 கிராம் எடுத்து அரை முதல் ஒரு பலம் சிற்றாமணக்கெண்ணெயில் கலந்து கொடுக்க, வயிற்றுவலி, சூதகத்தடை, சூதகக்கட்டு, முறைச்சுரம், பிரசவ வேதனை இவைகளை நீக்குவதுடன், குடற்புழுக்களையும் வெளிப்படுத்தும்.

வேர்

வேரை அரைத்து அரை முதல் ஒரு வராகனெடை கொடுக்க பாம்பு நஞ்சு முறியும் மற்ற நஞ்சுகளும் நீங்கும்.

வேர்த்தூளை ஒன்று முதல் ஒன்றரை வராகனெடை வீதம் தனியேயாவது வெந்நீருடன் கலந்தாவது கொடுக்க பிரசவ வேதனையைத்தூண்டும்.

முழுப்பூண்டு

இப்பூண்டுரசத்துடன் அதே அளவில் நல்லெண்ணெய் கூட்டி எரித்து பக்குவப் பாகத்தில் எடுத்து வடிகட்டி, கரும்படை, கரப்பான் இவைகளுக்குப்பூசி வரலாம்.

பூண்டைச் சேர்த்தரைத்து சற்றேறக் குறைய தேங்காயளவு எடுத்துருட்டி ஒருபடி நல்லெண்ணெயிலிட்டுக் காய்ச்சி, வடிகட்டி, கால் அல்லது அரைப்பலம் வீதம் ஐந்தைந்து நாட்களாக 40 நாள் கொடுத்துவர, பெருநோய்த் தடிப்பு நீங்கும் உப்பும் புளியுமாகா[9].

(அனுபவம்)

சேரும்பிறமருந்துகள்:

- **தாபவனலச் சூரணம்**[10]**:**

தீரும்நோய்கள்:

அசீரணம், சூலை, கரப்பாண், திமிர், வாயு, வாந்தி, சன்னி, சுவாசகாசம் சயம், இருமல், பேதி, கிராணி, குன்மம், காமாலை, வாயுறல், மூலம்

- **யோகராஜ குக்குலு**[11]

தீரும் நோய்கள்:

நடுக்குவாதம், மதவாதம், சிரோவாதம், சோனிதவாதம், மூத்திரக்கரிச்சரம்

சூலை, வில்வாதம், திமிர்வாதம், புறவீச்சு

- **நாகராதிச்சூரணம்**[12]

தீரும்நோய்கள்:

அதிசாரம், கிராணி, குன்மம்

- **லிங்ககட்டு**[13]

தீரும்நோய்கள்:

சுரம், சன்னி

- **புளியாரை கிருதம்**[14]**:**
- **தீரும்நோய்கள்:**

அதிசாரம், கிராணி

- **பாலசுரஹரமாத்திரை**[15]**:**

தீரும்நோய்கள்:

குழந்தையின் சுரம், விஷம்

பாம்பு நெருங்காமற் தடுக்கும் மனை மூலிகைகள்:

காரமா மூலியடா பங்கம்பாளை

கொடியவிட மணுகாது குடியோடப்போம்

நன்றான நாகதாளிக்கிழங்கு தானும்

நன்மனையிலிருக்கவிடம்நாடாதப்பா

அன்றான ஆகாசக்கருடன் மூலி

அம்மனையிலிருக்கவிட மற்றுப்போமே[16]

ஒவ்வொரு வீட்டிலும் மூலிகைகளை வைத்து வளர்த்து வருதல்நலம் அவற்றில் சிறந்த இனங்கள் வீட்டில் வளர்க்கப்படுவதால் பாம்பு போன்ற உயிர்கள்வராது. அம்மூலிகைகள் பின்வருமாறு:

ஆடுதீன்னாபாளை

நாகதாளிக்கிழங்கு

ஆகாசகருடன்கிழங்கு

நிலவேம்பு

3.1.2. MODERN ASPECT:

Medicinal plants occupy a distinct place in the life of human, right from the primitive till today. Use of plants as a source of medicine has been inherited and is an important component of health care system in India. India has more than 3000 years of medicinal heritage based on medicinal plants. Medicinal plants are widely used by all sections of the population either directly as folk remedies or indirectly in the preparation

of modern pharmaceuticals.

India is endowed with a rich wealth of medicinal plants; microbes are closely associated with the health and welfare of human beings. Some are beneficial and some are detrimental. Plants produce a diverse extent of bioactive molecules, making them wealthy source of various types of medications. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products and used in the traditional systems of medicine. A larger part of the total population in developing countries still uses traditional folk medicine obtained from plant resources [17].

About 80 percentages of individuals from developed countries use traditional medicine which has compounds derived from medicinal plants. In the last few years, a number of reported have been conducted in various countries to manifest such ability. Numerous plants have been used because of their antimicrobial traits, which are chiefly synthesized during secondary metabolism of the plant. Therefore, such plants should be examined to better understand their activities, trustworthy and efficiency [18].

Aristolochia is a large plant genus with over 500 species, belongs to the family Aristolochiaceae. In the indigenous system of medicine, the plant was used for the treatment of skin diseases, inflammation and purgative. Root extract was accounted to have anti-bacterial activity. Aristolochia species has been used extensively in the traditional

Aristolochia bracteolata also known as 'worm killer' in English due to its anthelminthic activity and trypanocidal effect is a perennial herb growing from 10–60 cm tall. The plant is important in traditional medicine in Africa, India and the Middle East [19]

Aristolochia bracteolata has been used in traditional medicine in Nigeria, India, and Ethiopia as an infusion of dried leaves to treat intestinal worms, skin itch, or insect bites [21, 22].

Habitat:

Aristolochia bracteolata is a climbing or prostrate perennial herb with an unpleasant smell, stems 10–60 cm tall from an underground rhizome. The leaves are ovate 1.5–8 X 1.5–7 cm with a petiole 0.5 cm–4.5 cm long. Flowers are dark purple, 0.5–5 cm tubular, with trumpet shaped mouth. Capsules are oblong-ellipsoid, 1.5–2.5 cm. Aristolochia bracteolata has been observed to have 2–3 flowers per leaf axil in Somalia however outside Somalia the plant seems to have solitary flowers [20].

Parts Used: Whole plant and Rhizome

Odour : Pungent

Taste : Bitter.

TAXONOMICAL CLASSIFICATION [23]:

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Magnoliids

Order: Piperales

Family: Aristolochiaceae

Genus: Aristolochia

Species: A. bracteolata

Synonyms:

Tamil: Aduthinnarppalai

English: Worm Killer, Dutchman's Pipe, Bracteated Birth Wort.

Sanskrit: Kitamari, Visanika

Hindi: Kitamar

Malayalam: Aduthinnappala, Karalakam

Kannada: Adu Muttada gida

Telugu: Gadide, Gadaparaku

Bengali: Kiramar, Patuvanga

Marathi: Kidamar

Gujarati: Kiramar

Oriya: Paniri

Action: Oxytocic, abortifacient, emmenagogue, antidote [24].

Types of Species in Genus Aristolochia[25]

Aristolochia abyssinica Klotzch - Africa

Aristolochia bracteata Retz.

Aristolochia crenata Ehreb. ex Duch

Aristolochia kotschyi Hoscht. ex A.rich

Aristolochia mauritiana Pers.

Einomeia bracteata (Retz.) Raf

Aristolochia maurorum Klotzsch

Aristolochia sempervirens Forssk

Ethno pharmacology/ Traditional uses [26]:

The uses of different parts of Aristolochia bracteolata in traditional system of medicine

Plant parts	**Traditional uses as/in**
Whole plant	Dermatitis, allergic disorder, leprosy, jaundice[29], worms, fever[30], Mosquito repellent [31], Anodyne, purgative, emmenagogue [32,33]
Leaves	Anti-inflammatory[34], dermatitis, rashes[30], skin disease, for scorpion sting[30], Antipyretic, snake bite[35], Antiulcer,amenorrhoea,antihelmintic[28], Antiplasmodial[36,37]
Seeds	Antibacterial, anti-inflammatory and analgesics, may toxic to goats[38]
Roots	Syphilis, gonorrhoea & skin diseases, eczema [27].

Phytochemistry:

The Phytochemical screening revealed the presence of alkaloids, triterpenoids, steroids and sterols, flavonoids, saponin, Phytosterols, carbohydrates, proteins, phenolic compounds and cardio glycoside[39,40].

Vaghasiya et al. carried out the analysis of phytoconstituent of A.bracteata in different extracts. The total phenolics content in the methanolic extract and acetone extract was found to be 59.22 ± 0.65 & 39.67 ± 0.92 respectively, were as the total

content of flavonoids were 36.06 ± 0.17 & 130.93 ± 2.58 in the methanolic and acetone extract respectively[41].

Chemical composition:

The root contains active alkaloids aristolochin, iso-aristolochic acid and allantoin[21].

MEDICINAL USES:

The paste of the leaf of Kitamari is applied over the wounds for helping in quick healing. The decoction of the leaf is consumed in a dose of 50 ml to treat dysmenorrhea and difficulty in labor. The decoction of the bark or whole plant is taken in a dose of about 40 ml to treat intestinal worm. The paste of the leaf of Kitamari is applied over eczema for its treatment. The powder of the seed is given with powder of black pepper to treat fever. The paste of the root is applied over the area over localized swelling for its treatment.

The whole plant is very bitter and has abortifacient, alterative, anthelmintic, antiperiodic, emmenagogue and purgative properties. The leaves and roots are used to rid the body of Guinea worm (a parasitic infection caused by a nematode)[42].

It should be used with great caution since the plant can be toxic to mammals. The stem and the root contain the alkaloid aristolochic acid. The dried, powdered root has been shown to increase the contractions of the uterus during labour. It has been used as a substitute for ergot. The powdered roots are combined with castor oil (from Ricinus communis) and used in the treatment of colic, amenorrhoea, dysmenorrhoea, intermittent fever and worms. Externally, its juice is applied to foul and neglected ulcers to destroy insect larvae [43].

The dried and powdered leaves, mixed with oil or blood, are emetic. They are used to treat breast diseases and are also applied topically to kill lice and ticks. It is also used to treat scorpion bites [44].

RESEARCH ARTICLES PUBLISHED IN JOURNALS:

- **Anti-Pyretic Activity:**

  Rajamanickam V et al. at 2009 was investigated for their anti-pyretic activity of *A. bracteolata*. Pet ether and acetone extracts of the plant *A. bracteolata* were prepared using Soxhlet extraction. Pyrexia produced in rats by injecting 20ml/kg (s.c) of 20% aqueous suspension of brewer's yeast suspension. Extracts at 250 mg/kg exhibited significant anti pyretic activity. Aspirin (300mg/kg) was used as standard for which Pet. Ether extracts was found to be more effective than acetone extract [45].

- **Anti-allergic activity:**

  Chitme H et al. at 2010 was evaluated anti allergic activity of *A. bracteolata* by using compound 48/80 induced anaphylaxis, dermatitis, rhinitis and pruritis, as a preclinical model for acute phase of hypersensitivity reactions. The late phase hypersensitivity was evidenced by considering toluidine diisocynate induced volume of broncho alveolar fluid secretion and its inhibition. The possible anti-allergic mechanism was evaluated by using compound 48/80 induced mast cell activation and estimated serum nitric oxide (NO), rat peritoneal fluid NO, broncho alveolar fluid NO and blood histamine levels. It has been reported that the chloroform extract of *A.bracteolata* had potent and significant inhibitory effect on compound 48/80 induced pruritis and dermatitis activity in Swiss albino mice. It showed significant effect in toluidine diisocynate induced rhinitis in Swiss albino mice. Mast cell membrane stabilization activity was also observed in compound 48/80 induced mast cell activation. A significant reduction was observed in serum nitrate levels, rat peritoneal fluid nitrate levels and BAL nitrate levels. The extract was also found to posses' significant inhibitory effect on blood histamine levels. It could be concluded that the chloroform extract Posses potent antiallergic activity, possibly through mast cell

membrane stabilization, inhibiting NO and histamine pathway [46].

- **Anti-inflammatory activity:**

Annie Shirwaikar et al. at 2003 were evaluated anti-inflammatory of *A. bracteolata*. The ethanollic extract of the shade dried leaves of *A.bracteolata* was evaluated anti-inflammatory activities in wistar rats by using the carrageenan induced left hind paw edema method. Significant reduction of edema volume was observed in the drug treated group when compared with the standard and untreated control. Antioxidant investigation of the ethanol extract along with its two successive fractions using nitric oxide and 1,1-diphenyl-2 picryl hydrazyl (DPPH)-induced free radical assay methods showed good free radical scavenging activity, thereby supporting its anti-inflammatory properties[47].

- **Anti-arthritis Activity:**

Havagiray R et al. at 2009 Anti-arthritic activity was demonstrated using Freund's complete adjuvant in rats. The results shows that, regular treatment of adjuvant induced arthritic rats with *A.bracteolata* extracts improves ESR, Hb value and also restores body weight. Significant ($P<0.01$) inhibitory effect was observed with *A. bracteolata* extract on Freund's complete adjuvant induced paw edema throughout the study ($P<0.001$). The latency to thermal stimuli and inhibitory effect on xylene induced ear edema was significantly ($P<0.05$) affected by oral treatment of *A. bracteolata*, irrespective of solvent used for extraction. Treatment of FCA induced rats with *A. bracteolata* extracts shown ($P<0.05$) increase in pain threshold, weight bearing ability, ambulation and also decline in scratching, defecation and urination, were observed as a sign of improvement in behavioural condition[48].

- **Anti-ulcer Activity:**

Mohamed Iyas K et al. at 2011 was evaluated anti-ulcer activity of A. bracteolata. The aqueous extract of leaves of *A. bracteolata* exhibited antiulcer

activity in rats. The anti-ulcer activity of *A. bracteolata* was evaluated against ethanol induced and pylorus ligation induced models, at two different dose levels of 400 and 800 mg/kg/body wt/day. The activity was compared with standard drug Ranitidine. Pre-treatment with the extract resulted in a significant decrease of the ulcerated area. The volume and acidity of the gastric juice decreased in the pre-treated rats. Among the two dose assessed, 800 mg/kg was found to have the significant activity than the lower dose [49].

- **Anti-bacterial Activity:**

Manikandar RV et al. at 2006 was evaluate the antibacterial activity of the aqueous leaf and aqueous root extract of the medicinal plant Aristolochia bracteolata using the standard disc diffusion method against four bacterial species, viz., Klepsiella pneumonia, Staphylococcus aereus, Escherichia coli and Pseudomonas flavus strains. The antibacterial activity revealed that the root extract has more effective than the leaf extracts. Staphylococcus aureus was the most sensitive organism among the tested organism [50].

Negi PS et al. at 2006 was investigated the antibacterial activity of *Aristolochia bracteolata* root extracts. Powdered Roots of *A. bracteolata* were extracted with ethyl acetate, acetone, methanol, and water for 8 hours each using a Soxhlet extractor. Antibacterial activity of dried extracts was evaluated by the pour-plate method against a few Gram positive and Gram-negative bacteria. All the crude extracts showed a broad spectrum of antibacterial activity among which ethyl acetate extract was found to be the most effective. This study shows the potential for replacement of synthetic preservatives by the use of natural extracts [51].

Kavitha D et al. at 2007 was conducted study, the different extracts (Aqueous, methanol and chloroform) of *A. bracteolata* were effective against the bacterial strains Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas fluorescens, Shigella flexneri, Proteus vulgaris and the fungal strains like Aspergillus Niger, Aspergillus terreus,

Penicillium notatum and Rhizopus stolonifer. Among the three extracts, methanol extract was found to have the significant activity followed by the chloroform extract against certain bacteria. Water extract did not have any activity against bacteria. Antifungal activity assessment indicated that the tested fungal strains are more susceptible to aqueous extract followed by methanol extract and chloroform extract[52].

- **Antifungal Activity:**

Ramasubramania Raja R et al. at 2011 was studied Antifungal activity of *A. bracteolata.* The ethanolic extracts of *A. bracteolata* using disc diffusion method and was found to have highest activity at minimum concentration. The study justify that the bioactive principles present in the extracts may be responsible in the treatment of ringworm infection. It was reported that Ethanolic extract effective against *Trichiophyton rubrum* and *Microsporum canis* [53].

Samia HA et al. at 2006 was evaluated and revealed that the Aristolochia Bracteata methanolic extract showed significant antibacterial and antifungal activity. The maximum zone of inhibition was against Bacillus subtilis (24 mm), Yersinia enterocholitica (22 mm) and Proteus vulgaris (20 mm) at the concentration of 5 mg/ml of extract. Methanolic extract showed MIC value of 250 Î¼g/ml against all the fungal pathogens. The methanol extract showed 78.27% of Î±-glucosidase inhibition[54].

- **Anti-plasmodial activity:**

Ahmed El-Tahir et al. at 1999 was evaluate the in vitro anti plasmodial activity against *Plasmodium falciparum* 3D7 (chloroquine sensitive) and Dd2 (chloroquine resistant and pyrimethamine sensitive) was investigated by Ramasubramania raja R *et al*. It was found that extract of A.bracteolata exerted activity on *P. falciparum* strain 3D7 with an IC_{50} less than 5 µg/mL. Phytochemical analysis indicated that the most active phase contained terpenoids and tannins and was devoid of alkaloids and saponins. The effect of plant

extracts on lymphocyte proliferation showed low toxicity to the human cells [55].

El-Hadi M et al. at 2010 was conducted another studies, shows that the plant extract of *A.bracteolata* has potent antimalarial activity (*in vitro*) against schizonts maturation of *Plasmodium falciparum*, the major human malaria parasite. The whole plant extracts of *Aristolochia bracteolata* produced 100% inhibition of the parasite growth at concentration ≤ 50 µg/ml. The two most active plants showed the presence of sterols, alkaloids and tannins [56].

- **Antioxidant properties:**

Thirugnanasampandan R et al. at 2008 was studied antioxidant and 2, 2-diphenyl picrylhydrazyl (DPPH) radical scavenging activities, reducing powers, and the amount of total phenolic compounds of the extracts on *A.bracteolata* [57].

Shahidi F et al. at 1992 was evaluating antioxidant activity of *A.bracteolata.* This antioxidative effect is mainly due to phenolic components, such as phenolic acids, and phenolic diterpenes[58].

Osawa T et al. at 1994 was revealed the antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [59].

Lee JC, Kim HR et al. at 2002 was determined the antioxidant activity by ammonium thiocyanate assay [60].

Blois MS et al. at 1951 2, 2-Diphenyl picrylhydrazyl (DPPH) free radicals scavenging activity was assessed according to Blois (1958)[61], with a slight weight modification. Absorbance was measured at 700 nm. Ascorbic acid solution was used for comparison. Increased absorbance of the reaction mixture indicated increased reducing power [61].

Kalpana Devi et al. at 2011 analysis of antioxidant property was carried out by two methods

i) Enzymatic method and ii) Non enzymatic method

i) Enzymatic assays are a) superoxide dismutase activity, b) catalase activity, c) peroxidase activity, d) ascorbate oxidase, e) glucose-6-phosphate-dehydrogenase activity.

ii) Non enzymatic assays are a) scavenging activity of DPPH, b) Determination of superoxide radical scavenging activity, c) Determination of reducing power. d) Hydroxyl radical scavenging activity, e) Total Phenol content, f) Ascorbic acid content. This Study is reveals that Aristolochia Bracteata plant is the source of natural antioxidant and some of the compounds have significant antioxidant property[62]

- **Antimicrobial Activity:**

Parekh J et al. at 2006 the antimicrobial activity of *A.bracteolata* was investigated by Parekh *et al*. The antimicrobial assay was done by both the agar disc and agar well diffusion method against six medically important microorganisms viz. Bacillus subtilis, Staphylococcus subfava, Alcaligenes fecalis, Proteus mirabilis, P. aeruginosa and Candida tropicalis. . According to the study, the methanol extract was found to be more effective than the aqueous extract [63].

