PRECLINICAL SAFETY EVALUATION OF NILAVEMBU KUDINEER – A SIDDHA POLY HERBAL FORMULATION

The Dissertation submitted by

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DECLARATION BY THE CANDITATE

I hereby declare that this dissertation entitled **"Preclinical Safety Evaluation of Nilavembu Kudineer – A Siddha Poly Herbal Formulation"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. V. Manjari M.D(S), Lecturer, Department of Nanju Maruthuvam, National Institute of Siddha, Chennai – 47,** and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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BONAFIDE CERTIFICATE

Certified that I have gone through the dissertation submitted by **Dr. M. R. Srinivasan (Reg.No: 321716205)** a student of first year M.D(S), Branch-VI, Department of NanjuMaruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai-47, and the dissertation work has been carried out by the individual only. This dissertation does not represent of reproduce the dissertation submitted and approved earlier.

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INDEX

INTRODUCTION

1. INTRODUCTION

The Siddha System of Medicine is one of the ancient systems contemporaneous with those of the submerged lands, Egyptian, Mesopotamian, Chinese and Grecian medicines. The unique nature of this system is its continuous service to humanity for more than five thousand years in combating diseases and in maintaining its physical, mental, and moral health. While many of its contemporaries had completed their courses long long ago, since its origin, development and ramifications have become obscure, any literary research on this subject, to be scientific and useful, should commence with a comparative study of the medicines of those ancient civilization, which will illuminate many of the dark corners of our systems. Siddha system, as it exists has much in common with those ancient medicines; the enormous pharmacopoeia containing herbals, animals, and minerals products at times, including substance of disgusting character; empirical treatments under the guise of magic exorcism, incantations, pilgrimage, peregrination, mountaineering and similar activities.

Application of heat and cold, ointment, potions and poultice, bloodletting, counter irritation, bath, suction, manipulative processes such as massage, concentration on hygiene and diet, periodical use of purgatives and emetics, and among drugs, honey, salt, sulphate of copper, mercury, alum; brains, liver, bones, blood, skull, horns of various animals, tissues of reptiles, and such detestable things, are common in these systems.

Siddhars were those who were not only a physician but also social reformers. They were well versed in the field of medicine, natural science, alchemy, astrology, etc. Siddhars were the persons who attained siddhi, that perfection and who had overcome death through these Siddha medicines.

They give many excellent medicines which cure many diseases, even simple fever to challengeable diseases like Tuberculosis, HIV and Cancers

Many awful viral fevers have been reported recently in India and other Asian countries. Mortality rate of some of dreadful viral fevers like Dengue, Chikungunya and Swine flu have been increased which creates panic among the people. Moreover children, old ages are getting affected more frequently. In Siddha clinical practice Nilavembu Kudineer (NK) a decoction based polyherbal Siddha formulation is prescribed for Suram

(fever). It is used as first line therapy and general remedy for various types of fever caused by unknown orgin[1].

Nilvembu Kudineer it has been used extensively in traditional Siddha medicine as it promotes health and treats various types of illnesses. As per literature, the ingredient of Nilavembu Kudineer is Nilavembu (Andrographis paniculata), Vettiver (Vertiveria zieanioides), Vilamichuver (Plectranthus vettiveroides), Santhana siraai (Santalum album), Pei pudal (Tricosanthes cucuerino), Parppadagam (Hedyotis corymbosa), Koraikkizhangu (Cyperus rotundus), Sukku (Zingiber officinale), Milaku (Piper nigrum). And Suram (Fever) is the only indication of the Nilavembu Kudineer.

Literature review revealed the combination of Nilavembu Kudineer and Adathodai Manappagu has showed good response in thrombocytopenia in dengue fever[2]. It is one of the important herbal medicines which have been used for centuries in Asia to treat various illnesses (Sattayasai et al., 2010). Phytochemical investigations of A. paniculata revealed the presence of diterpenes, flavonoids and polyphenols (Sareer et al., 2014). Extensive studies over the last decades have reported that this herbal plant is useful as an anti-inflammatory agent (Xia et al., 2004), anti- microbial activity (Mishra et al., 2009b), anti-thrombotic activity (Thisoda et al., 2006), anti- malarial (Mishra et al., 2009a), immunostimulant (Xu et al., 2007), hypotensive and hypoglycaemic agent (Zhang and Tan, 2000) and used for treatment of upper respiratory tract infection (Coon and Ernst, 2004).