Angalaparameswari S et al. at 2012 found that the aristolochic acid from the root of *Aristolochia bracteolata* poses significant antimicrobial activity. Aristolochic acid I was isolated from the methanolic & ethyl extract extracts of *Aristolochia bracteolata* and conformed through IR, NMR & MS. The percentage purity of aristolochic acid I was determined by UV & HPLC method. Antibacterial activity of extracts of *A.bracteolata* and the isolated compound was determined by disc diffusion method. Microbial assay of isolated compound (Aristolochic acid I) from ethyl acetate & ethanol extracts were shown good antimicrobial activity and the zone of inhibition of both at higher concentration 50µg/ml were similar with the standard aristolochic acid [64].

- **Wound healing activity:**

Kumar B et al. at 2005 was evaluated the wound healing properties of *A. bracteolata*. By the ethno botanical knowledge base for treatment of cuts and wounds which includes a usage of plants/plant extracts/decoctions or pastes, methods employed by tribals and folklore practices prevailing in India have been analysed [65].

Shirwaikar A et al. at 2003 the ethanol extract of the leaves of *Aristolochia bracteolata Lam.* was studied for its effect on wound healing in rats, using incision, excision and deadspace wound models, at two different dose levels of 400 and 800 mg/kg/body wt /day. The plant showed a definite, positive effect on wound healing, with a significant increase of the level of two powerful antioxidant enzymes, super oxide dismutase and catalase, in the granuloma tissue [66].

- **Anti angiogenetic activity:**

Marina G et al. at 2007 Petroleum ether extract of *Aristolochia bracteolata* Lam (Aristolochiaceae) roots was screened for the activity against cutaneous melanoma using Chicken Chorioallantoic Membrane (CAM) Assay has been studied. Angiogenesis and melanoma cell survival were visualized and recorded using dissecting microscope and imaging system. Concentrations of the extracts ranging from 10mg to 50mg were screened. Chemical tests of the extract revealed the presence of alkaloids, triterpenes and steroids. It was observed that 30mg dose notably reduced the proliferation of blood vessels and reduced survival rate of melanoma cells in CAM. Cultured melanoma cell lines were obtained from cancer research centre, Hyderabad. It was concluded that the root extract of *Aristolochia bracteolata* possess inhibitory effect on proliferation of melanoma cells and its topical application may be more advantageous to treat cutaneous melanoma, since topical treatments have advantages for rapid, effective and natural healing of cancers targeting the cancer site with much higher doses than could ever be achieved with oral treatments alone [67].

- **Trypansocidal effect:**

Samia HAR et al. at 2006 *Aristolochia bracteolata* was evaluated for its *in vivo* activity against trypanosome evansi infection in the rats. Six groups of 10 rats each aged 5-7 weeks, average weight 150grams were used. Both the chloroformic and methanolic extract of the plant extract was administered orally at dose rates of 250 and 500mg/kg BW. The activity was compared to cymelarsan which was given at a dose rate of 2.5mg/kg BW subcutaneously. Results showed that plant extract gave a promising trypansocidal effect. The chloroformic extract gave better result than that of the methanolic extract with both doses [68].

- **Anti-implantation & Abortifaciant activity:**

Sathish Kumar Muthureddy Nataraj et al. at 2007 Ethyl acetate soluble fraction of the ethanolic extract of *Aristolochia bracteolata* was tested for precoital & postcoital anti-implantation test & abortifacient activities in female albino rats. In the precoital study, the treatment at 20 & 40 mg/kg body weight showed significant & dose related anti-implantation & abortifaciant properties. In the post coital study of 20, 30 & 40 mg/kg body weight doses similar results were observed. The total anti fertility activity of 40 mg/kg body weight was found to be comparable to the standard ethinyl oestradiol given for the similar period [69].

Khare C. et al. at 2007 study was conducted in *A. bracteolata*. That reveals that leaves and fruit contain ceryl alcohol, aristolochic acid and beta-sitosterol. Aristolochic acid is insecticidal, poisonous, nephrotoxic. Leaf juice - vermifuge. Seeds - strong purgative. Products containing aristolochic acid are banned in the U.S., Canada, Great Britain, European countries and Japan. The seed compounds containing aristolochic acid and magnoflorine, induce contractions in the isolated uterus of pregnant rat and stimulate the isolated ileum of guinea pig. They also activate the muscarinic and serotonergic receptors in a

variety of organs. [70].

- **Antidote activity:**

Alagesaboopathi C. et al. at 2009 Field survey was undertaken in Kumaragiri Hills of Salem District of Tamilnadu, India in the topic of Ethnomedicinal plants and their utilization by villagers noted that Aristolochia bracteata Retz. (Aristolochiaceae). Vernacular name: Aduthinnapalai. Decoction of the whole plant mixed with castor oil, which cures fever and worms. Leaves paste applied externally in skin diseases and snake bite [71].

- **HEPATO-PROTECTIVE:**

Ratna manjula R et al. at 2011 The survey was carried out on Ethnomedicinal plants used to cure jaundice in Kammam District of Andhra Pradesh, India yielded 28 species belonging to 27 genera and 20 families used for curing jaundice by the aborigines of the district. Half of them are herbs Aristolochia bracteolata plant is one among that Twenty ml of leaf juice is administered twice a day for 5 days [72].

MATERIALS & METHODS

4.1 COLLECTION:

The Aadutheendapaalai (*Aristolchia bracteoalta*) were collected from land at Valayamadevi village, Cuddalore district.

4.1.1 AUTHENTICATION:

The Herbal drug Aadutheendapaalai is identified and authenticated by Assistant Professor, Department of Medicinal Botany, National Institute of Siddha, Chennai-47.

4.1.2 PREPARATION OF TEST DRUG:

SELECION OF TEST DRUG:

The test drug Aadutheendapaalai ver was selected for the evaluation of toxicity studies in Wister albino rats.

INGREDIENTS:

1. Aadutheendapaalai ver (Root of *Aristolchia bracteoalta*)

Figure 2: Root of Aadutheendapaalai ver

METHOD OF PURIFICATION AND PREPARATION:

Decayed parts or the mud sticking to the Aadutheendapaali are removed and then cut the root from whole plant. Due to purification again the root is allow to soak in water, wash well and dried in sunshade. Then it was grounded well to obtain fine powder. After this process, the dried powder was sieved through white cloth and it was stored in an air tight container and it was labelled as *Aadutheendapaalai ver Chooranam.*

THERAPEUTIC DETAILS OF AAGASAGARUDAN KIZHANGU CHOORANAM:

Form of the Medicine: Chooranam (Powder)
Route of Administration: Enteral
Clinical dose: Half to One Varaagan (2-4 grams)
Adjuvant: water
Indication:

Toxic bite (Nanju kadigal)
Intestinal worms (Kudarpuzhu)
Skin diseases (Pandri mamisa thol pondra padai)

4.2 QUALITATIVE ANALYSIS

4.2.1PHYSICO CHEMICAL ANALYSIS

The *Aadutheendapaalai ver chooranam* was studied by physicochemical parameters. This study was done at Noble research institute Chennai-600032.

1. Loss on drying of the sample at 105ºC

4g of test drug was weighed in a previously weighed 100ml beaker and heated in an oven at 105ºC for 5hours. Cooled in a dessicator and weighed. Repeated the procedure till constant weight was obtained. The percentage loss in weight of the test drug was calculated by the following formula.

Calculation:

$$\text{Percentage of loss on drying at } 105^{\circ}C = \frac{\text{Loss in weight of test drug}}{\text{Weight of test drug taken}} \times 100$$

2. Ash content

a. Total ash content

4g of test drug was weighed accurately in a previously ignited and tarred silica dish. The material was evenly spread and ignited in a muffle furnace at 400^{0}C until it became white indicating the absence of carbon. The dish was cooled in a desiccator and weighed. As carbon free ash cannot be obtained in this manner, the dish was cooled and the residue moistened with sufficient quantity of water. Dried on a water bath and then ignited in the electric furnace to get the constant weight. Cooled the dish in a desiccator and then weighed. The percentage of total ash of air-dried materials was calculated as per the formula given below.

Calculation:

$$\text{Percentage of total ash} = \frac{\text{Weight of the ash}}{\text{Weight of test drug taken}} \times 100$$

b. Acid-insoluble ash

The total ash of the test drug was found out as described above. To the dish containing the total ash was added 25 ml of dilute hydrochloric acid. Boiled gently for 6 minutes and filtered. Collected the insoluble matter on an ash less

filter paper and washed with distilled water until the residue was free from acid. Transferred the filter paper containing the insoluble matter to the original dish. Dried and ignited to the constant weight. Cooled the dish in a desiccator, and then weighed. Calculated the percentage of acid-insoluble of the air-dried material by the given following formula

Calculation:

$$\text{Percentage of acid-insoluble ash} = \frac{\text{Weight of the acid-insoluble residue}}{\text{Weight of test drug taken}} \times 100$$

i. Extractive of the test drug

a. Water-soluble extractive of the test drug

4 g of the test drug was weighed accurately in a glass stoppered flask. Add 100 ml of distilled water and shakened occasionally for 6 hours and then allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent and pipetted out 25 ml of the filtrate in a reweighed 100 ml beaker and evaporated to dryness on a water bath. Kept in an air oven at 105ºC for 6 hours. Cooled in a dessicator and weighed. Repeated the experiment twice, and taken the average value. The percentage of water soluble extractive was calculated by the formula given below.

Calculation:

$$\text{Percentage of water soluble extract} = \frac{\text{Weight of the extract}}{\text{Weight of sample taken}} \times \frac{100}{25} \times 100$$

b. Alcohol-soluble extractive of the sample

4 g of the sample was weighed accurately in a glass stoppered flask. Added 100 ml of distilled alcohol (approximately 95%) and shaken occasionally for 6 hours

and then allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent and pipetted out 25 ml of the filtrate in a pre-weighed 100 ml beaker and evaporated to dryness on a water bath. Kept in an air oven at 105ºC for 6 hours and cooled in a desiccator and weighed. Repeated the experiment twice, and taken the average value. The percentage of alcohol soluble extractive was calculated by the formula given below.

Calculation:

$$\text{Percentage of alcohol soluble extract} = \frac{\text{Weight of the extract}}{\text{Weight of sample taken}} \times \frac{100}{25} \times 100$$

ii. Determination of pH

The pH of the Aadutheendapaalai ver was estimated as per the method prescribed in the Indian standard (IS) - 6940(1982). One gram of the test drug was taken into a 100ml graduated cylinder containing about 50 ml of water. The cylinder was shaken vigorously for two minutes and the suspension was allowed to settle for hour at 25ºC to 27ºC, then 25 ml of the clear aqueous solution was transferred in to a 50 ml beaker and tested for pH using digital pH meter[73,74].

4.2.2 BIO-CHEMICAL ANALYSIS:

The bio-chemical analysis of **Aadutheendaapaalai ver chooranam** was done at Biochemistry lab, National Institute of Siddha, Chennai-47.

Experimental procedures of Chemical analysis

S.No	EXPERIMENT	OBSERVATION	INFERENCE
1.	Appearance of sample	Pale brown in color	

2.	**Test for Solubility:** a. A little (500mg) of the sample is shaken well with distilled water. b. A little (500mg) of the sample is shaken well with con. HCl/Con. H2So4	Sparingly soluble Completely soluble	Presence of Silicate Absence of Silicate
3.	**Action of Heat:** A small amount (500mg) of the sample is taken in a dry test tube and heated gently at first and then strong.	White fumes evolved	Presence of Carbonate
4.	**Flame Test:** A small amount (500mg) of the sample is made into a paste with Con.HCl in a watch glass and introduced into non-luminous part of the Bunsen flame.	Bluish green flame not appeared	Absence of Copper

Preparation of Extract:

5gm of ***Aadutheendaapaalai ver chooranam*** is weighed accurately and placed in

a 250ml clean beaker and 50ml of distilled water was added with it. Then it was boiled well for about 10 minutes. Then it was allowed to cool and filtered in a 100ml volumetric flask and made up to 100ml with distilled water [73]

Experimental procedures of Biochemical analysis

S.No	EXPERIMENT	OBSERVATION	INFERENCE
I.	**Test For Acid Radicals**		
1	**Test For Sulphate:** 2ml of the above prepared extract was taken in a test tube and 2ml of 4% dil. ammonium oxalate solution was added	No Cloudy appearance	Absence of Sulphate
2	**Test For Chloride:** 2ml of the above prepared extracts was added with 2ml of dil-HNO3 until the effervescence ceases off. Then 2 ml of silver nitrate solution was added.	No Cloudy appearance	Absence of Chloride
3	**Test For Phosphate:**		

	2ml of the extract was treated with 2ml of con.HNo3 and 2ml of dil.ammonium molybdate solution.	No Yellow precipitate	Absence of Phosphate
4	**Test For Carbonate:** 2ml of the extract was treated with 2ml dil. magnesium sulphat solution	Cloudy appearance present	Presence of Carbonate
5	**Test For Nitrate:** 1ml of the substance was heated with copper turning and concentrated H2SO4 and viewed the test tube vertically down.	Brown gas was not evolved	Absence of Nitrate
6	**Test For Sulphide:** 1ml of the substance was treated with 2ml of con. HCL	No Rotten Egg Smelling gas was evolved	Absence of Sulphide
7	**Test For Fluoride & Oxalate:** 2ml of extract was added with 2ml of dil. Acetic acid and 2ml dil.calcium chloride solution and heated**.**	No Cloudy appearance	Absence of fluoride and oxalate

8	**Test For Nitrite:** 3drops of the extract was placed on a filter paper, on that-2 drops of dil.acetic acid and 2 drops of dil.Benzidine solution were placed.	Characteristic changes not appeared	Nitrite absent
II. Test For Basic Radicals			
1.	**Test For Lead:** 2ml of the extract was added with 2ml of dil.potassium iodine solution.	No Yellow Precipitate is obtained.	Absence of Lead
2.	**Test For Copper:** One pinch (50mg) of substance was made into paste with con. HCl in a watch glass and introduced into the non-luminuous part of the flame.	No Blue colour precipitate formed.	Absence of Copper
3.	**Test For Aluminium:** To the 2ml of extract dil.	No characteristic	Absence of

	sodium hydroxide was added in 5 drops to excess.	changes	Aluminium
4.	**Test For Iron:** a.To the 2ml of extract add 2ml of ammonium thiocyanate solution. b.To the 2ml of extract 2ml ammonium thiocyanate solution and 2ml of con HN03 is added	Presence of mild red color appearance Presence of mild Red colour was formed	Presence of Iron Presence of Iron
5.	**Test For Zinc:** To the 2ml of the extract dil.sodium hydroxide solution was added in 5 drops to excess and dil.ammonium chloride was added.	No White precipitate formed	Absence of Zinc
6.	**Test For Calcium:** 2ml of the extract was added with 2ml of 4% dil.ammonium oxalate solution	Cloudy appearance was formed	Presence of Calcium

7.	**Test For Magnesium:** 2ml of the extract dil.sodium hydroxide solution was added in drops to excess.	white precipitate not formed	Magnesium absent
8.	**Test For Ammonium:** To 2ml of the extract 1 ml of Nessler's reagent and excess of dil.sodium hydroxide solution were added.	Brown colour formed	Presence of Ammonium
9.	**Test For Potassium:** A pinch (25mg) of substance was treated with 2ml of dil.sodium nitrite solution and then treated with 2ml of dil.cobalt nitrate in 30% dil.glacial acetic acid.	Mild Yellowish red precipitate formed	Presence of Potassium
10.	**Test For Sodium:** 2 pinches (50mg) of the substance was made into paste	Yellow colour flame	Sodium absent

	by using HCl and introduced into the blue flame of Bunsen burner.	not appeared	
11.	**Test For Mercury:** 2ml of the extract was treated with 2ml of dil.sodium hydroxide solution.	Yellow precipitate not formed	Mercury absent
12.	**Test For Arsenic:** 2ml of the extract was treated with 2ml of dil.sodium hydroxide solution.	Brownish red precipitate not formed	Arsenic absent

III. Other constituents			
1.	**Test For Starch :** 2ml of the extract was treated with weak dil.iodine solution	Blue colour developed	Presence of Starch
2.	**Test For Reducing Sugar:** 5ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2	The was no specific change in colour	Absence of Reducing sugar

	minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes.		
3.	**Test For The Alkaloids**: a) 2ml of the extract is treated with 2ml of dil.potassium lodide solution. b) 2ml of the extract is treated with 2ml of dil.picric acid	Reddish brown precipitation formed Yellow precipitation formed	Presence of Alkaloid Presence of Alkaloid
4.	**Test For Tannic Acid:** 2ml of extract was treated with 2ml of dil.ferric chloride solution.	No Black precipitate obtained	Tannic acid absent
5.	**Test For Unsaturated Compound:** To the 2ml of extract 2ml of dil.Potassium permanganate solution was added.	Potassium permanganate was not decolourised	unsaturated compounds absent

6.	**Test For Amino Acid:** 2 drops of the extract was placed on a filter paper and dried well, then 20ml of Biurette reagent was added in it.	Violet colour not developed	Amino acids absent
7.	**Test For phenols:** 2ml of the extract was treated with 2 ml of dil.ferric chloride solution.	No specific colour formation	Phenols absent

4.2.3 PHYTOCHEMICAL ANALYSIS

Test for alkaloids:

Mayer's Test: To the test sample, 2ml of mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

Test for coumarins:

To the test sample, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow color.

Test for saponins:

To the test sample, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of Saponins.

Test for tannins:

To the test sample, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

Test for glycosides- Borntrager's Test

Test drug is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of choloroform is added and shaken, choloroform layer is separated and 10% ammomia solution is added to it. Pink colour indicates presence of glycosides.

Test for flavonoids:

To the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Appearance of yellow color indicates the presence of Flavonoids.

Test for phenols:

Lead acetate test: To the test sample; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Test for steroids:

To the test sample, 2ml of chloroform was added with few drops of conc. Sulphuric acid (3ml), and shaken well. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Triterpenoids

Liebermann–Burchard test: To the chloroform solution, few drops of acetic anhydride was added then mixed well. 1 ml concentrated sulphuric acid was added from the sides of the test tube, appearance of red ring indicates the presence of triterpenoids.

Test for Cyanins

A. Aanthocyanin:

To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100^{o}C. Formation of bluish green colour indicates the presence of anthocyanin.

Test for Carbohydrates - Benedict's test

To the test sample about 0.5 ml of Benedic's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

Proteins (Biuret Test)

To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins [75].

5. QUANTITATIVE ANALYSIS

5.1.1 ANALYSIS OF PHYTOCOMPOUNDS

The analysis of Phytocompounds is performed by using Thin layer chromatography (TLC), High performance thin layer chromatography. The Experimental Procedure was done at Noble Research Solution, Chennai

TLC Analysis

Test sample was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Micro pipette were used to spot the sample for TLC applied sample volume 10-micro liter by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber

with the specified solvent system after the run plates are dried and was observed using visible light Short-wave UV light 254nm and light long-wave UV light 365 nm [76].

High Performance Thin Layer Chromatography Analysis

HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. Thus this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of phyto therapeutics.

Chromatogram Development

It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analyzed. After elution, plates were taken out of the chamber and dried.

Scanning

Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phyto constituents present in each sample and their respective Rf values were tabulated [77].

5.1.2 PESTICIDE RESIDUAL

Extraction

Test sample were extracted with 100 ml of acetone and followed by homogenization for brief period. Further filtration was allowed and subsequent addition of acetone to the test mixture. Heating of test sample was performed using a rotary evaporator at a temperature not exceeding 40ºC until the solvent has almost completely evaporated. To the residue add a few milliliters of toluene and heat again until the acetone is completely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter[78,79]

5.1.3 STERILITY TEST BY POUR PLATE METOD

Objective

The pour plate techniques were adopted to determine the sterility of the product. Contaminated / un sterile sample (formulation) when come in contact with the nutrition rich medium it promotes the growth of the organism and after stipulated period of incubation the growth of the organism was identified by characteristic pattern of colonies. The colonies are referred to as Colony Forming Units (CFUs).

Methodology

Test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45^{o}C were added. Agar and sample were mixed thoroughly by tilting and swirling the dish. Agar was allowed to completely gel without disturbing it. (about 10 minutes). Plates were then inverted and incubated at 37^{o} C for 24-48 hours and further extended for 72 hrs for fungal growth observation. Grown colonies of organism was then counted and calculated for CFU.

5.1.4 Aflatoxin Assay by TLC (B1, B2, G1, G2)

Standard:

Aflatoxin B1

Aflatoxin B2

Aflatoxin G1

Aflatoxin G2

Solvent:

Standard samples were dissolved in a mixture of chloroform and acetonitrile (9.8: 0.2) to obtain a solution having concentrations of 0.5 µg per ml each of aflatoxin B1 and aflatoxin G1 and 0.1 µg per ml each of aflatoxin B2 and aflatoxin G2.

Test solution: Concentration 1 µg per ml

Procedure

Standard aflatoxin was applied on to the surface to pre coated TLC plate in the volume of 2.5 µL, 5µL, 7.5 µL and 10 µL. Similarly the test sample was placed and Allow the spots to dry and develop the chromatogram in an unsaturated chamber containing a solvent system consisting of a mixture of chloroform, acetone and isopropyl alcohol (85: 10: 5) until the solvent front has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent front and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm [80].

6. Toxicity Studies of *Aadutheendapaalai Ver Chooranam*

To evaluate the safety profile of *Aadutheendapaalai ver chooranam* with acute and sub-acute toxicity study carried out as per OECD Test Guidelines 423 & 407.

Principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved the use of animals and the study design. Institutional Animal Ethical Committee number: (IAEC). (NIS/IAEC-VII/28082018/16) dated 28.8.2018.

1. Acute Toxicity Study of *Aadutheendapaalai Ver Chooranam*

Species: Wister albino Rats

Sex: Male and Female

Age/weight at start of test: 6 weeks/140-160g b.wt

Acclimatization Period: 7 days prior to dosing

Housing: Polypropylene cages

Husbandry: 12-h light/12-h dark artificial photoperiod/ Room temperature 22°C (±3°) and relative humidity 30–70%

Feed and Water: Rodent pelleted feed RO purified water *ad libitum*

Identification: Animals will be kept in individual cages and numbered

Experimentation Details of Acute Toxicity Study:

Groups/Treatment regimen: Grouped by randomization

Test Guideline: OECD-423

Length of exposure to test substance: Once single dose

No of Animals: 3 Female/ group

Control group: Vehicle (water)

Test groups: 2000 mg/kg.b.wt

The Female Albino Rats of weighing 150-200g were obtained from authorized animal breeders of the animal laboratory in TANUVAS, Madhavaram, and Chennai and stocked in the animal house at National Institute of Siddha,

Chennai. Animals were housed in a cage at 22°C ±3°C and relative humidity 30–70% and have free access to standard rat pellet diet (Sai Meera Foods Pvt. Ltd., Bangalore). The animals were treated with ***Aadutheendapaalai ver chooranam*** by oral route for one day and monitored for behavioral parameters for the first 4 hours (1/2 hr, 1hr, 2 hr, 3 hr,4 hr) after drug administration. Body weight of the animal will be monitored at weekly intervals. The animals that the die within this period will be subjected to necropsy. Remaining animals will be weighed and sacrificed under the injection of ThioPentothal Sodium on the 15th day of the Study period. The toxicological effect was assessed on the basis of mortality.

Preparation of Test Drug Doses:

Groups	Test drug	No. of Rats
Group I:	Vehicle control (Water)	3Female
Group II:	Test drug (AVC) – 2000 mg/kg b.wt	3 Female

Route of administration

Oral route was selected because it is the normal route of clinical administration.

Administration of Dose

The animals were fasted (only food was withheld) for 12hrs and weighed prior to dosing. Three animals were used for each step. A single dose of the solution (2000mg/kg) was consecutively administered by oral gavage using intubation cannula. The food was withheld for another 4hrs after dosing and administration of the drug. As per the guideline, the starting dose level was taken as 2000 mg/kg body weight.

Observations:

Observations were made and recorded systematically and continuously observed after the substance administration as per the guidelines.

- ✓ ½ hour, 1 hour, 2 hours, 4 hours and up to 24 hours observation
- ✓ All rats were observed twice daily for 14 days
- ✓ Body weight were Calculated weekly once
- ✓ Feed & water intake were Calculated daily

Cage side observation

The animals were monitored for behavioral parameters like Alertness, Aggressiveness, piloerection, Grooming, Gripping, Touch Response, Motor Activity, Tremors, Convulsions, Muscle Spasm, Catatonia, Muscle relaxant, Hypnosis Analgesia, Lacrimation, Exophthalmos, Diarrhea, Writhing, Respiration, Mortality

Gross necropsy:

At the end of the 14th day, all the animals were sacrificed by using the injection of Pentothal sodium Gross necropsy includes examinations of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents. Brain,eye, lungs, heart, spleen, liver, kidneys, adrenals, uterus, of all animals.

2. Repeated Dose 28-Day Oral Toxicity Study of *Aadutheendapaalai Ver Chooranam*

Experimental Animals:

Species: Wistar Albino Rats

Sex: Male and Female

Age/weight at start of test: 6 weeks/140-160g b.wt

Acclimatization Period: 7 days prior to dosing

Housing: Polypropylene cages with bedding with Husk

Husbandry: 12-h light/12-h dark cycle/

Room temperature: 22°C ± 3°C and

Relative humidity: 30–70%

Feed and Water: Rodent pelleted feed

RO purified water ad libitum

Identification: Animals will be kept in Polypropylene cages and Numbered

Experimentation Details of Repeated dose 28 days Toxicity Study:

Groups/Treatment regimen: Grouped by randomization

Test Guideline: OECD-407

Length of exposure to test substance: 28 days

No of Animals: 5 Female+5 Male / group

Control group: Vehicle (Water)

Test groups: Aadutheendapaalai ver chooranam (Control, Low dose, Mid dose, High dose)

The 40 Wistar albino rats of both sexes selected randomly. The animals were divided into four groups. Each group consists of 5 animals. The first group treated as vehicle control and second, third, fourth group were treated with Aadutheendapaalai ver chooranam Low-dose, Mid dose, High-dose respectively. The control animals were administered with water as a vehicle. The other animals treated with ***Aadutheendapaalai ver chooranam*** which was mixed with water at the dose levels of Low dose 360mg/ kg b.wt, Mid dose720 mg/kg b.wt and High dose 1440 mg/kg b.wt For 28 days. The administration was given by oral, once daily for 28 consecutive days. The animals were observed the behavioural parameters for the study period. Body weight of the animal was being monitored at weekly intervals. Food & water intake were Calculated daily. All the animals were sacrificed at the end of the study (28 days) by using the injection of

Pentothal Sodium. Blood was collected from the anesthetized animals from the abdominal aorta for the following investigations like Haematology, Biochemical analysis. Gross pathological changes were monitored the animals and then the organs were studied by histopathological examination. The doses (Low, Mid, High dose) were fixed from the result from the acute toxicity study

Groups	Test drug	No.of Rats
Group I	Vehicle control (water)	10(5M+ 5F)
GroupII	Test drug (AVC)- low dose(360 mg/Kg b.wt)	10(5M + 5F)
GroupIII	Test drug(AVC) - Mid dose (720 mg/Kg b.wt)	10(5M +5 F)
GroupIV	Test drug(AVC) High dose (1440 mg/Kg b.wt)	10(5M +5 F)

Observations:

Experimental animals were kept under observation throughout the course of study for the following

- ✓ All rats were observed twice daily for 28 days
- ✓ Body weight were Calculated weekly once
- ✓ Feed & water intake were Calculated daily

Cage side observation

The animals were monitored for behavioral parameters like, Alertness, Aggressiveness, piloerection, Grooming, Gripping, Touch Response, Motor Activity, Tremors, Convulsions, Muscle Spasm, Catatonia, Muscle relaxant, Hypnosis, Analgesia, Lacrimation, Exophthalmos, Diarrhea, Writhing, Respiration, Mortality.

Laboratory Investigations:

On the 29th day, the animals were fasted overnight, then anesthetized to collect blood samples from the abdominal aorta in two tubes: one with EDTA for hematological parameters, another one without any anticoagulant and was centrifuged at 4000 rpm at 4°C for 10 minutes to obtain the serum for biochemical parameters.

Hematological Investigations:

Blood samples of control and experimental rats were analyzed for haemoglobin (Hb), total red blood corpuscles (RBC), white blood corpuscles (WBC) count, Platelet, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), were calculated by auto analyzer.

Biochemical Investigations:

Serum samples of control and experimental animals were analyzed for, Bilirubin, BUN, Creatinine, Triglyceride, Total Cholesterol, HDL, LDL, VLDL, using standard methods. Activities of glutamate oxaloacetate transaminase/ Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine aminotransferase (GPT/ALT) were estimated as per the colorimetric procedure.

Necropsy:

All the animals were sacrificed on the 29th day and sub-acute group were sacrificed on after 28 days. Gross necropsy includes examinations of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents. Brain, eye, lungs, heart, spleen, liver, kidneys, adrenals, sex organs, of all animals were recorded.

Histopathology:

The organs included liver, kidneys, spleen, brain, heart, lungs and stomach of the animals were preserved, and they were subjected to histopathological examination. Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of all the animals (low, mid, high) a n d satellite group were preserved and fixed in 10% formalin for 24 hrs. Samples were dehydrated in an auto technic and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin 50^0C and then in a cubical block of paraffin made by the "L" molds. It was followed by microtome and the slides were Prepared then stained with Haematoxylin-eosin.

Statistical analysis:

Findings such as body weight changes, food consumption, water intake, hematology and biochemical analysis were subjected to One-way ANOVA Dunnet's test using a computer software program followed by D *Graph Pad Instat-3*

RESULTS

The prepared drug analyzed by various analytical procedures and studied by acute and sub-acute toxicity studies through OECD guidelines. The results are given by the following tabulation, graphs and microscopical slides.

RESULTS

The prepared drug analyzed by various analytical procedures and studied by acute and long term toxicity studies through OECD guidelines. The results are given by the following tabulation, graphs and microscopical slides.

4.1 Qualitative Analytical Studies on *Aadutheendaapaalai Ver Chooranam*

4.1.1 Physico-chemical Analysis

Figure 3: Colour and Nature of Aadutheendaapaalai ver chooranam (AGC)

State	Solid
Nature	Pale whitish brown
Odor	Mild
Touch	Dry
Flow Property	Free flowing
Appearance	Pale whitish brown

Table 1: Physico-chemical properties of Aadutheendapaalai ver chooranam (APC)

S.no	Parameter	Mean (n=3) SD
1.	Loss on Drying at 105^0C (%)	7.867 +/- 0.8327
2.	Total Ash (%)	8.9 +/- 0.2646
3.	Acid Insoluble Ash (%)	0.2733 +/- 0.04619
4.	Water Soluble Extractive (%)	11.57 +/- 6.825
5.	Alcohol Soluble Extractive (%)	3.6 +/- 2.234

4.1.2 Biochemical Analysis

Biochemical analysis of Aadutheendaapali ver chooranam (AGC)

The bio-chemical analysis of **Aadutheendaapaalai ver chooranam** was done at Biochemistry lab, National Institute of Siddha, Chennai-47.

Table 2: Biochemical analytical report of Aadutheendapaalai ver chooranam (APC)

S.NO	I. TEST FOR RADICALS	RESULT
1	Test for Sulphate	+
2	Test for Chloride	-
3	Test for Phosphate	+
4	Test for Carbonate	+
5	Test for Nitrate	-

6	Test for Sulphide	+
7	Test for Fluoride & oxalate	-
8	Test for Nitrite	-
9	Test for Borate	-

S.NO	III. MISCELLANEOUS	RESULT
1	Test for Starch	+
2	Test for Reducing sugar	-
3	Test for the Alkaloids	+
4	Test for Tannic Acid	-
5	Test for Unsaturated compound	-
6	Test for Amino acid	+

S.NO	II. TEST FOR RADICALS	RESULT
1	Test for Lead	-
2	Test for Copper	-
3	Test for Aluminium	+
4	Test for Iron	+
5	Test for Zinc	+
6	Test for Calcium	+
7	Test for Magnesium	-
8	Test for Ammonium	-
9	Test for Potassium	-
10	Test for Sodium	-
11	Test for Mercury	-
12	Test for Arsenic	-

RESULTS OF BIOCHEMICAL ANALYSIS

The Biochemical analysis shows the presence of Sulphate, Phosphate, Carbonate, Sulphide, Iron, Calcium, Zinc, Aluminum, Starch, Alkaloids, Amino acid in Aadutheendapalai ver Chooranam.

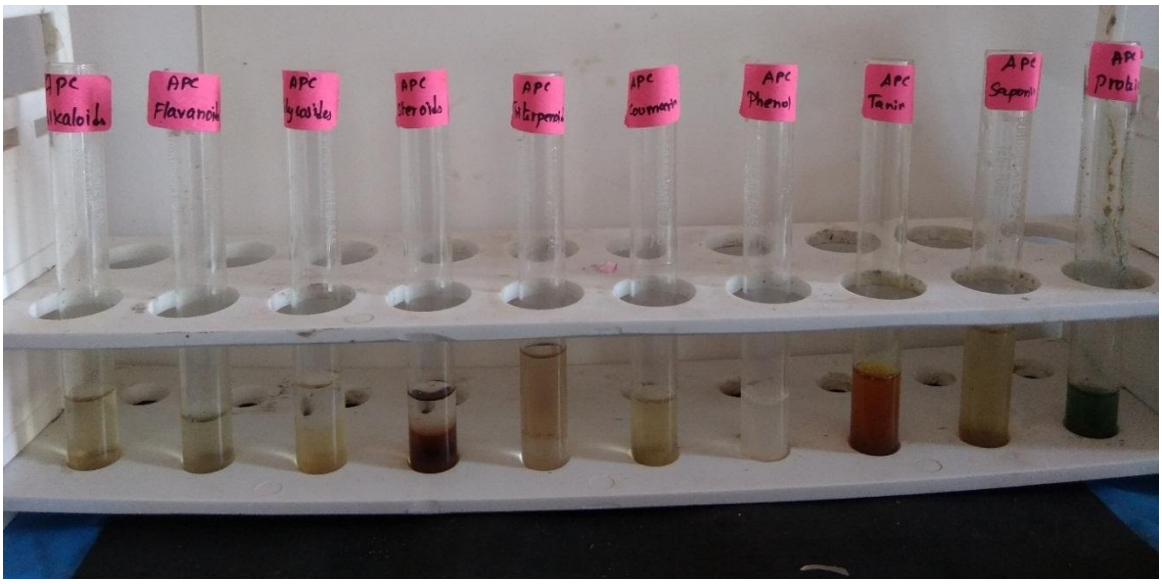
4.1.3 Phytochemical Analytical Report

Table 3: Phyto-chemical Analytical report of Aadutheendapaalai ver chooranam (APC)

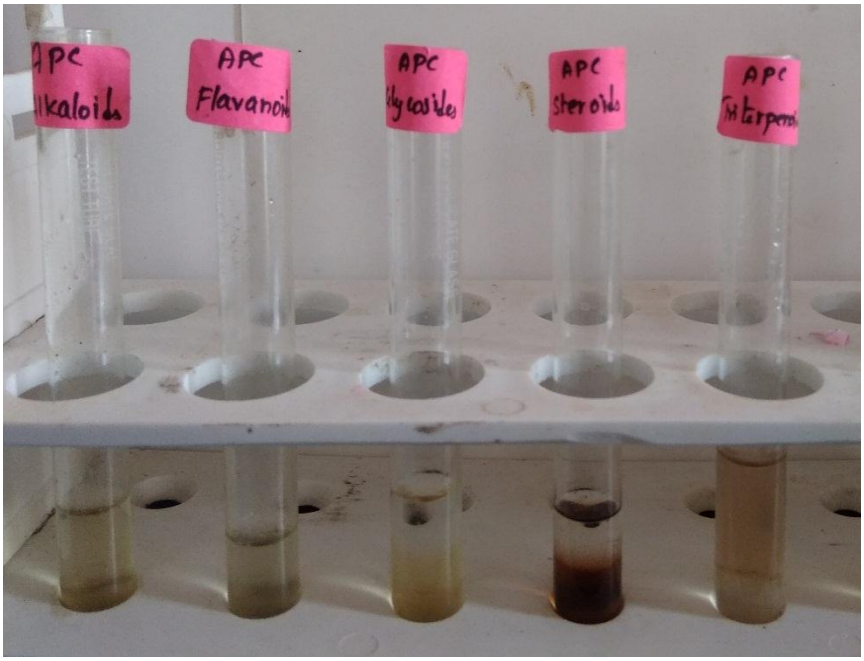
S.NO	TEST	OBSERVATION
1	**ALKALOIDS**	+
2	**FLAVANOIDS**	_
3	**GLYCOSIDES**	_
4	**STEROIDS**	+
5	**TRITERPENOIDS**	+
6	**COUMARIN**	_
7	**PHENOL**	+
8	**TANIN**	+
9	**PROTEIN**	_
10	**SAPONINS**	+
11	**SUGAR**	+
12	**ANTHOCYANIN**	_
13	**BETACYANIN**	_

+ -> Indicates Positive and - -> Indicates Negative

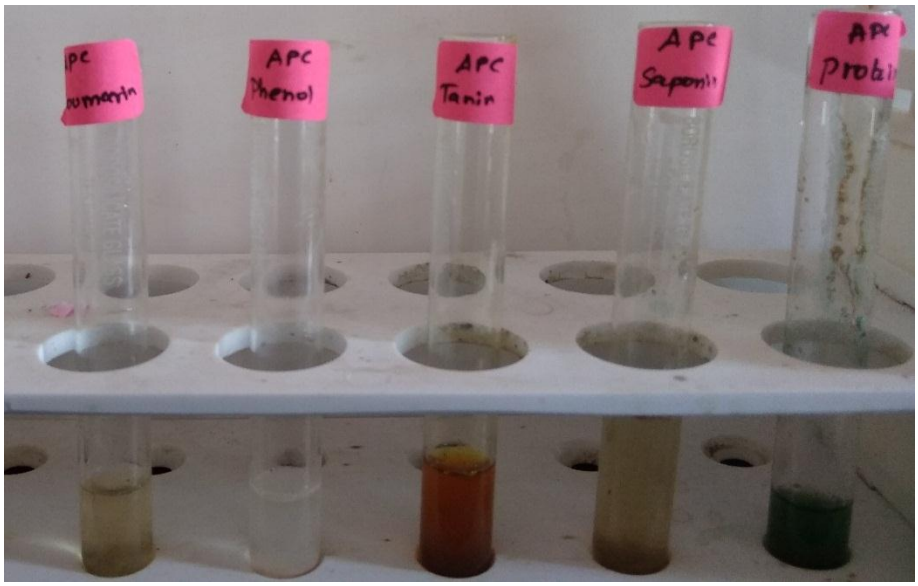
Qualitative Phytochemical Investigation



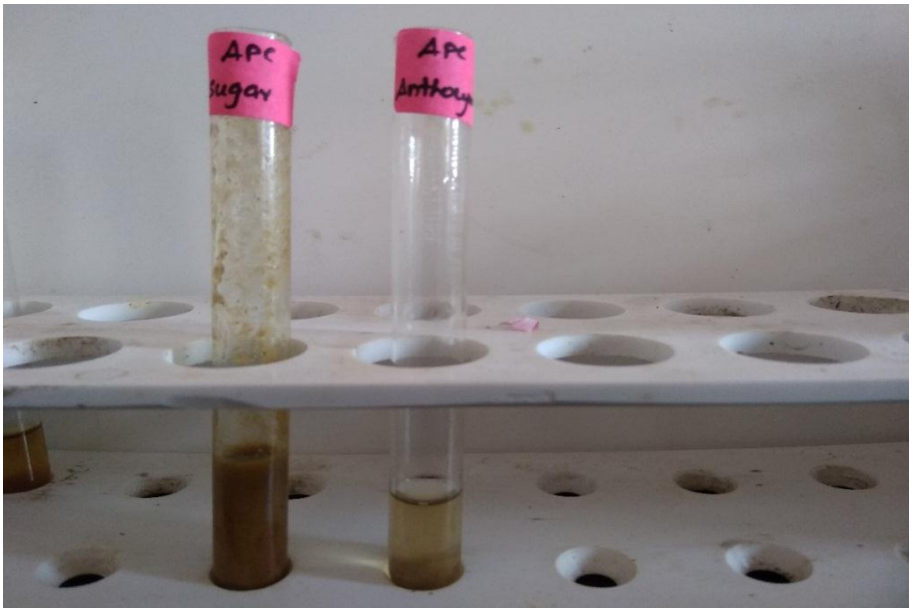
Test for Alkaloids, Flavonoids, Glycosides, Steroids and Triterpenoids