A study by Nagalekshmi et al. (2011) found that the extracts of A. paniculata offered protection against hepatotoxicity induced by paracetamol. In another study, the anti-fertility effect of 2 g/kg dried A. paniculata for 6 weeks in both male and female rats was confirmed (Zoha et al., 1989). Akbarsha et al (1990) reported that A. paniculata caused arrest in spermatogenesis, decreased sperm count and motility and also morphology abnormalities. Another study reported that the administration of andrographolide from A. paniculata (25 mg/kg and 50 mg/kg for 48 days) has been demonstrated to have anti-fertility property and reproductive toxicity (Akbarsha & Murugaian, 2000). In female rats, A. paniculata aqueous extract (1 g/kg for 4, 6 and 8 weeks) caused infertility by lowering the reproductive hormone such as Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), oestrogen and progesterone (Sakilah et al., 2009). Other then Nilavembu, So many pharmacological activities done in

the ingredients of the Nilavembu Kudineer, they are extensively revealed in literature review topic.

Till now the safety of Nilavambu Kudineer no proved scientifically through the animal models. The Acute safety profile of Nilavembu Kudineer is only scientifically validated. So my study was designed to investigate the safety profile of Nilavembu Kudineer. This investigation is extremely important in order to evaluate the safety of Nilavembu Kudineer consumption. The author selected and studied the safety profile of Nilavembu Kudineer through Acute and 28 days repeated oral toxicity studies as per OECD guideline 423 and 407.

AIM & OBJECTIVES

2. AIM AND OBJECTIVE

TITLE OF THE RESEARCH:

Preclinical safety Evaluation of "Nilavembu Kudineer" – A Siddha Poly Herbal Formulation.

AIM

To evaluate the safety profile of "**Nilavembu Kudineer"** through Acute and 28 days Repeated dose oral Toxicity studies in Wistar Albino rats.

OBJECTIVES

- Collect the literature review of ingredients of Nilvembu Kudineer
- Purification and Preparation of the Medicine as per - literature
- To Study the quality parameters for "Nilavembu Kudineer"
- To do Acute oral toxicity study and 28 days repeated oral toxicity study of "Nilavembu Kudineer" as per OECD Guideline 423 & 407.
- Evaluation of safety of the Nilavembu Kudineer.

REVIEW OF LITERATURE

3. REVIEW OF LITERATURE

3.1. NILAVEMBU – Andrographis paniculata

Taxonomic Classification:

Kingdom : Plantae

Subkingdom : Tracheobionta (Vascular Plants)

Superdivision : Spermatophyta (Seed Plants)

Division : Magnoliophyta (Flowering Plants)

Class : Magnoliopsida (Dicotyledons)

Subclass : Asteridae

Order : Scrophulariales

Family : Acanthaceae

Genus : Andrographis Wall. ex Nees

Species : Andrographis paniculata (Burm.f.) Wall. ex Nees

Vernacular Name

Sans.	:	Bhunimba, Mahatikta (King of Bitter), Kirata
Eng.	:	The creat, King of Bitters, Chiretta
Hind.	:	Kiryat, Mahatita
Tam.	:	Nilavembu
Mal.	:	Nilacaepu, Kiriyat
Tel.	:	Nelavemu
Can.	:	Nelabaevu

Habitat:

This annual is common in hedgerows throughout the plants of India, cultivated in gardens from Lucknow to Assam, especially in Bengal.

Constituents:

Dy-mock and his co-workers found that an aqueous infusion of the herb was intensely bitter and acid and thought that the bitterness was due an indifferent, non-basic principle. Gorter, (1911) thought that the bitter substance in the leaves was a lactone 'andrographolid' of the formula $C_{20}H_{30}O_5$. Later investigations by Bhuduri (1914) showed that the leaves contained two bitter substance and trace of an essential oil. The first bitter principle obtained as intensely bitter yellow crystals with formula $C_{19}H_{28}O_5$. It did not respond to any tests form alkaloids and glucosides. The second bitter substances was obtained in an amorphous form and was named 'Kalmeghin' $C_{19}H_{51}O_5$,.The plant as a whole contained a biter principle and the ash a considerable quantity of sodium chloride and potassium salts. The plant is very rich in Chlorophyle. A green resinous extract is obtained by extraction with alcohol which is believed to be the active principle, called 'Kalmeghin' (Kalmegh Resin) and contains 0.6% alkaloid of the crude plant (Dr. K. C. Bose et al.).

Phytochemical Constituents:

- ✓ Andrographolide,
- ✓ Neoandrographolide,
- ✓ Favonoids (7-O-methylwogonin, apigenin, onysilin and 3,4-dicaffeoylquinic acid)[3,4]

Phytochemical attributes

The characteristic secondary metabolites encountered in the plant have considerably enhanced its importance in the arena of medicinal plants and medicines. It is specifically rated very high in therapeutic action in curing liver disorders and common cough and cold in humans. A number of diterpenoids and diterpenoid glycosides of similar carbon skeleton have been isolatedfrom *Andrographis*, mainly the most bitter compounds among them are andrographolide, neoandrographolide, deoxyandrographolide.

The aerial parts of the plant (leaves and stem) are used to extract the active phytochemicals (Mishra *et al.*, 2007).

Previous investigations on the chemical composition of *Andrographis paniculata* showed that it is a rich source of deterpenoids and 2'-oxygenated flavonoids including andrographolide, neoandrographolide, 14-deoxy-11, 12-didehydroandrographolide, 14-deoxyandrographolide, isoandrographolide and 14-deoxyandrographolide 19 β-D-glucoside, homoandrograholide, andrographan, andrographosterin, stigmasterol (Pholphana *et al.*,2004; Chen and Liang, 1982; Pramanick *et al.*,2006; Kanokwan and Nobuo, 2008)

Andrographiside, deoxyandrographiside, homoandrographolide, andrographan, andrographon, andro-graphosterin (Mishra *et al.,* 2007).

The bioactive compound of the medicinal plant *Andrographis paniculata* is andrographolide. Andrographolide has highly bitter taste, is colorless crystalline in appearance, and possess a "lactone function" (Mishra *et al.,* 2007).

The leaves of *Andrographis* contain the highest amount of andrographolide (2.39%), the most medicinally active phytochemical in the plant, while the seeds contain the lowest (Sharma *et al.,* 1992).

The molecular formula of andrographolide is $C_{20}H_{30}O_5$, Andrographolide can be easily dissolved in methanol, ethanol, pyridine, acetic acid and acetone, but slightly dissolved in ether and water.

Parts Used:

Whole plant

Action

Roots and Leaves are,

Stomachic

Tonic,

Antipyretic,

Anthelmintic,

Febrifuge,

Cholagogue

Uses:

Decoction or Infusion of the leaves has been used with satisfactory results in sluggish liver, neuralgia, certain forms of dyspepsia associated with gaseous distention of the bowels (Gouty dyspepsia) In general debility, in convalescence after fevers and in advanced stages of dysentery. During epidemics of influenza a tincture of the plant is highly efficacious in arresting the progress of the diseaseVery useful in intermittent and remittent fevers, especially when combined with arsenic

Decoction or Strong infusion of the root-stalks and leaves is a household febrifuge, bitter tonic, alterative, anthelmintic and anti-periodic useful in ague or intermittent fever [6]

Phytochemical constituents in *Andrographis paniculata* Burm.f. Nees

Table 1 Phytochemical constituents in *Andrographis paniculata* Burm.f. Nees

Constituents	**Class**	**Bioactivity**	**Reference**
Andrographolide	Diterpene	Anti-inflammatory, anti-cancer, anti-microbial and hepatoprotective	Levita, *et al.*, 2010 Shen, *et al.*, 2009
Bis-andrographolide	Terpene	Anti-HIV	Reddy, et al, 2005
14-deoxy-11,12-didehyro andrographolide	Terpene	Anti-fungal	Sule, *et al.*, 2012
14-deoxyandrographolide	Terpene	Anti-fungal	
Neoxyandrographiside	Terpene	Anti-fungal	

Ninandrographolide	Terpene	Immunostimulant	Puri, *et al.*, 1993
Oxygenated flavones	Flavonoids	Anti-bacterial	Xie, *et al.*, 2015
Oroxylin A	Flavone	Anti-cancer	Li, *et al.*, 2009
Wogonin	Flavone	Anti-inflammatory, anti-cancer, anti-oxidant	Lin, *et al.*, 1996 Li-weber, *et al.*, 2009
Carvacrol	Phenol	Anti-microbial	Didry, et al., 1994 (24)
Eugenol	Ether-alcohol	Anti-septic activity	Ali, *et al.*, 2005
Myristic acid	Fatty acid	Anti-bacterial activity	Agoramoorthy, *et al.* 2007
Chlorogenic acid	Phenolic acid	Anti-nociceptive effect	Bagdas, *et al.*, 2014
Hentriacontane	Alkanes	Anti-plasmodial and larvicidal	Sowmiya, *et al.*, 2017
Tritriacontane	Hydrocarbon	Anti-oxidant	Takaba, *et al.*, 1997
Caffeic acid	Polyphenol	Clastogenic, anti-oxidant, antiviral, anti-cancer and anti-thrombosis	Hanham, *et al.*, 1983 Jiang, *et al.*, 2005
Dicaffeoylquinic acid	Carboxylic acid	Anti-oxidant	Danio, *et al.* 2009
B-sitosterol-D-glucoside	Phytosterol	Anti-inflammatory	Deepak, *et al.*, 2000

Pharmacological Evaluation:

Antidiabetic activity:

Antidiabetic property of *A paniculata* 0.4 g/kg/b.wt was confirmed by Borhanuddin *et al*. (1994) and Husen *et al.* (2004) in aqueous extract and 400 mg/kg in ethanolic extract by Zhang *et al* .(2000).

Along with antihyperglycaemic property, the ethanolic extract 400 mg/kg of *A. paniculata* may also reduce oxidative stress in diabetic rats as studied by Zhang *et al.* (2000).