Test for Coumarin, Phenol, Tanins, Saponin, Proteins



AnthoCyanin and carbohydrates

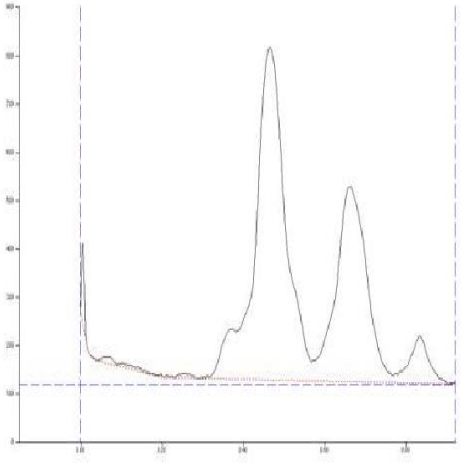


5. QUANTITATIVE ANALYSIS

5.1.1 ANALYSIS OF PHYTOCOMPOUNDS

TLC Visualization of APC - TLC plate visualization at 366

HPTLC finger printing of Sample APC



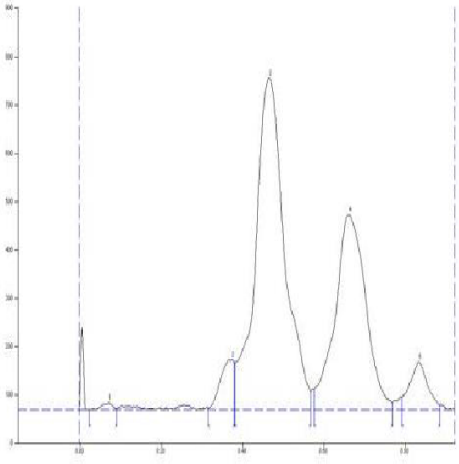


Table 4: HPTLC Peak table of Aadutheendapaalai ver chooranam (APC)

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.02	0.7	0.07	15.2	1.16	0.09	2.3	302.8	0.46
2	0.32	4.3	0.37	104.4	7.97	0.38	98.4	2626.3	3.97
3	0.38	98.5	0.47	686.7	52.44	0.57	40.5	36998.3	55.89
4	0.58	43.5	0.66	404.2	30.87	0.77	15.5	22985.0	34.72
5	0.79	25.5	0.84	99.0	7.56	0.89	10.9	3288.9	4.97

REPORT

HPTLC finger printing analysis of the sample reveals the presence of five prominent peaks corresponds to presence of five versatile phyto components present with in it.

Rf value of the peaks ranges from 0.02 to 0.79. Further the peak 3 occupies the major percentage of area of 55.89 which denotes the abundant existence of such compound.

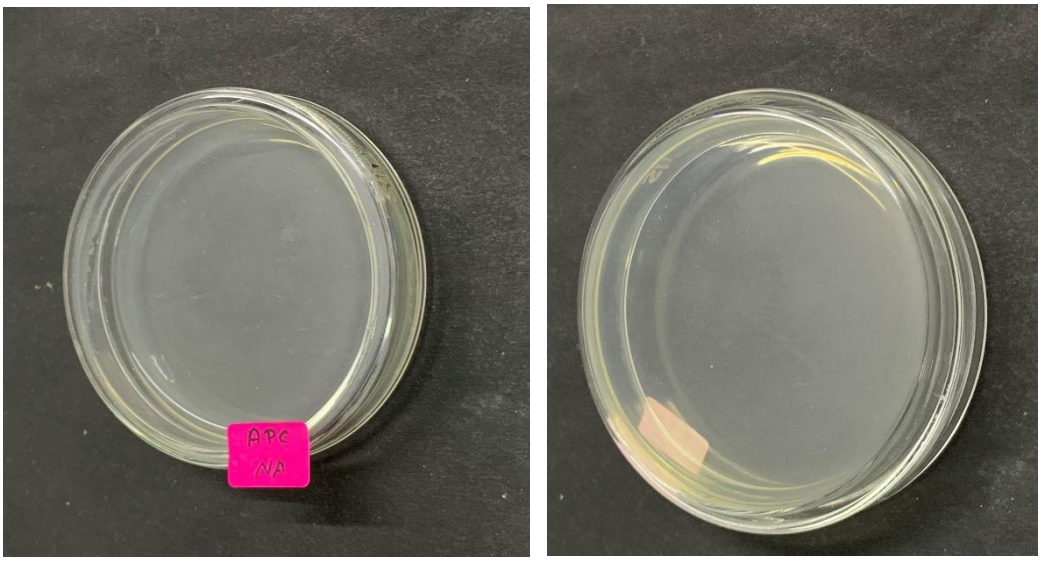
5.1.2 PESTICIDE RESIDUAL

Table 5: Pesticide residual report of Aadutheendapaalai ver chooranam (APC)

Test Result Analysis of the Sample APC Pesticide Residue	**Sample APC**	**AYUSH Limit (mg/kg)**
I.Organo Chlorine Pesticides		
Alpha BHC	BQL	0.1mg/kg
Beta BHC	BQL	0.1mg/kg
Gamma BHC	BQL	0.1mg/kg
Delta BHC	BQL	0.1mg/kg
DDT	BQL	1mg/kg
Endosulphan	BQL	3mg/kg
II.Organo Phosphorus Pesticides		
Malathion	BQL	1mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorovos	BQL	1mg/kg
III. Organo carbamates		
Carbofuran	BQL	0.1mg/kg
III.Pyrethroid		
Cypermethrin	BQL	1mg/kg

The results showed that there were no traces of pesticides residues such as Organo chlorine, Organo phosphorus, Organo carbamates and pyrethroids in the sample provided for analysis.

5.1.3 STERILITY TEST BY POUR PLATE METOD



Observation

No growth was observed after incubation period. Reveals the absence of specific pathogen

Result

No growth / colonies were observed in any of the plates inoculates with the test sample.

Table 6: Sterlity Test report of Aadutheendapaalai ver chooranam (APC)

Test	**Result**	**Specification**	**As per AYUSH/WHO**
Total Bacterial Count	Absent	NMT 10^5CFU/g	As per AYUSH specification
Total Fungal Count	Absent	NMT 10^3CFU/g	

5.1.4 Aflatoxin Assay By TLC (B1, B2, G1, G2)

Table 7: Aflatoxin report of Aadutheendapaalai ver chooranam (APC)

Aflatoxin	Sample APC	AYUSH Specification Limit
B1	Not Detected - Absent	0.5 ppm
B2	Not Detected - Absent	0.1 ppm
G1	Not Detected - Absent	0.5 ppm
G2	Not Detected - Absent	0.1 ppm

The results shown that there was no spots were been identified in the test sample loaded TLC plates when compare to the standard, which indicates that he sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2.

6. Acute Oral Toxicity Study of Aadutheendapaalai Ver Chooranam

Acute toxicity study carried out as per OECD guidelines, there were no treatment related death or signs of toxicity developed in wister albino rats at dosage of 10 times the therapeutic dose (2000 mg/kg b.wt) throught out the study period. Further, no gross pathological changes have been seen in the internal organs of both control and treated groups.

S. No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	Control	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	Test group therapeutic dose (2000 mg/b.wt)	+	-	-	+	+	+	--	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 6: Effect of Aadutheendapaalai ver chooranam on behavioural signs of acute toxicity study.

1.Alertness, 2.Aggressiveness, 3. Pile erection, 4.Grooming, 5.Gripping, 6.Touch Response, 7.Motor Activity, 8.Tremors, 9.Convulsions, 10.Muscle Spasm, 11.Catatonia, 12.Muscle relaxant, 13.Hypnosis, 14.Analgesic, 15.Lacrimation, 16.Exophthalmos, 17. Diarrhea, 18.Writhing, 19.Respiration, 20.Mortality.

+ Presence of Activity - Absence of Activity

1.All the data were summarized in the form of table revealed that there was no abnormal signs and behavioural changes in all animals at the dose level of 2000 mg/kg body weight administered orally, during the study period.

2. There was no morbidity and mortality rate were observed in all test animals. Body weight of all test drug treated animals were gradually increased .

3. There was no necropsy findings seen of all the orifices and vital organs of 2000 mg/kg.b.wt of Aadutheendapaalai ver chooranam treated animals in acute toxicity study.

There were no changes in skin and fur, eyes and mucous membranes of all animals. The eating, drinking habit, sleep pattern, locomotion were normal in all animals and no changes in body weight as compared to control group. At the end of the 14th day, necropsy was performed and there was no abnormality seen in test groups as compared to control group during the examination.

SUB ACUTE TOXICITY STUDY OF AADUTHEENDAPAALAI VER CHOORANAM

Sub-acute oral toxicity study carried out as per OECD guideline for a period of 28 days. The changes observed in the food intake, water intake, body weight changes, haematological and biochemical parameters were mentioned in below table.

Table 7: Effect of Aadutheendapaalai ver Chooranam on Body Weight changes of wister albino rats in Sub-acute toxicity study.

BODY WEIGHT	CONTROL	LOW	MID	HIGH
DAY 1	184.4±20.71	180.5±24.54	189.3±26.21	188.5±26.46
DAY 7	205.3±28.77	197±24.17	210.1±32.45	206±32.38
DAY 14	216.9±39.66	214±27.46	225.3±41.81	219.9±40.41
DAY 21	235.2±41.79	232±30.84	230.9±33.55	237.9±41.92
DAY 28	250.9±46.25	250±39.44	256±47.12	254±47.65

Data expressed as Mean ± SD for N = 10 rats in control group and N = 10 in Low, Mid, High dose groups, one – way ANNOVA followed by Dunnett's test. Significant indicates that *P<0.05, ** P<0.01

Graph 2: Effect of Aadutheendapalai ver chooranam on Body weight (gm) changes of Wister albino rats in Sub-acute toxicity study

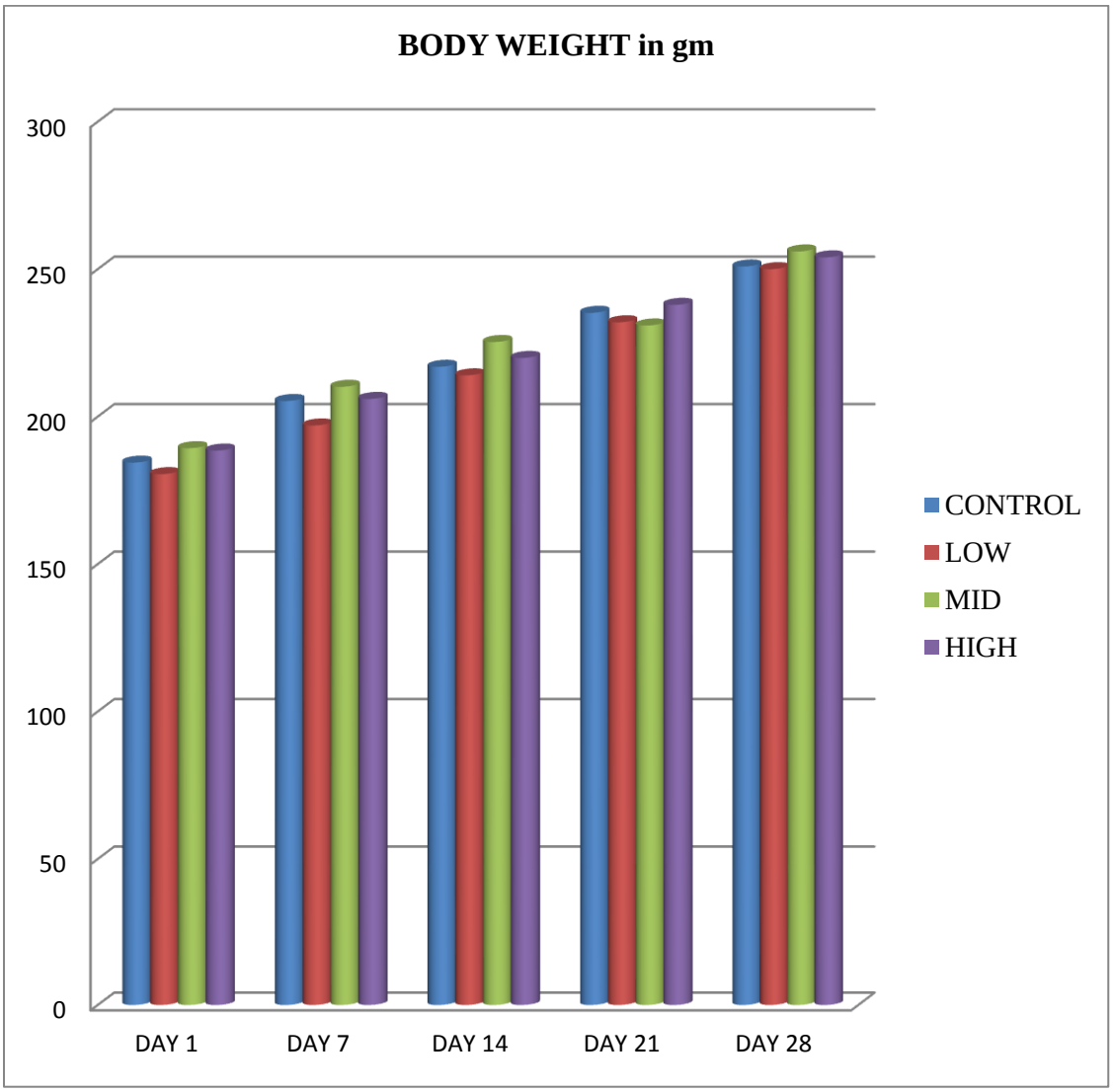


Table 8 : Effect of Aadutheendapaalai ver Chooranam on Water intake of wister albino rats in Sub-acute toxicity study.

WATER	DAY 1	DAY 7	DAY 14	DAY21	DAY 28
CONTROL	71±1	76±2	81±3	87.5±2.5	91.5±1.5
LOW	74±1	77±2	82±2	86.5±0.5	91±1
MID	73.5±1.5	76.5±1.5	81±1	84±1	87±2
HIGH	71.5±1.5	75±1	79±1	83±1	86.5±1.5

Data expressed as Mean ± SD for N = 10 rats in control group and N = 10 in Low, Mid, High dose groups, one – way ANNOVA followed by Dunnett's test. Significant indicates that *P<0.05, ** P<0.01

Graph 2: Effect of Aadutheendapaalai ver Chooranam on Water intake of wister albino rats in Sub-acute toxicity study.

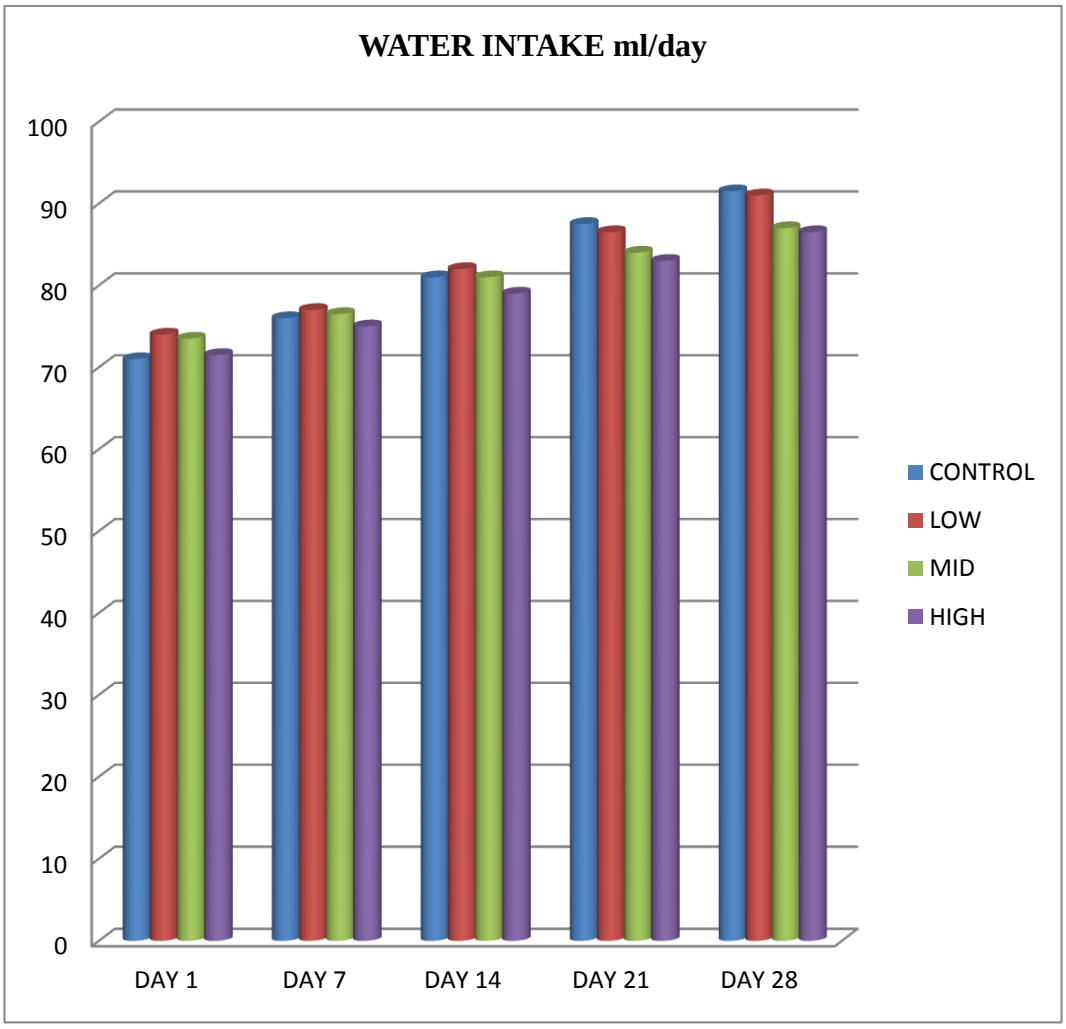


Table 9: Effect of Aadutheendapaalai ver Chooranam on Food intake of wister albino rats in Sub-acute toxicity study.