Further, it was concluded by Yu BC *et al.* in 2003 1.5 mg/kg of the andrographolide was responsible for the antihyperglycemic activity. Finally in 2006, dosage of hot water (0.8 g/kg b.w.) and ethanol extracts of A. paniculata (2 g/kg b.w.) was found to restore impaired estrous cycle in alloxan-induced diabetic rats (Reyes *et.al*. 2006).

Anti-inflammatory activity:

A. paniculata can also inhibit the production of inflammatory mediators and alleviate acute hazards at its optimal dosages of 2, 2.2, and 2.4 microg/mL (Chao *et.al*. 2011).

It was also found to inhibit the tumor-specific angiogenesis by regulating the production of various pro and antiangiogenic factors by *in vivo* and *in vitro* studies in the dosage of *A. paniculata* extract 10 mg/dose/animal for 5 days (Sheeja *et.al*. 2007).

Antoxidant activity:

Sheeja *et al*. (2006) concluded that the methanolic extract of *A. paniculata* (10 mg/dose/animal) was found to inhibit the formation of oxygen derived free radicals such as superoxide (32%) hydroxyl radicals (80%), lipid peroxidation (80%), and nitric oxide (42.8%) in *in vitro* system.

Trivedi et al. studied the effect of the *A. paniculata* on antioxidant activity in mice by using the enzymes y-Glutamyl transpeptidase, glutathione-S-transferase, and lipid peroxidation compared to Benzenehexa Chloride (BHC). The activities of antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase, and the levels of glutathione were decreased following the BHC effect.

Hepatoprotective activity:

In 1993, Visen *et al.* found that andrographolide (0.75 - 12 mg/kg/p.o) had a significant dose-dependent protective activity against paracetamol-induced toxicity on ex vivo preparation of isolated rat hepatocytes.

Kapil *et al*. (1993) proved effects of *A. paniculata* on hepatotoxicity induced in mice by carbon tetrachloride in the dosage of 50, 100, 200 mg/kgb.wt of *A. paniculata* extract.

Immuno-modulatory activity

In 1993, Puri *et al.* reported that the ethanolic extract (10 micro g / mL in vitro or 1 mg/kg in vivo) and purified diterpene andrographolides of *A. paniculata* (Acanthaceae) induced significant stimulation of antibody and delayed type hypersensitivity (DTH) response to sheep red blood cells (SRBC) in mice.

Rajagopal *et al.* in 2003 suggested that andrographolide 10 μg/kg was an interesting pharmacophore with anticancer and immunomodulatory activities.

Further, in 2004, a positive anticancer and immunomodulatory activity of the methanolic extract of 10 μg/kg *A. paniculata* were screened Kumar *et al.* for human cancer and immune cells.

In 2005 Cheung *et al.* carried out the in vitro cytotoxicities of the ethanolic extract of *A. paniculata* (APE) and its main diterpenoid components evaluated in various cancer cells. APE was found to be significantly growth inhibitory to human acute myeloid leukemia HL- 60 cells with an IC (50) value of 14.01 ug/ml after 24 hours of treatment.

Wiart *et al.* found that some isolated compounds, i.e. Andrographolide, neoandrographolide, and 14-deoxy-11, 12- didehydroandrographolide, ent-labdene diterpenes showed viricidal activity against herpes simplex virus 1 (HSV-1).

Antivenom Activity

Further, aqueous extracts of A. *paniculata* were expected to be scorpion venom antidotes with low cytotoxicity. The production of interleukin-2 and interferon-gamma in normal and Ehrlich ascites carcinoma-bearing animals was elevated. latest by 2006 in an experimental study by Zhou *et al.*, it showed that the key mediators in relaying the cell death signaling initiated by Andrographolide. The facts that andrographolide was a nontoxic substance was evident from a recently reported toxicological study, 32 and was reconfirmed by the observations made in the pilot experiment described in this

communication. By contrast, even the lowest andrographolide dose tested (3 mg/kg/d) completely prevented the daily handing- and intermittent foot-shock-triggered body weight losses, and the slight elevation of core body temperatures within physiological ranges observed in control animals. Moreover, dose- and treatment regimen-dependent partial protection against transient foot-shock-triggered hyperthermia, with ED50 values around 10 mg/kg/d, was observed in the pilot as well as in both the confirmatory experiments. Because the dose and duration of treatment dependence of these observed effects of andrographolide were not identical, it seems reasonable to assume that different physiological thermoregulatory mechanisms and biological process were involved in its diazepam-like beneficial effects observed only after their fairly low daily oral doses.