FEED	DAY 1	DAY 7	DAY 14	DAY21	DAY 28
CONTROL	42±1	45±4.24	48.5±4.94	53.5±2.12	56.5±2.12
LOW	46±2.82	49±1.41	51±1.41	53.5±2.12	59±1.41
MID	44.5±0.70	46.5±0.70	49±1.41	52±2.82	54.5±0.70
HIGH	47±1.41	49±1.41	51±1.41	53.5±0.70	56.5±2.12

Data expressed as Mean ± SD for N = 10 rats in control group and N = 10 in Low, Mid, High dose groups, one – way ANNOVA followed by Dunnett's test. Significant indicates that *P<0.05, ** P<0.01

Graph 3: Effect of Aadutheendapalai ver chooranam on Food intake changes of Wister albino rats in Sub-acute toxicity study

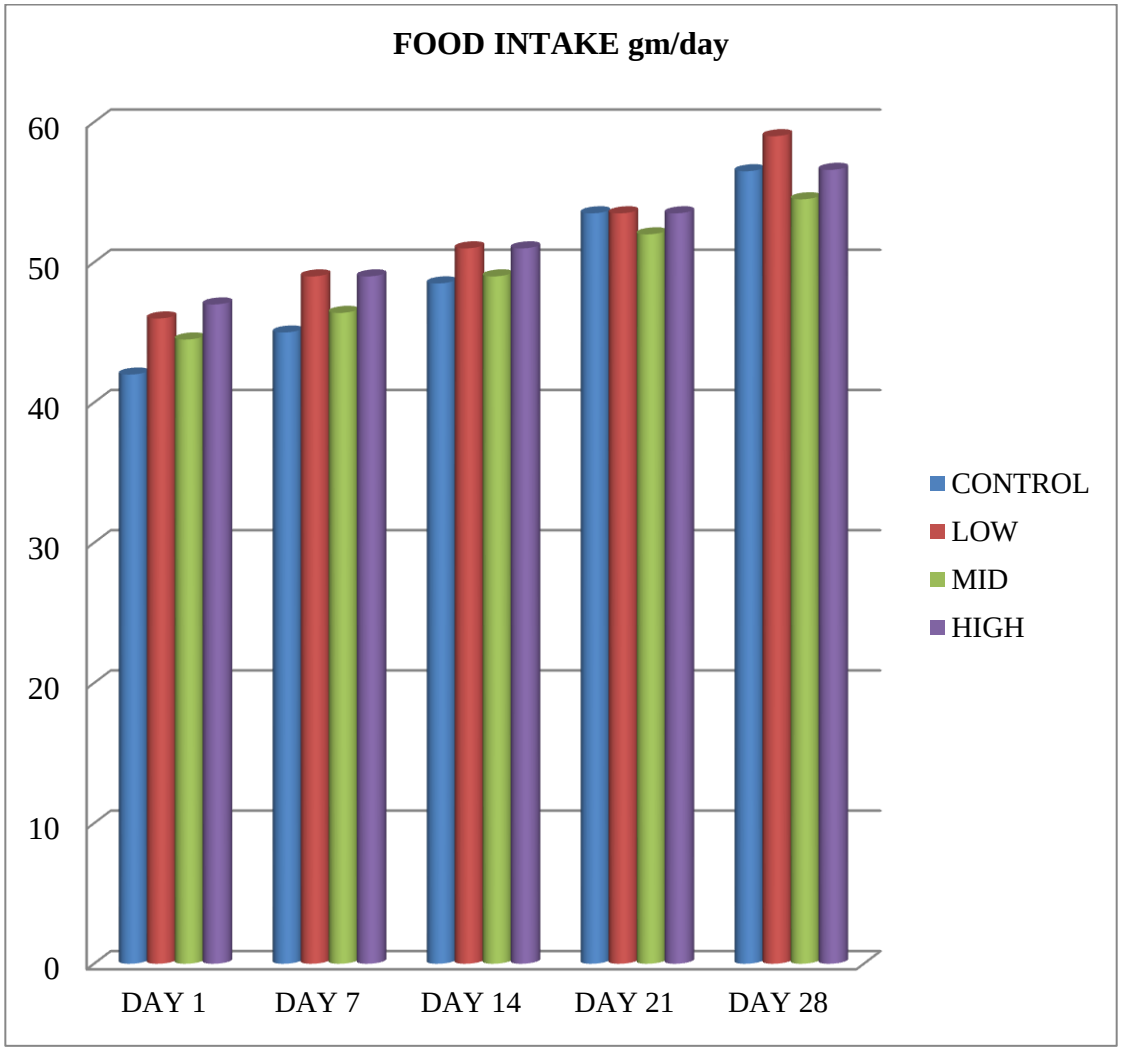
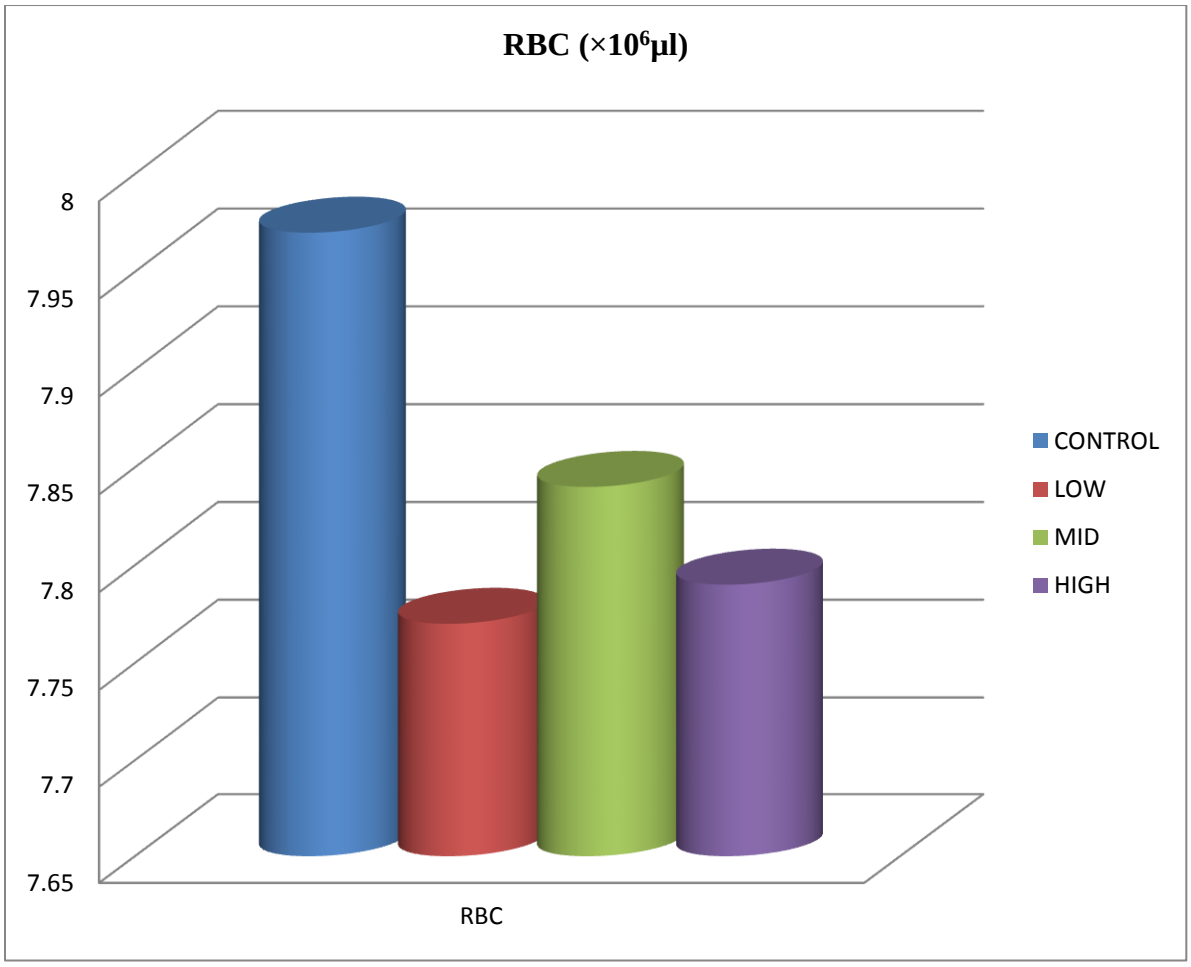


Table 10: Effect of Aadutheendapaalai ver Chooranam on food intake of wister albino rats in Sub-acute toxicity study.

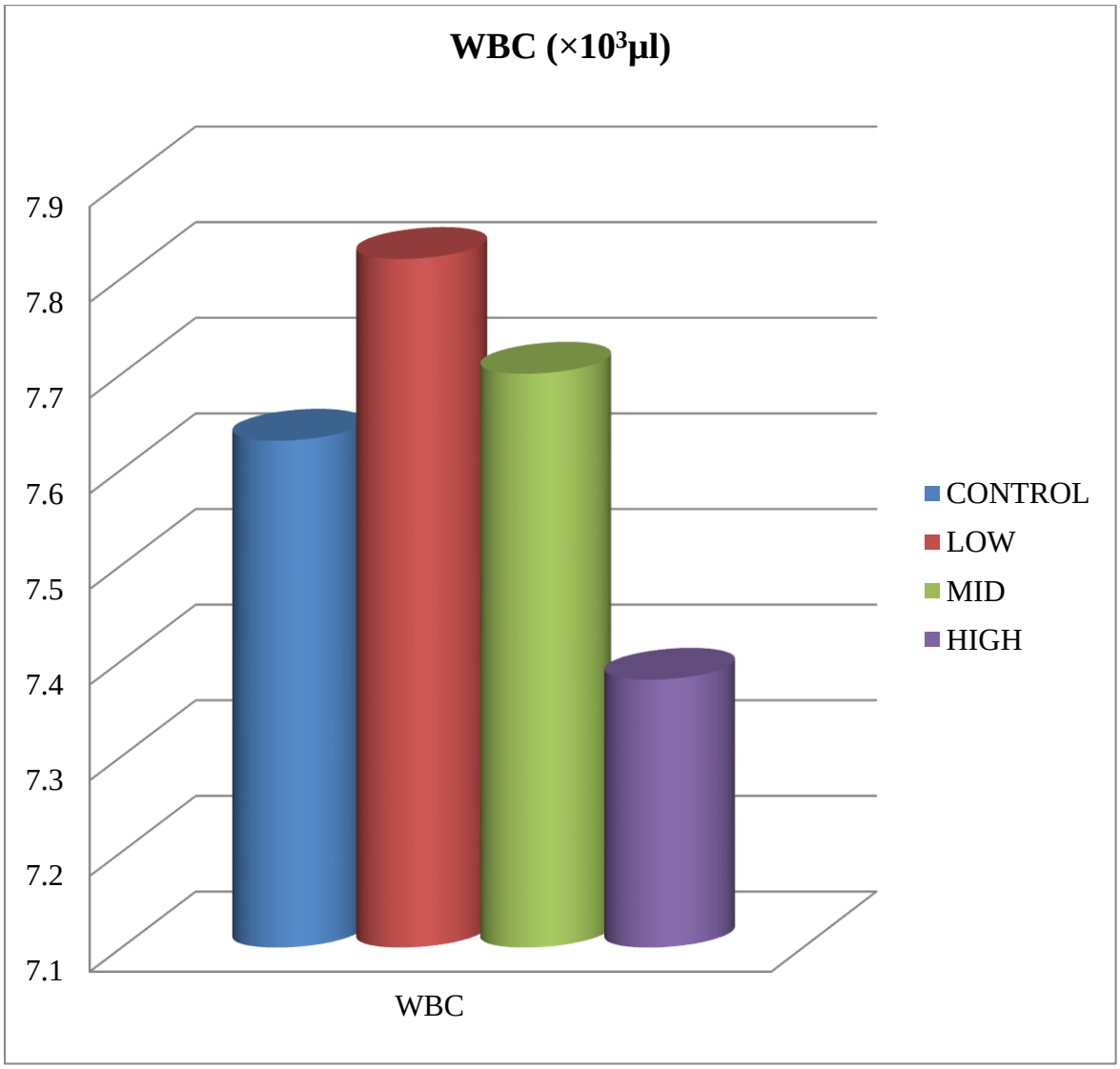
HEMATOLOY	CONTROL	LOW	MID	HIGH
RBC ($\times 10^6$µl)	7.97±0.64	7.77±0.68	7.84±0.65	7.79±0.65
WBC ($\times 10^3$µl)	7.63±1.09	7.82±0.93	7.7±0.88	7.38±0.56
PLATELET ($\times 10^3$µl)	752.7±136.38	743±130.64	766±81.98	739.5±125.23
HB (g/dl)	12.68±1.49	13.55±1.60	13.75±1.84	12.58±1.14
MCH (Pg)	16.69±1.29	16.89±1.04	16.91±1.11	16.36±1.19
MCV (Fl)	55.82±6.43	60.02±7.43	59.92±7.13	57.3±5.94
N (%)	2.99±0.71	2.74±0.31	2.74±0.31	3.1±0.59
E (%)	1.44±0.27	1.481±0.21	1.461±0.19	1.51±0.26
B (%)	0.2±0.42	0.2±0.42	0.2±0.42	0.2±0.42
L (%)	72.3±7.66	71.5±5.87	71.4±5.81	71.9±7.79
M (%)	3.29±1.46	3.32±1.10	3.22±0.95	3.26±1.50
BUN (mg/dl)	13.3±2.54	13.4±2.27	13.6±2.06	13.3±2.62
CREATININE (mg/dl)	0.6±0.14	0.58±0.14	0.54±0.11	0.6±0.14
BILIRUBIN (mg/dl)	0.31±0.13	0.3±0.12	0.27±0.11	0.31±0.13
SGOT(U/L)	82±4.92	79.8±2.52	79.1±2.68	79.8±3.82
SGPT (U/L)	15.6±2.67	15.4±2.45	29.1±44.28	14.5±2.17
T.CHO (mg/dl)	129.5±16.98	127.5±10.95	124.9±9.58	132.8±12.14
HDL (mg/dl)	53.3±6.66	55.2±6.51	56.3±5.51	53.2±5.41
LDL (mg/dl)	70.07±14.10	67±12.80	67.25±13.5	64±13.57
VLDL (mg/dl)	14.89±3.19	14.98±3.20	15.25±2.87	14.53±3.31
TG (mg/dl)	36.1±10.87	38.1±8.25	38.8±10.03	37±10.07

Data expressed as Mean ± SD for N = 10 rats in control group and N = 10 in Low, Mid, High dose groups, one – way ANNOVA followed by Dunnett's test. Significant indicates that *P<0.05, ** P<0.01

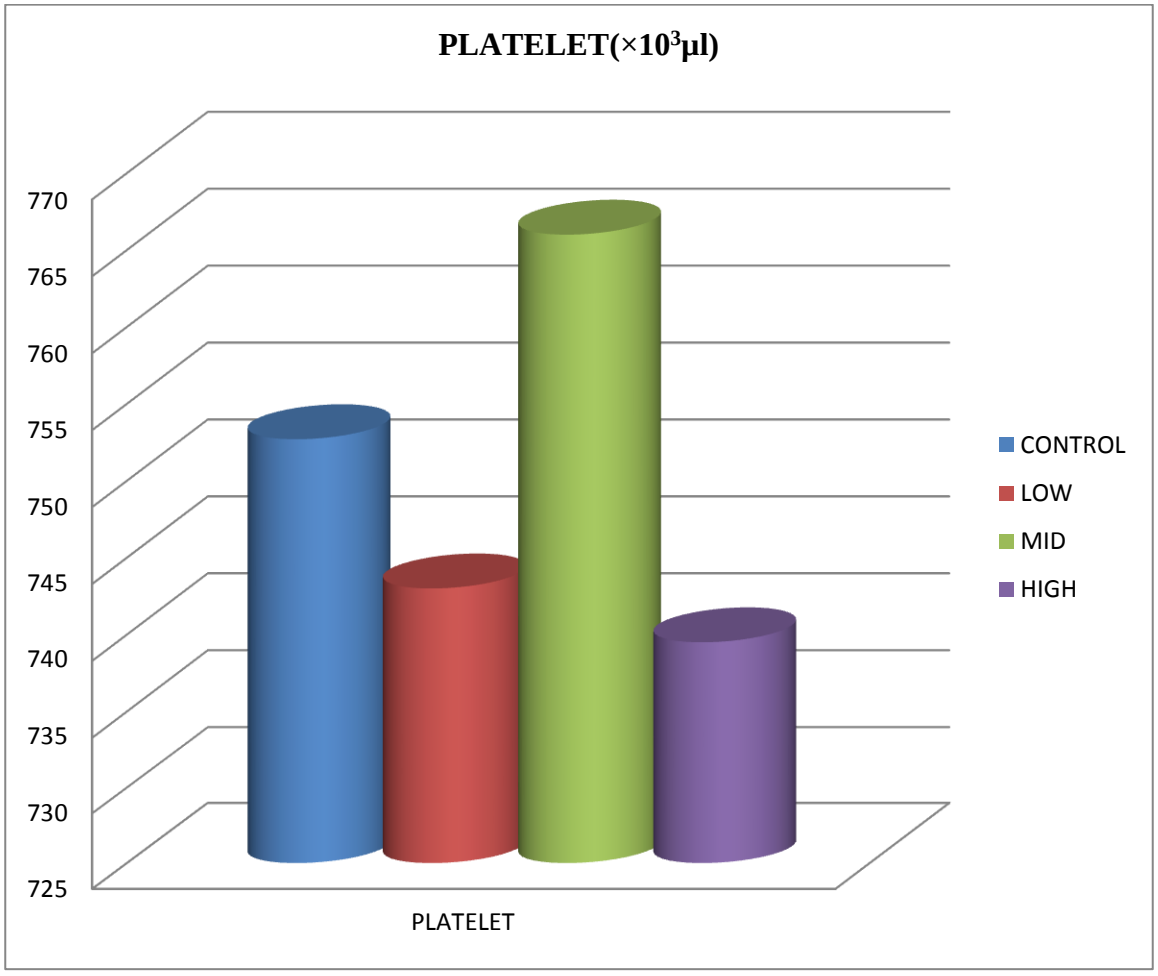
Graph 4: Effect of Aadutheendapaalai ver chooranam on Hematological parameters – RBC of Wister albino rats in Sub-acute toxicity study