3.2. VETTIVER – Vetivera zizanioides

Taxonomic Classification

Kingdom	**:**	Plantae – Plants
Subkingdom	**:**	Tracheobionta – Vascular plants
Superdivision	**:**	Spermatophyta – Seed plants
Division	**:**	Magnoliophyta – Flowering plants
Class	**:**	Liliopsida – Monocotyledons
Subclass	**:**	Commelinidae
Order	**:**	Cyperales
Family	**:**	Poaceae – Grass family
Genus	**:**	Vetiveria Bory – vetivergrass
Species	**:**	Vetiveria zizanioides (L.) Nash – vetivergrass

Vernacular Names:

Sans.	**:**	Usheera, Veeranam, Amranalam,
Eng.	**:**	Cuscus grass
Hind.	**:**	Khus, Khas bena.
Tam.	**:**	Vettiver
Mal.	**:**	Ramachham
Tel.	**:**	Kuruvaeru, Vetti-vellu, Vetti-veru
Can.	**:**	Lavanchi

Habitat:

Coromandel Coast, Mysore, Bengal, Rajputana and Chota Nagpur

Parts Used:

Fibrous wiry roots from the rhizome.

Constituents:

A volatile essential oil, resin, colouring matter, a free acid, a salt of lime, oxide of iron and woody matters

Action:

Tonic,

Refrigerant,

Stomachic,

Stimulant,

Antispasmodic,

Diuretic

Emmenagogue.

Uses:

Being a cooling medicine it is in the form of infusion a grateful refreshing drink in fevers, inflammations and irritability of the stomach.

Externally a paste of root is rubbed on the skin to remove oppressive heat or burning of the body. By mixing it with red sandalwood and a fragrant wood called padmakasta to a tub of water an aromatic bath is prepared. Its essence or oil or otto is given in two minimum doses to check the vomiting of cholera, and is used in perfumery. Grass used in the form of cigarettes and smoked with benzoin relieves headache.

Phytochemical constituents in Vertivera zizanioides

Table 2 Phytochemical constituents in Vertiveria zizanioides, L.

Constituents	**Class**	**Bioactivity**	**References**
Vetivone	Sesquiterpene	Anti-bacterial	Dos Santose, *et al.*, 2014
α-cadinene	Sesquiterpene	Anti-microbial	De Falco, *et al.*, 2013
α-calacorene	Sesquiterpene		
Epikhusinol	Sesquiterpene alcohol	Anti-fungal	Kaushal, *et al.*, 2001
Khusol	Sesquiterpene alcohol		
Khusenic acid (zizanoic acid)	Sesquiterpene	Anti-bacterial	Dwivedi, *et al.*, 2013
Zizanol	Sesquiterpene alcohol	Repellent	Khallil, *et al.*, 2011

Pharmacological Activity:

Antioxidant activity:

The essential oil of vetiver root had been showed to posses antioxident activity. Recently antioxidant activity of vetiver oil has been attribute to β-vetinine, β-vetinone and α-vetinone. Vetiveria zizanioides useful in the rehabilitation of metalliferous mine wastelands. As the presence of Pb and Zn greatly enhanced the activity of superoxidase dismutase(SOD), peroxidase (POD), catalase (CAT) 4 implying different mechanism to detoxify active oxygen species exist in different part of the plant. These result showed that vetiver oil and some of its component can be potential alternative natural antioxidants.[7]

Antibacterial Activity:

The test organisms used were *Escherichia coli* NCIM 2118; *Bacillus subtilis* NCIM 2063, *P. aeurogenosa* NCIM 2036 and *Staphylococcus aureus* NCIM 2079.*Vetiveria zizanioides* (vetiver) against *Staphylococcu saureus, Streptococcus pyogenes, Escherichia coli* and *Corynebacterium ovis* were evaluated. Against *S.aureus,* vetiver oil was superior to the other two oils in the pure state and diluted with dimethyl sulphoxide 1:10, 1:100, 1:1000 and 1:10000; inhibition by the pure oil was 60-70% that by penicillin or streptomycin.

The test organisms used were *Asperigulls nigar, Asperigulls clavatus and Candida albicanus.* All the stock cultures were obtained from Microbiology department Karpagam University, Coimbatore, India.[7]

Antitubercular activity:

Extracts and fractions were evaluated for antimycobacterial activity against Mycobacterium tuberculosis H(37) Rv and H(37)Ra strains using radiometric BACTEC 460 TB system. The ethanolic extract of intact as well as spent root were showed potent antituberculosis activity at a minimum concentration of 500μg/mL.[7]

3.3. VILAMICHU VER - Plectranthus vettiveriodes

Taxonomic Classification

Kingdom **:** Plantae

Subkingdom **:** Tracheobionta

Division **:** Magnoliophyta

Subclass **:** Lamials

Family **:** Lamiaceae

Genus **:** Plectranthus

Species **:** vettiveroides *(*Jacob*)* N.P.Singh & B.D.Sharma

Pharmacological Activity

Digestive Conditions:

Disorders of the digestive system are treated using *Plectranthus vettiveroides* species of *Plectranthus. Plectranthus vettiveriodes* are used to treat stomach pain, nausea, vomiting, diarrhoea, mouth and throat infections and are used as purgatives, carminatives and as antihelmintics. *Plectranthus vettiveroides* is also used in India to treat stomachaches, dyspepsia, nausea, and vomiting (Dash and Kashyap, 1987) and also used for hair tonic, emmenagogue in india. (Yoganarasimhan, 2000).

Antioxidant Activity:

The present *in-vivo* antioxidant activity revealed that administration of methanolic extract of *Plectranthus vettiveroides* Jacob extract showed a significant decrease in thiobarbituric acid reactive substances (TBARS) levels. The treatment also resulted in a significant increase in liver GSH, SOD, CAT, GPX levels when compared with diabetic control rats. It clearly suggest that the methanolic extract of *Plectranthus vettiveroides* Jacob treated group may effectively normalize the impaired antioxidant status in Streptozotocin induced diabetic treated groups.[8]

Antioxidant activity of ethanolic extract of C*oleus vettiveroides* was assessed by three different in-vitro model of measuring antioxidant profile *i.e.* total antioxidant

activity, FRAP assay and estimation of total flavonoid. Significant total antioxidant activity was found in ethanolic extract of C*oleus vettiveroides*. The IC50 values of the ethanolic extract of *Coleus vettiveroides* and ascorbate were found to be 180µg/ml and 410µg/ml respectively. The ethanolic extract of C*oleus vettiveroides* also shows significant result in FRAP assay method. High flavonoid content was found in ethanolic extract of C*oleus vettiveroides.* The high antioxidant capacity observed for ethanolic extract of C*oleus vettiveroides* suggests that this plant could be used as an additive in the food industry providing good protection against oxidative damage.[9]

Anti - diabetic Activity:

Methanolic extract of *Plectranthus vettiveroides* was administered to normal and experimental diabetic rats for 15 days. Significant reduction in fasting blood glucose levels was observed in the methanolic extract is treated diabetic animals from day 7 onwards. In oral glucose tolerance test, reduction in fasting blood glucose a level was noted after 60 min of extract administration. After 15 days of treatment with extracts the maximum reduction in fasting blood glucose was observed in diabetic rats treated with methanolic extract of *Plectranthus vettiveroides* (200 mg/kg & 400 mg/kg body wt) and the loss of body weight was controlled in treated rats as compared to diabetic control. The extract treatment also showed a significant decrease in level of urine sugar level of sugar level. Streptozotocin treatment leads to elevated levels of Serum Amylase, Triglycerides, Total Cholesterol, SGPT, ALP, Bilirubin, Blood Urea and Creatinine and decreased the levels of Liver Glycogen and Total Protein. However, treatment with methanolic extract of *Plectranthus vettiveroides* significantly reversed the above changes compared to the control group as observed in the streptozotocin-induced rats. Microscopically examined pancreas section of rats treated with methanolic extract showed normal architecture of pancreas. It was suggest demonstrate that methanolic extract of *Plectranthus vettiveroides* possesses significant anti-diabetic activity.[10]

3.4. SANTHANAM – Santalum album.Linn.

Taxonomic Classification

Kingdom : Plantae – Plants

Subkingdom : Tracheobionta – Vascular plants

Superdivision : Spermatophyta – Seed plants

Division : Magnoliophyta – Flowering plants

Class : Magnoliopsida – Dicotyledons

Subclass : Rosidae

Order : Santalales

Family : Santalaceae – Sandalwood family

Genus : Santalum L. – sandalwood

Vernacular Name:

Sans. : Srigandha, Sewt Chandan, Chandanam, Gandashrah, Bhadra Shree

Eng. : White Sandalwood Tree

Hind. : Safed Chandan, Sufeed Sandal

Tam. : Shandanakkattai

Mal. : Chandana maram

Tel. : Gandhapuchekka

Can. :Shrigandhadamara

Habitat:

This small evergreen tree grows wild or is cultivated in Mysore state and Coorg grown also in Coimbatore, Salem and Southern parts of Madras.When grown away from its natural habitat it tends to lose much of its essential oil for which it is esteemed in

medicine. The trees growing on hard rocky ferruginous soils are richer in oil the those growing on fertile tracts.

Parts Used:

Wood and Volatile oil.

Constituents of oil:

Santalol a body or a mixture of isomers or sesquiterpene alcohols with different boiling points, is the principal constituents of the oil, occurring therein to the extent of 90% or more. It is a mixture of two isomers known as A-Santalol and B-Santalol. The rest is composed of aldehydes santalol and ketones. E.g. isovalernic aldehyde, santonone, santalone esters, free acids etc.

Actions:

Wood is bitter, cooling, sedative and astringent

Oil is astringent and disinfectant to the mucous membranes of the genito urinary and bronchial tracts also diuretics, expectorant and stimulant.

Uses:

Sandalwood is useful in biliousness, fever and thirst. Applied externally in the form of a paste with water or rose water to scorpion stings, inflamed swellings, to prickly and skin eruptions, to the temples in headaches, and fevers and hemicrania and to skin diseases to allay itching inflammation head and pruritus.