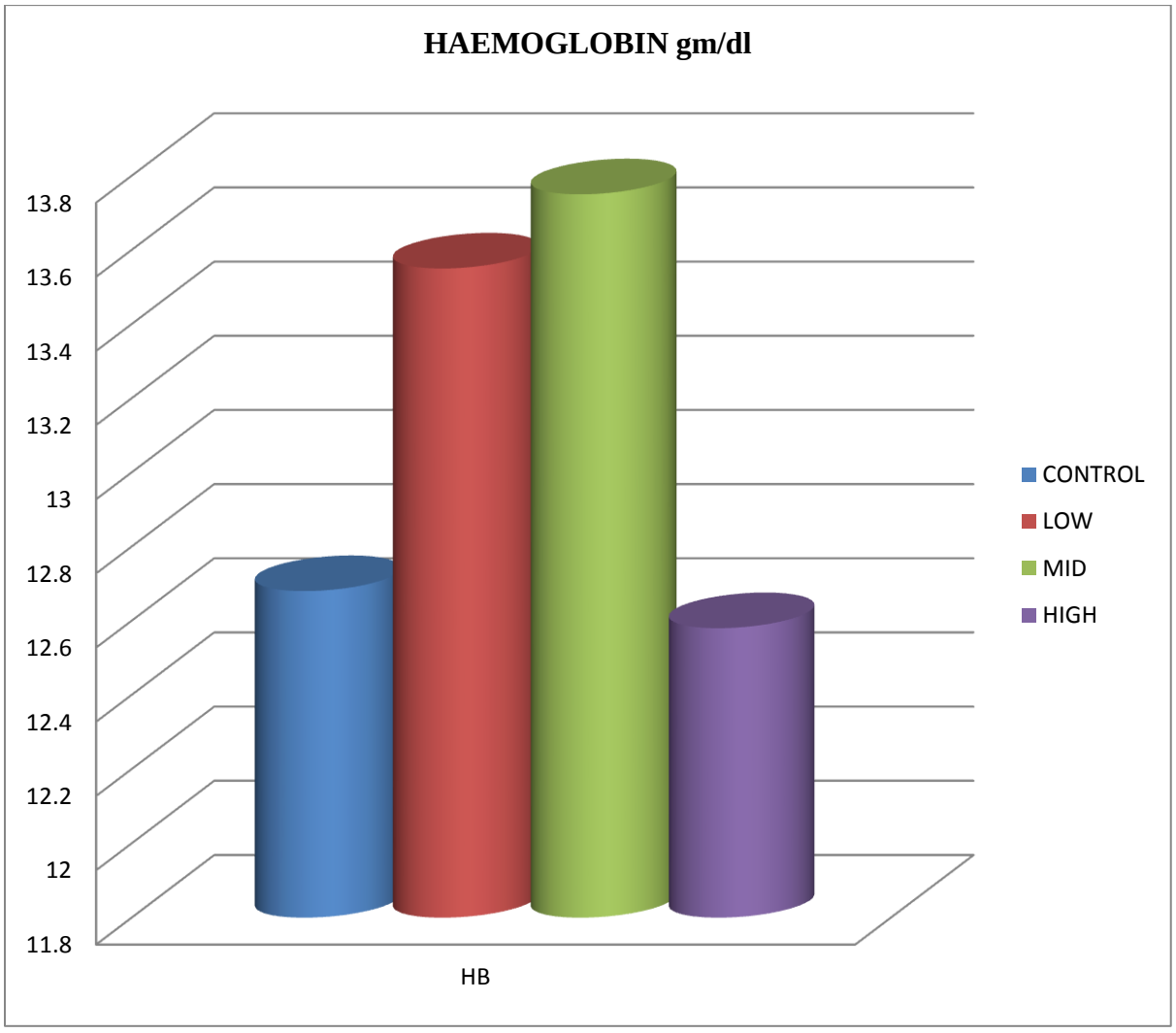
Graph 5: Effect of Aadutheendapaalai ver chooranam on Hematological parameters – WBC of Wister albino rats in Sub-acute toxicity



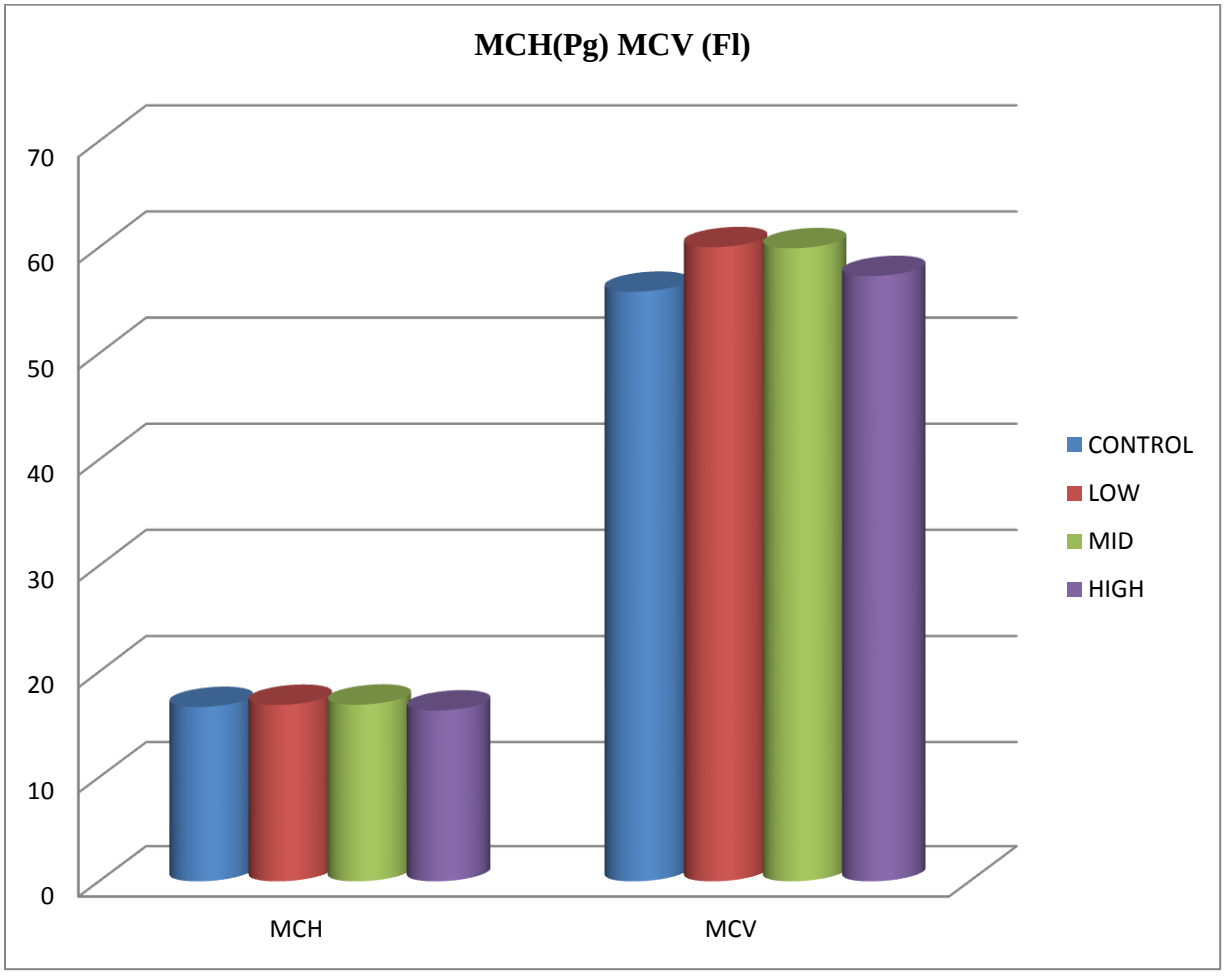
Graph 6: Effect of Aadutheendapaalai ver chooranam on Hematological parameters – Platelet of Wister albino rats in Sub-acute toxicity study



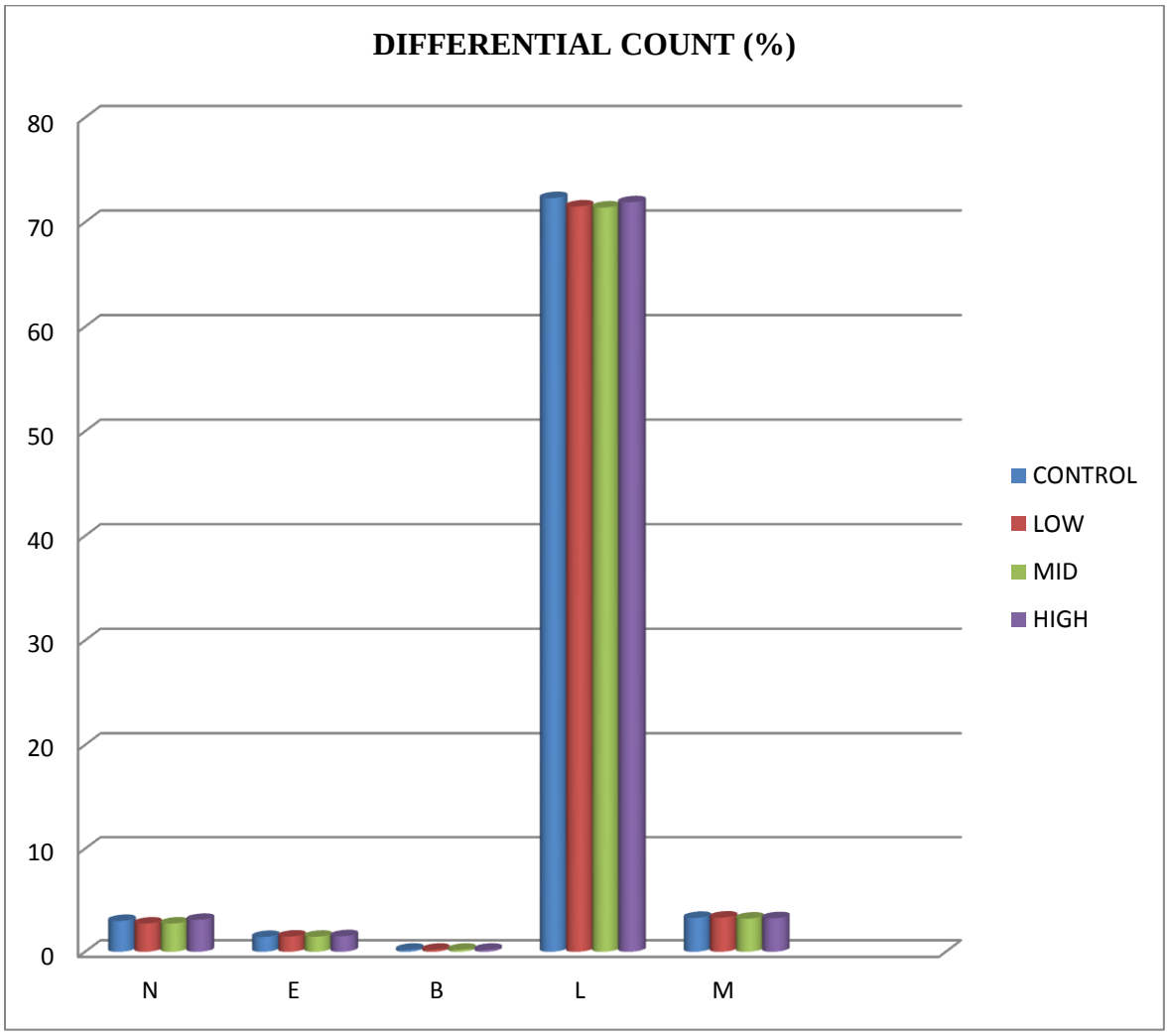
Graph 5: Effect of Aadutheendapaalai ver chooranam on Hematological parameters – HB of Wister albino rats in Sub-acute toxicity study



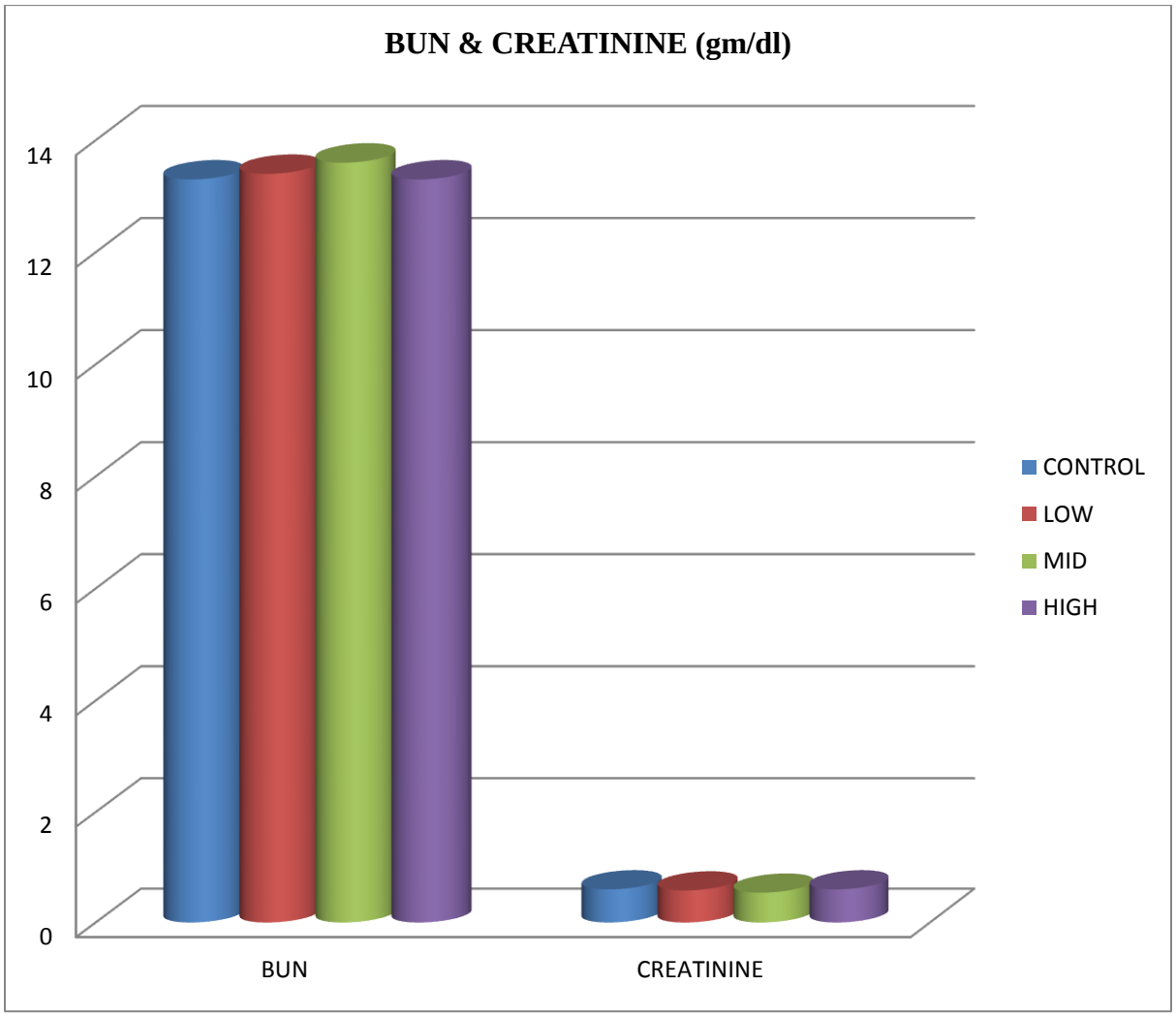
Graph 5: Effect of Aadutheendapaalai ver chooranam on Hematological parameters – MCH & MCV of Wister albino rats in Sub-acute toxicity study



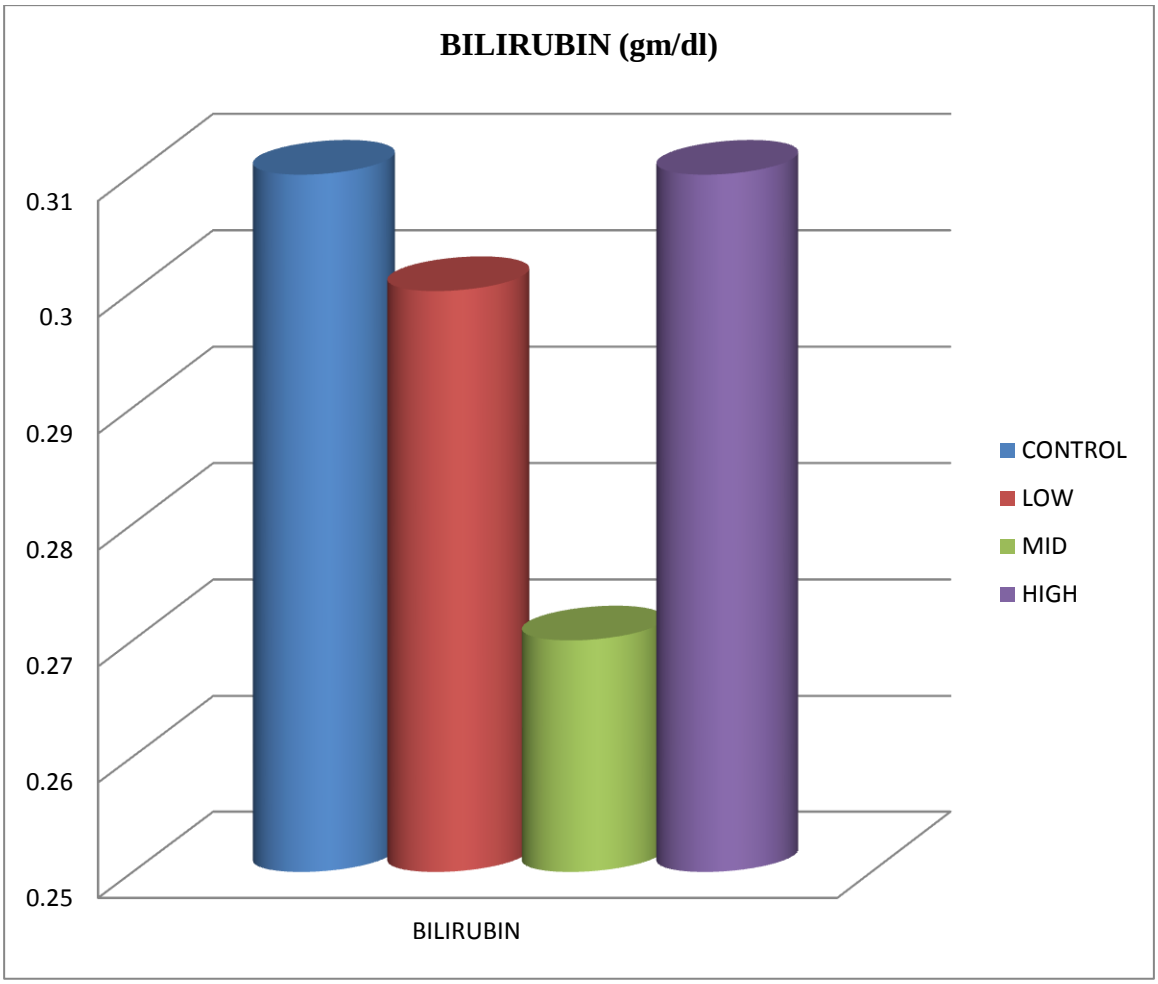
Graph 6: Effect of Aadutheendapaalai ver chooranam on Hematological parameters – Differential Count of Wister albino rats in Sub-acute toxicity study



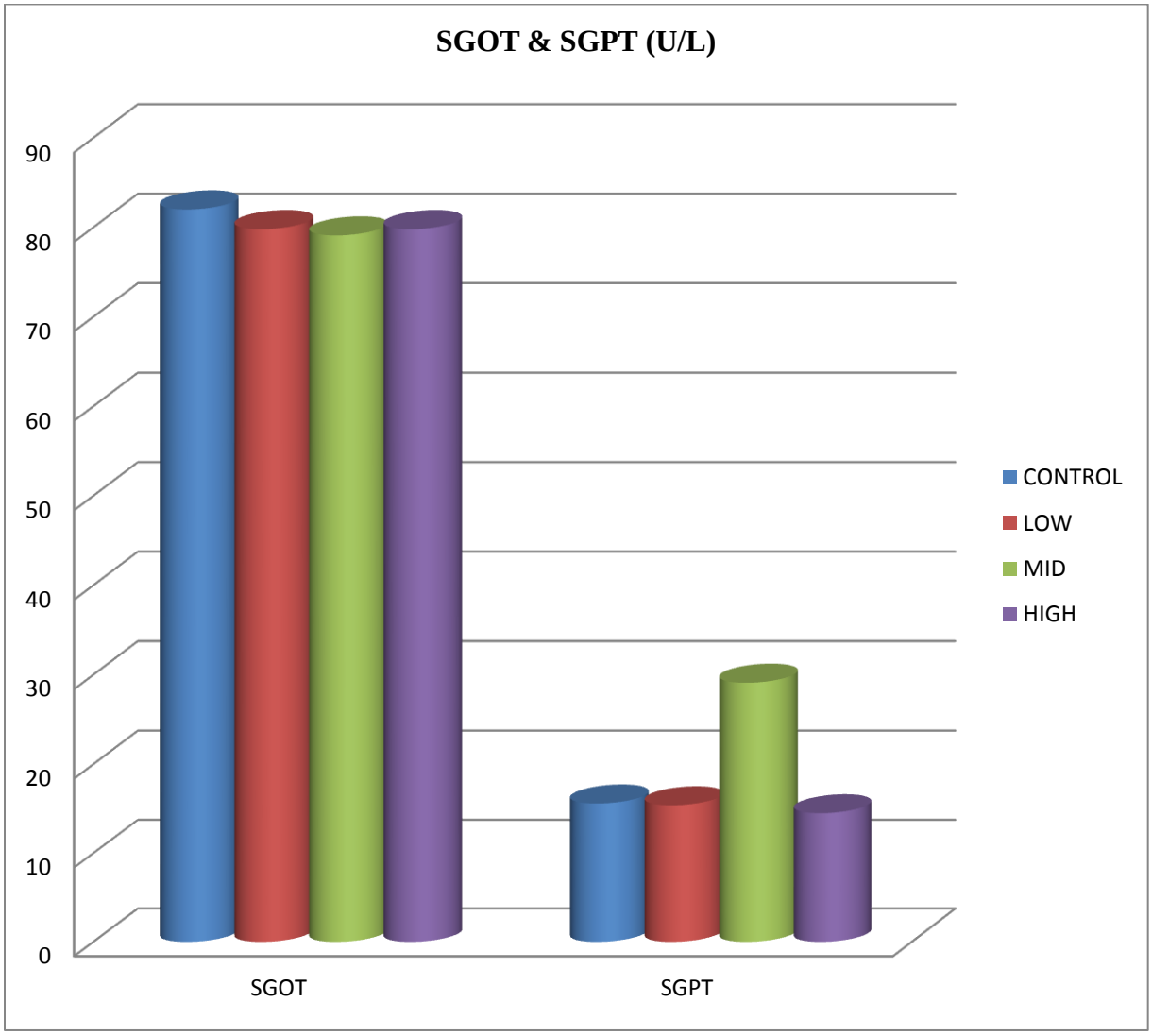
Graph 7: Effect of Aadutheendapaalai ver chooranam on Hematological parameters – BUN and Creatinine of Wister albino rats in Sub-acute toxicity study