An emulsion of the wood is used as a cooling application to the skin in erlysipelas, prurigo and sudamina (Chakaradatta). It has also been used as a diaphoretic and as an aphrodisiac. In case of Morbid thirst the power of the wood is taken tin coconut water. Two tolas of the watery emulsion of sandal wood, with the addition of sugar, honey and rice water is given to check gastric irritability and dysentery and to relieve thirst and heat of body.[11]

Power of Sandal wood made into pills or in cow milk is administered for gonorrhoea. Locally applied the powder allays prickly heat and checks copious perspiration. A powder of the sandal wood and some other ingredients smeared in ghee

and allowed to smoulder in fire and to permit the smoke to spread in all the corners of the house wards off plague attacks.[11]

Dr Henderson of Glasgow was the first to direct the attention of the European physicians to the use of the oil as a remedy for gonorrhoea and since his time it has been employed internally in many cases where copaiba and cubebs had previously failed. It is preferable to copaiba as it does not communicate an unpleasant odour to the urine nor does it so readily produce untoward effects.[11]

The famous German medicine "Salvarsaan" is said to be a preparation of the essential principles of sandal oil. Sandal oil is a popular remedy in gonorrhoea, chronic foetid bronchitis and cystitis, gleet, urethral haemorrhage and kindred affections and in pyelitis and chronic cystitis.

A mixture of the oils of sandal, of cubbs, and copaiba is generally recommended for gonorrhoea. Dose is 7 drops on sugar. In remittent fevers the oil acts as a diaphoretic. It diminished the rapidity of the hearts action.

Externally the oil is an excellent application in scabies in every stage and forms. Sandal oil mixed with its lobule the quantity of mustard oil is a good application for pimples on the nose.[11]

Phytochemical Constituents in *Santalum album* L.

Table 3 Phytochemical Constituents in *Santalum album* L.

Constituents	**Class**	**Bioactivity**	**References**
α, β-santalol	Sesquiterpenes	Chemo - prevention and antifungal activity	Kim, *et al.*, 2017
α, β-santalals, α, β-santaldiol	Sesquiterpenes		Kim, *et al.*, 2017
10(Z)-sandalnol	Sesquiterpenes	Anti-cancer activity	Kim, *et al.*, 2017
α-santalenoic acid	Sesquiterpenes	Anti-cancer	Kim, *et al.*,

		activity	2017
Vanillic acid 4-O-Neohesperidoside	Sesquiterpenes	Anti-cancer activity	Kim, *et al.*, 2017
2α,12-dihydroxy10(Z)-Campherene	Sesquiterpenes	Antifungal and cytotoxic activity	Kim, *et al.*, 2017
2β,12-dihydroxy10(Z)-Campherene	Sesquiterpenes	Antifungal	Kim, *et al.*, 2017
2,12,13-trihydroxy-10-Campherene	Sesquiterpenes	Antifungal and cytotoxic activity	Kim, *et al.*, 2017
(Z)-lanceol	Sesquiterpenes	Anti-microbial activity	Ochi, *et al.*, 2005
(Z)-7-hydroxynuciferol	Sesquiterpenes	Anti-microbial activity	Ochi, *et al.*, 2005
Eugenol-4-O-rhamnosyl glucoside	Glycoside	Anti-cancer activity	Kim, *et al.*, 2017
Methoxyeugenol-4-O-rhamnosyl Glucoside	Glycoside	Anti-cancer activity	Kim, *et al.*, 2017
2*R*-(*Z*)-campherene-2,13-diol	Sesquiterpenes	Anti-bacterial activity	Ochi, *et al.*, 2005
(*Z*)-2β-hydroxy-14-hydro-β-santalol	Sesquiterpenes	Anti-bacterial activity	Ochi, *et al.*, 2005
(*Z*)-2α-hydroxy-albumol	Sesquiterpenes	Anti-bacterial activity	Ochi, *et al.*, 2005
(*Z*)-1β-hydroxy-2-hydrolanceol	Sesquiterpenes	Anti-bacterial activity	Ochi, *et al.*, 2005

Pharmacological Activity

Hepatoprotective activity:

Hydro-alcoholic extract of 200 and 400 mg/kg of *S. album* showed significant hepatoprotective activity against CCl4 and paracetamol induced hepatotoxicity by decreasing the activities of serum marker enzymes, bilirubin and lipid peroxidation and significant increase in the levels of glutathione, superoxide dismutase, catalase and protein in a dose dependent manner, which was further confirmed by the decrease in the total weight of the liver and histopathological examinations.[12]

CNS Effects

Santalum album L. was found to possess memory enhancement potency.[13,14] Studies on sedative effect showed that inhalation of East Indian sandalwood oil decreased the motility of mice to an extent of 40-78% compared with 0% control.[15]