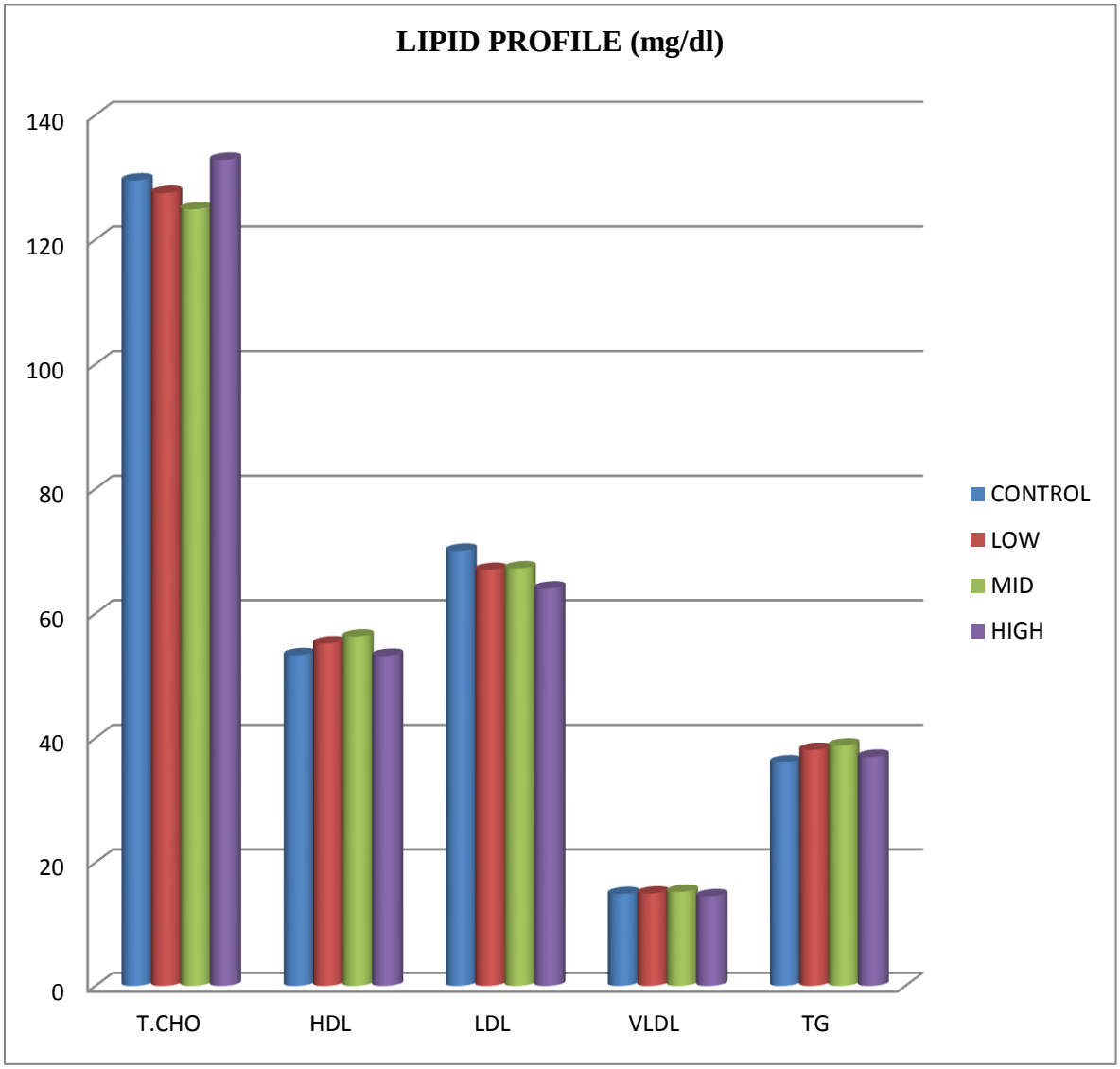
Graph 7: Effect of Aadutheendapaalai ver chooranam on Hematological parameters – Bilirubin of Wister albino rats in Sub-acute toxicity study



Graph 8: Effect of Aadutheendapaalai ver chooranam on Hematological parameters – SGOT & SGPT of Wister albino rats in Sub-acute toxicity study



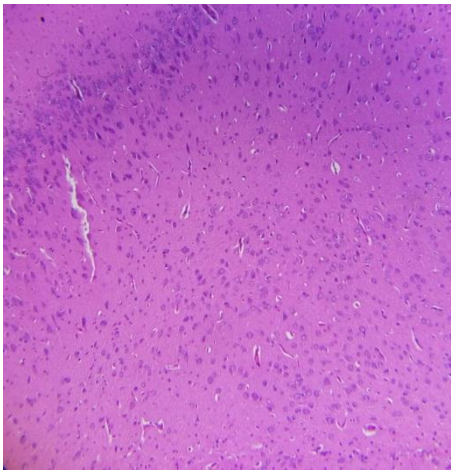
Graph 9: Effect of Aadutheendapaalai ver chooranam on Hematological parameters – Lipid profile of Wister albino rats in Sub-acute toxicity study

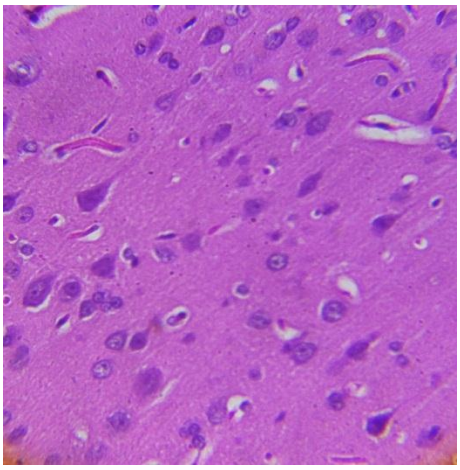


28 Day Repeated Dose Oral Toxicity Study

Histopathology of Brain

CONTROL HIGH DOSE





BRAIN

CONTROL:

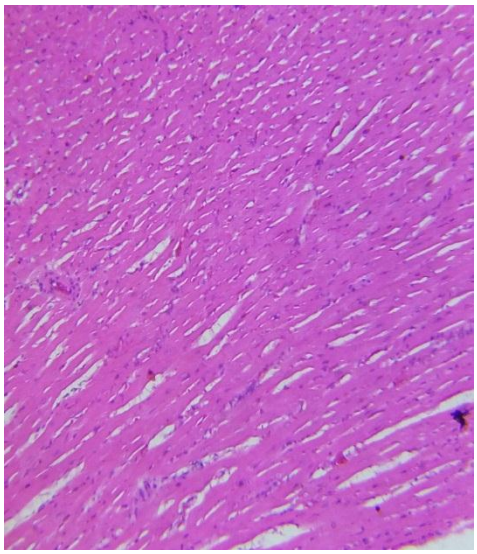
Arrangement of the neurons appears intact with no signs of degeneration or apoptotic changes in both the sample so cortex region showed normal neurons with polygonal to round cell bodies containing dense cytoplasm.

HIGH DOSE:

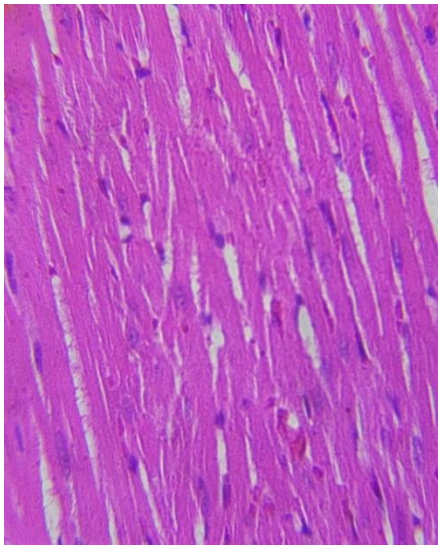
Appearance of Hippocampal neurons was normal with dense network no Signs of ischemic changes in the cerebral hemisphere

Histopathology of Heart

CONTROL



HIGH DOSE



HEART

CONTROL:

Perfectly -arranged myocardial fibers, clear transverse striation and normal structure were observed.

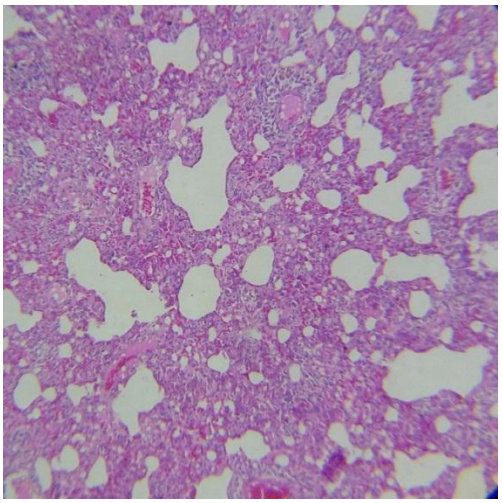
Appearance of cardiomyocyte was normal with dark nuclear region. The nuclei of muscle fibers appear oval arrangement

HIGH DOSE:

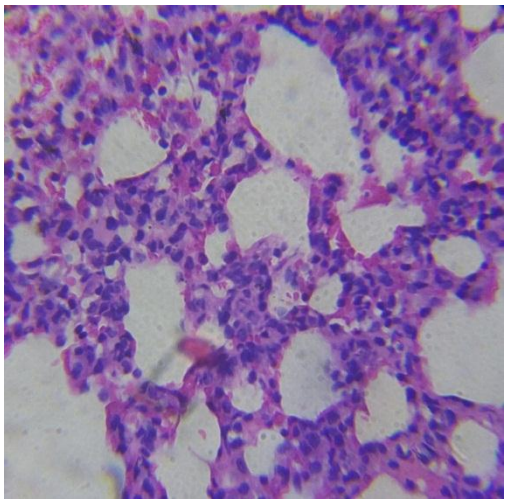
Myocardial cells appears normal with well-defined mycoplasma and prominent nucleus and nucleolus

Histopathology of Lung

CONTROL



HIGH DOSE



LUNGS:

CONTROL:

Bronchial opening appears regular with no signs of infiltration

Appearance of alveolar network was normal

Nucleus of type I and II alveolar cells looks normal

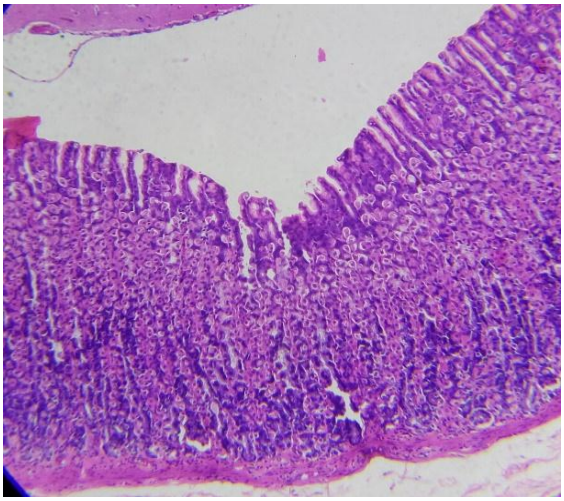
HIGH DOSE:

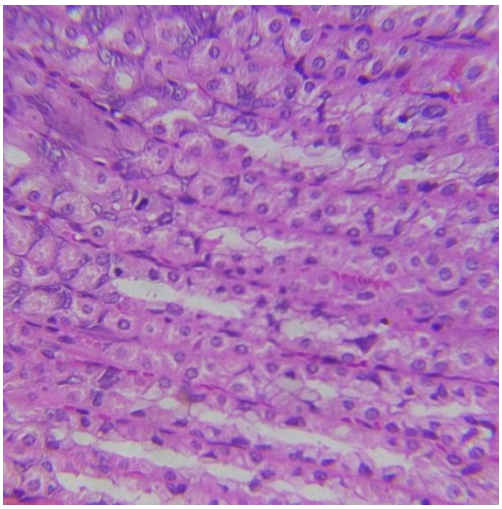
Perivascular region appears normal, Alveolar septa and wall appeared widen and normal

No signs of lymphocyte cuffing

Histopathology of Stomach

CONTROL HIGH DOSE





STOMACH:

CONTROL:

Gastric glands, gastric glands including secretary sheath appears normal

Normal gastric mucosa containing intact gastric gland cells, parietal cells which are spherical cell with deeply stained dark nucleus

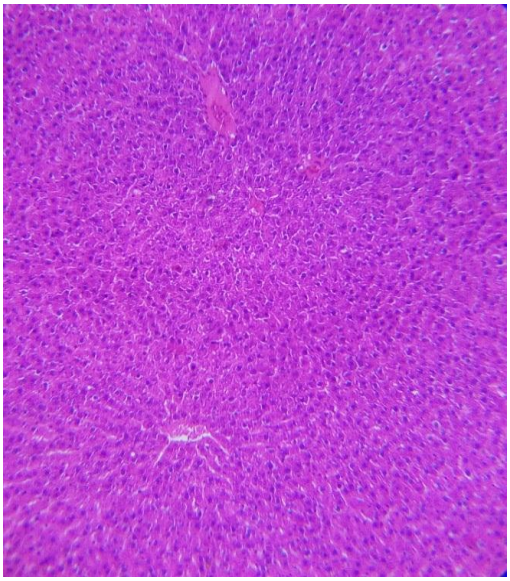
HIGH DOSE:

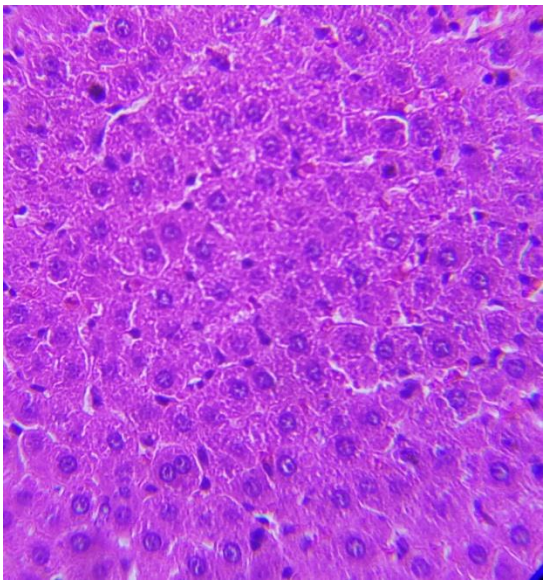
No signs of ulcer and glandular degeneration were observed

Appearance of Sub-mucosa and gastric glands appear normal

Histopathology of Liver

CONTROL HIGH DOSE





LIVER:

CONTROL:

Rare appearance of Kupffer cells with no evidence of phagocytosis in

Intra cytoplasmic region

Liver parenchyma appears normal with no evidence of necrosis

Appearance of terminal hepatic venules (central veins) to the portal tracts was Normal

HIGH DOSE:

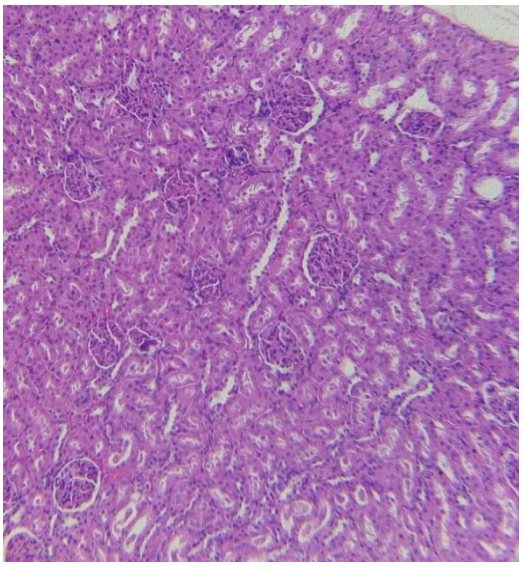
Apparent loss of liver parenchyma were observed

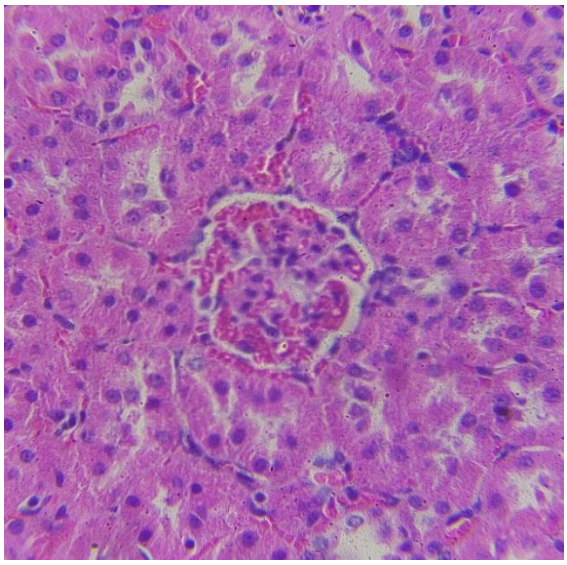
Increase distant of liver sinusoids were observed

Occasional presence of Kupffer cells with no evidence of phagocytosis in intracytoplasmic region

Histopathology of Kidney

CONTROL HIGH DOSE





KIDNEY:

CONTROL:

Appearance of Podocytes and parietal epithelium in the glomeruli appears normal

Proximal and distal convoluted tubule appears normal

No signs of lesion or inflammation were observed

No signs of cellular necrosis

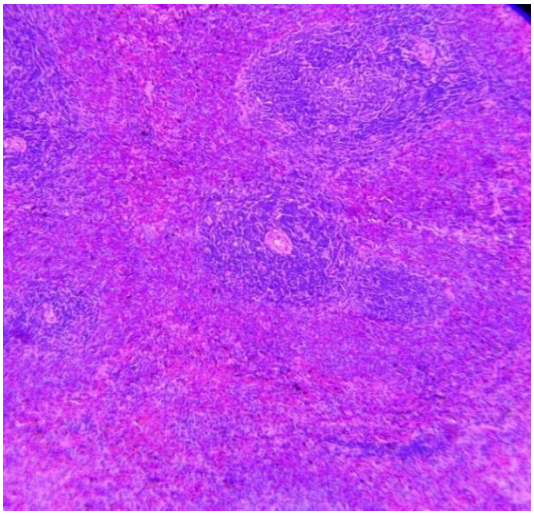
HIGH DOSE:

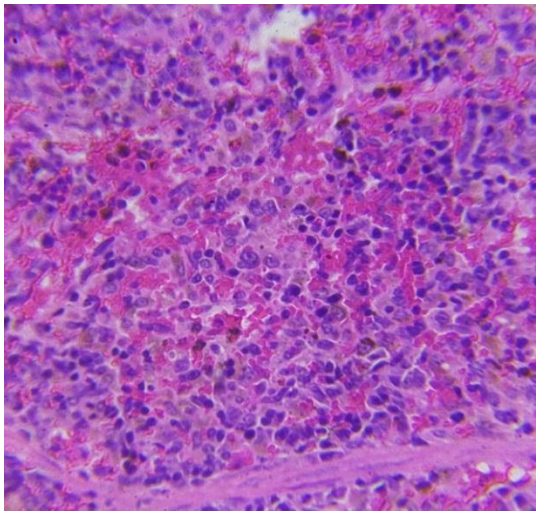
Some renal tubules appears hypertrophic

Appearance of Podocytes and parietal epithelium in the glomeruli appears normal

Histopathology of Spleen

CONTROL HIGH DOSE





SPLEEN:

CONTROL:

No signs of perivascular inflammation

Appearance of splenic sinuses, Splenic cord and endothelial orientation was normal

Appearance of LF – lymphoid follicle; PALS – periarterial lymphoid sheath was normal with no significant signs of enlargement

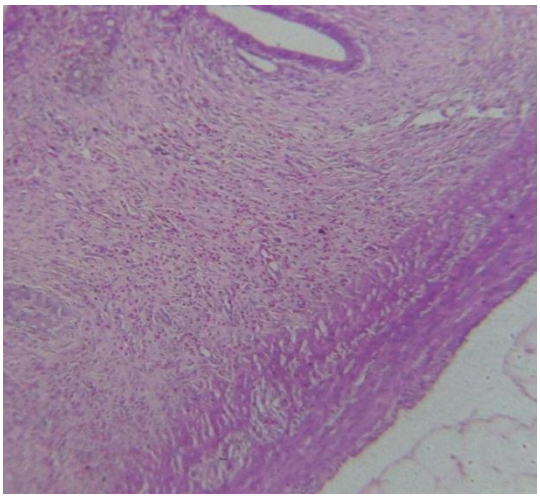
HIGH DOSE:

Marginal vascular zone radiated in between red and white pulp

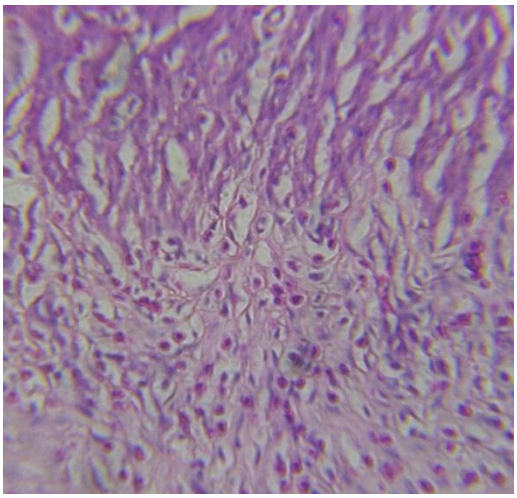
Appearance of spleenic red pulp was normal

Histopathology of Uterus

CONTROL



HIGH DOSE



UTERUS

CONTROL:

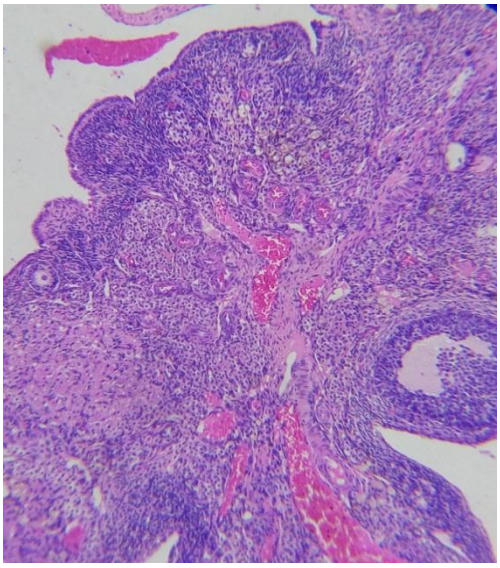
Appearance of endometrium, myometrium and uterine glands was normal.

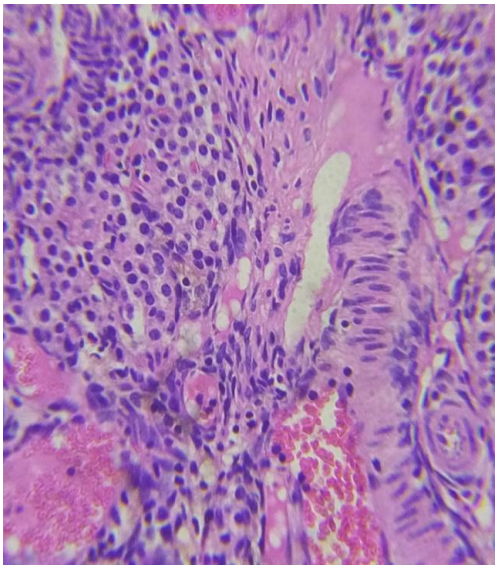
HIGH DOSE:

Appearance of endometrium, myometrium and uterine glands was normal

Histopathology of Ovary

CONTROL HIGH DOSE





OVARY

CONTROL:

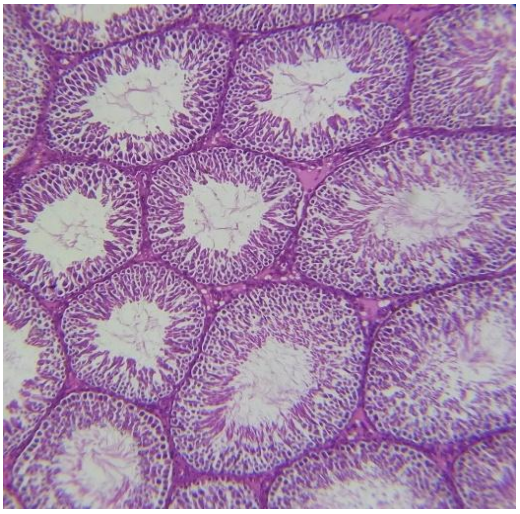
Histopathological analysis of ovary showing normal corpus luteum (CL) and primordial follicles with few mature ovarian follicles with no signs of abnormality.

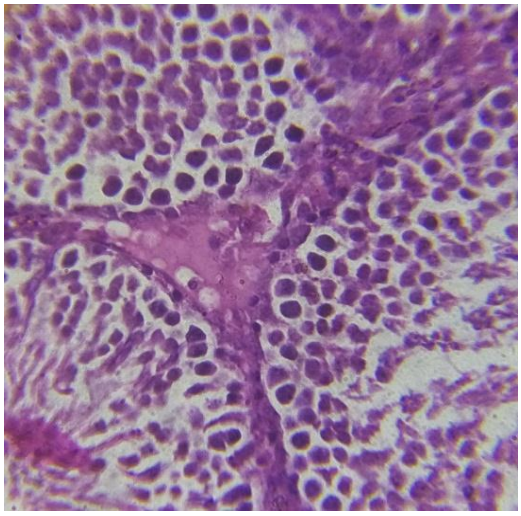
HIGH DOSE:

Appearance of antral follicle, primary oocyte and secondary follicles all were normal

Histopathology of Testes

CONTROL HIGH DOSE





TESTES:

CONTROL:

Histocytology of testicular tissue shows well differentiated germ cells with respect of spermatogonia includes spermatid and sperm were observed Appearance of leydig cells, interstitial tissue, seminiferous tubule, Sertoli cells and spermatogonia were normal

HIGH DOSE:

Histo cytology of testicular tissue shows well differentiated germ cells with respect of spermatogonia includes spermatid and sperm were observed Appearance of leydig cells, interstitial tissue , seminiferous tubule, Sertoli cells And spermatogonia were normal

DISCUSSION

7. DISCUSSION

I have selected ***Aadutheendapalai ver chooranam(AVC)*** to evaluate the safety profile. First with the test drug I have done the process of standardization with qualitative and quantitative analysis**.** The following are the analysis

- Physico-chemical analysis
- Biochemical Analysis
- TLC Analysis
- HPTLC Analysis
- Pesticide residual
- Sterility test
- Aflatoxin assay

The safety profile is evaluated by Acute and Long Term toxicity studies on Wister Albino rats as per OECD guideline.

The **Physico-chemical analysis** of AVC (Table: 1) concludes the following results. The pH of AVC is 6.5. Being weak acidic, the drug is more readily absorbed in an acid medium like stomach which enhances the bioavailability of the drug. The loss on drying test is designed to measure the amount of water and volatile matters in a sample when the sample is dried under specified condition. Low moisture content is always desirable for higher stability of drugs. The percentage of loss on drying of AVC was 7.86% (Normal range: 1-20%). So, Low moisture of AVC could get maximum stability and better shelf life.

The Ash limit Tests are designed to measure the amount of the residual. A high ash value is indicative of contamination, substitution, adulteration or carelessness in preparing the drug. The total Ash content and Acid Insoluble Ash values of AVC were

8.9% (Normal range: 1-25%) and 0.27% (Normal range: 0.1 – 10%). This indicates the purity of the test drug.

Extraction value determines the amount of active constituents in a given amount of the formulation when extracted with a solvent media such as water and alcohol. The water soluble and alcohol soluble extract values provide an indication of the extent of polar and non-polar compounds respectively present in AVC .The extract values of Alcohol in AVC is 3.6% and water is 11.57%. Decreased Water soluble ash value (2.8 %) indicates easy facilitation of diffusion and osmosis mechanisms.

Biochemical Analysis of Aadutheendapaalai ver chooranam for Acid radicals, Basic radicals, and other constituents demonstrates the presence of Sulphate, Phosphate, Carbonate, Sulphide, Zinc, Iron, Calcium, Aluminum, Starch, Alkaloids, Amino acid (Table 2)

Phosphate is a charged particle that contains the mineral phosphorus. The mineral phosphorus is primarily used for growth and repair of body cells and tissues. It reduces the histamine release by activated mast cells

Zinc is deeply involved in regulation of immune system. Deficiency of zinc leads to development of inflammatory and autoimmune disorders. So the presence of zinc in AVC cures the inflammatory disorders thereby regulating the immune system.

The **Phytochemical Analysis** of Aadutheendapaalai ver chooranam results showed that the Alkaloids, Steroids, Triterpenoids, Phenol, Tanin, Saponin and Sugar it reveals that the therapeutic potent of the drug (Table 3)

The **Quantitative Analysis** of Aadutheendapaalai ver chooranam through **TLC & HPTLC** results showed that the HPTLC finger printing analysis of the sample reveals the presence of five prominent peaks corresponds to presence of five versatile phyto

components present with in it. Rf value of the peaks ranges from 0.02 to 0.79. Further the peak 3 occupies the major percentage of area of 55.89 which denotes the abundant existence of such compound (Table 4)

The **Qualitative Analysis** of Aadutheendapaalai ver chooranam through **Pesticide residual test** results showed that there were no traces of pesticides residues such as Organo chlorine, Organo phosphorus, Organo carbamates and pyrethroids in the sample provided for analysis it reveals that the plant did not contaminate with pesticides (Table 5)

The **Qualitative Analysis** of Aadutheendapaalai ver chooranam through **Sterility Test By Pour Plate Method** showed that were no growth was observed after incubation period reveals that the absence of specific pathogen. (Table 6)

The **Qualitative Analysis** of Aadutheendapaalai ver chooranam through **Aflatoxin Assay By TLC (B1, B2, G1, G2)** showed that was no spots were been identified in the test sample loaded TLC plates when compare to the standard, which indicates that he sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2. (Table 7)

In **Acute toxicity study** there was no abnormal signs reported at the dose level of (2000 mg/kgb.wt) within 24hours in Wistar Albino Rats. No mortality and No pathological changes have been seen in the internal organs of both control and treated groups in the 14 days study period. And the Body weight, food intake and water intake of animals are normal.

Sub-acute Toxicity Study was conducted for about 28 days as per OECD guideline in 3 doses Low dose (360mg/kg b.wt), Mid dose (720mg/kg b.wt), High dose (1440mg/kg b.wt). Animals were observed throughout the period. There was no significant change in body weight (Table: 7), water intake (Table: 8), and food intake (Table: 9). After 28 days

animals were sacrificed and blood samples were collected and investigated. The results revealed that there were no significant in RBC, WBC, DC, Platelet count and no significant changes in Haemoglobin. In hepatic parameter there were no significant changes in SGOT and SGPT. In Biochemical parameter there were no significant changes in BUN, Creatinine and Lipid profile (Table: 10). The histopathological study on the organs such as brain, heart, lungs, kidney, spleen, liver, stomach, uterus, ovary and testes was normal in control, low dose, mid dose and high dose groups.

After 28 days repeated oral toxicity study upto 2000 mg there were no mortality and morbidity observed in animals and also there were no changes in Haematology, Serology and Histopathology

SUMMARY

8. SUMMARY

Aadutheendapaalai ver chooranam was taken as dissertation drug for the evaluation of its toxicity profile. This is a single drug formulation of ***Aadutheendapaalai ver*** administered with Water as adjuvant. The drug was chosen from the Siddha text Gunapadam mooligai vaguppu. The above drug is indicated for Toxic bite (Nanju kadigal), Intestinal worms (Kudarpuzhu) And Skin diseases (Pandri mamisa thol pondra padai).

The raw drug were collected from land at Valayamadevi village, Cuddalore district and the drugs were identified and authenticated by Asst.Professor of Botany, National Institute of Siddha. The drug was underwent physicochemical, biochemical analysis, Phytocompound analysis using TLC and HPTLC techniques, Pesticide residual, Sterility by pour plate test and Aflatoxin assay. Acute and sub-acute toxicity studies were conducted as per OECD guideline.

The weak acidic pH of AGC enhances the bioavailability of the drug. Decreased Water soluble ash value indicates easy facilitation of diffusion and osmosis mechanisms. The Ash value of the test drug shows the purity.

The Biochemical analysis of drug contains Sulphate, Phosphate, Carbonate, Sulphide, Iron, Zinc, Calcium, Aluminum, Starch, Alkaloids, Amino acid.

HPTLC finger printing analysis of the drug reveals the presence of five prominent peaks corresponds to presence of five versatile phyto components present with in it. Rf value of the peaks ranges from 0.02 to 0.79. Further the peak 3 occupies the major percentage of area of 55.89 which denotes the abundant existence of such compound

The Pesticide residual, Sterility and Aflatoxin assay of drug reveals the purity.

The Acute toxicity study results revealed that no mortality in dose level 2000mg/kg body weight group animals. It concludes that LD 50 cut-off of ***Aadutheendapaalai ver chooranam*** may be above 2000mg//kg body weight as per OECD guideline. Long term toxicity study was conducted for about 28 days as per the OECD guideline.

The different dose levels of ***Aadutheendapaalai ver chooranam*** selected were Low dose (360mg/kg b.wt), Mid dose (720mg/kg b.wt) and High dose (1440mg/kg b.wt) respectively. The control animals were administered normal saline only. The haematology and biochemical parameters were subjected to one way ANOVA analysis which shows no significant changes in CBC, Lipid profile, RFT and LFT in all test groups compared to control group in blood. The histopathology report of organs reveals that all organs such as Brain, Heart, Lung, Stomach, Liver, spleen, kidney, testes, uterus and ovary were normal in low, mid and high dose groups when compared to control group.

CONCLUSION

9. CONCLUSION

From the results of this study, the qualitative analysis of (AVC) reveals the Purity and Bioavailability of the drug.

Toxicity studies states that there was no mortality and signs of toxicity observed for acute oral administration of AVC with the therapeutic dose (2000 mg/kg b.wt) in the prescribed manner. In sub-acute toxicity study there was no significant changes in haematological, biochemical parameter in AVC treated groups when compared to control group. The histopathology report also confirms that there are no remarkable cellular changes at all the dose levels. It clearly demonstrates that there was No Observed Adverse Effect Level (NOAEL) up to the high dose level (1440 mg/kg b.wt).

Based on these results it can be conclude that, the dose level of Aadutheendapaalai ver chooranam 2 to 4gm (Arai muthal oru varaagan alavu) mentioned in the Siddha literature Gunapadam mooligai vaguppu is safe dosage for human consumption.

In upcoming days it should be carried out to study the pharmacological activity and clinical trial to prove the efficacy of the drug

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ANNEXURE

(16)

CERTIFICATE

This is certify that the project title Preclinical Safety Evaluation of **"Aadutheendapalaiver chooranam**– A Siddha formulation **has been approved by the IAEC.** Total Number of animals approved: 46 Rats (23M+23F). Approval No: NIS/IAEC-VII/28082018/16

V. Banumathi

Prof. Dr. V. Banumathi MD(S),
Chairman IAEC,

Prof .Dr. K. Nachimuthu
CPCSEA Nominee

Chairman/Member Secretary of IAEC: **CPCSEA Nominee:**

Name of the principle investigator: Dr. L Sakthimanipriya
Department of Nanjumaruthuvam

Name of the Guide : Dr.R.Madhavan MD(S)
Lecturer ,

NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

BOTANICAL CERTIFICATE

Certified that the following plant drug used in the Siddha formulation **"Aadutheendapalai Ver Chooranam"** taken up for Dissertation studies by **Dr.L.Sakthimanipriya** M.D.(S), II year, Department of Nanju Maruthuvam, 2019, are identified through Visual inspection, Experience, Education & Training, Organoleptic characters, Morphology and Taxonomical methods as

Aristolochia bracteata Retz. (Aristolochiaceae), Root

MEDICINAL BOTANY * CHENNAI 600 047 * National Institute of Siddha

Certificate No: NISMB3872019

Date: 12-05-2019

Authorized Signatory

Dr. D. ARAVIND, M.D.(S),M.Sc.,
Assistant Professor
Department of Medicinal Botany
National Institute of Siddha
Chennai - 600 047, INDIA

CERTIFICATE

Ministry of AYUSH

NATIONAL INSTITUTE OF SIDDHA

Ministry of AYUSH, Government of India

Tambaram Sanatorium, Chennai - 600 047.

WORKSHOP ON RESEARCH METHODOLOGY & BIOSTATISTICS

This is to certify that

Dr. L. SAKTHI MANI PRIYA

has participated in the above Workshop held from 16.04.2018 to 20.04.2018 conducted by the Dept. of Noi Naadal, at National Institute of Siddha, Tambaram Sanatorium, Chennai-600 047.

Dr. G.J. Christian
Coordinator
HoD, Dept. of Noi Naadal,
National Institute of Siddha

Prof.Dr.V.Banumathi
Director,
National Institute of Siddha
Chennai – 600 047.

NATIONAL INSTITUTE OF SIDDHA

An Autonomous Body under Ministry of AYUSH
Govt. of India

Workshop on

Laboratory Animal Care and Basic Research Techniques

(11-15 February, 2019)

CERTIFICATE

This is to certify that

Dr. L.Sakthimanipriya

has participated as Trainee in the workshop on

"Laboratory Animal Care and Basic Research Techniques"

held between 11.02.2019 & 15.02.2019 at National Institute of Siddha, Tambaram Sanatorium, Chennai.

Dr.V.Suba
Organising Secretary

Dr.B.R.Senthilkumar
Coordinator

Prof.Dr.V.Banumathi
Chairperson / Director, NIS