Sedative effect of sandalwood oil and aqueous extract had already been proved.[16,17] Sandalwood oil was reported to had a relaxing effect on the nerves and used for hot or agitated emotional states leading to headaches, insomnia and nervous tensions.[18]

Bioactive constituent, Santalols were reported to have central nervous system (CNS) depressant effects hence demonstrate implication in patients having sleep disorders.[19] In a first of its kind study, olfactory receptor neurons were identified that were specifically stimulated by four synthetic sandalwood compounds and oil.[20]

Furthermore, solvent extracts of heartwood were shown to have neuroleptic property in mice. Alpha and santalols significantly increased the levels of homovanillic acid, 3, 4-dihydroxyphenylacetic acid and/or 5-hydroxyindoleacetic acid in the brain of mice upon intra-gastric and intra-cerebro-ventricular routes of administration.[16]

Alpha-santalol was shown to be a strong antagonist of dopamine D2 and serotonine 5 HT2A receptor binding. In addition, the effect of alpha-santalol, was the same as that of chlorpromazine as an antipsychotic agent.[21] Alpha-santalol caused significant physiological changes such as relaxing and sedative effects, whereas sandalwood oil provoked physiological deactivation but behavioural activation after transdermal absorption.[22] Recently, TLC254 bioautographic assays indicated that alpha-santalol, the major constituent of the oil, was a strong inhibitor of both tyrosinase and

cholinesterase *in vitro*, and hence there was a great potential of the essential oil for use in the treatment of Alzheimer's disease as well as in skin-care.[23]

Anti-ulcer Activity

Oral treatment of 500 mg/kg of *S. album* stem hydroalcoholic extract had been reported to demonstrate good level of gastric protection in rats by effectively inhibiting physically (stress) and chemically (both Local Irritant and Drug-NSAID) induced gastric ulceration.[24]

Antibacterial activity

Several studies had focused on the antimicrobial properties of East Indian sandalwood oil [25] while many other studies focused on the Australian sandalwood oil.[26]

A comparative study conducted with 26 essential oils screened for antibacterial activities against axilla bacteria demonstrated strongest activities for sandalwood oil and their synthetic analogues.[27] Sandalwood oil was an effective antibacterial agent against Methicillin resistant *Staphylococcus aureus* and antimycotic resistant *Candida* species.[28]

Crude extract as well as α- and β-santalol compounds of sandalwood oil exhibited antibacterial activity against *Helicobacter pylori* a Gram-negative bacterium which was strongly linked to the development of duodenal, gastric and stomach ulcers.[29]

Sandal wood oil also showed activity against *Herpes simplex* virus Type 1[30] and santalol had anti-influenza activity against H3N2 virus. In another study, maximum inhibitory actions of sandalwood oil were recorded against *Bacillus mycoides* and *Escherichia coli*.[31] Methanol extract of *S. album* reported to be effective against *Bacillus subtilis, Salmonella typhi, Staphylococcus aureus* and *Pseudomonas aeruginosa* and highly active against *Candida albicans*.[32]

Sandal wood oil showed anti-dermatophytic activity against *Microsporum canis, Trichophyton rubrum* and *Trichophyton mentagrophytes*.[33] Besides, the sandalwood oil constituents, and santalol were active against *Salmonella typhimurium* and *Staphylococcus aureus* whereas episantalene was found to be active against *S. typhimurium*.[33] Santalbic acid (trans-11-octa-decen-9-ynoic acid), a major constituent of the seed, was found to inhibit gram positive bacteria and several pathogenic fungi.[34]

Santalols in high and/or medium concentrations found to be active against yeast, gram positive and negative bacteria, showed better antimicrobial efficacy even in low concentrations.[25] Further, immature tree shoots were also shown to be antibacterial against 13 bacterial strains.[35]

Antifungal Activity

Sandalwood oil was reported to possess anti-fungal activity against *Microsporum canis, Trichophyton mentagrophytes* and *T. rubrum*.[36] Sandalwood oil was found to be effective against human pathogenic fungal strains *Microsporum canis, Trichophyton mentagrophytes* and *T. rubrum* but was ineffective against *Candida albicans, Aspergillus niger* and *A. fumigates*.[37]

Antiviral Activity

The anti-viral activity of sandalwood had also been established through biological studies. Sandalwood oil had been shown to be used in prevention and treatment of warts, skin blemishes and other viral induced tumours on skin.[38,39] Traditional medicine system including Ayurvedic and Chinese medicine also mention about the antiviral potency sandalwood oil.[40]

In an *in vitro* study, 0.0015% sandalwood oil demonstrated antiviral activity against *Herpes simplex* viruses (HSV)-1 & 2 in a dose-dependent manner through inhibition of viral replication. It was further assumed that sandalwood oil helped protect the cells by modulating liver's gluthatione, S-transferase and levels of acid-soluble sulfhydryl.[41] Sandalwood oil showed *in vitro* inhibitory effect against herpes simplex virus type 2 (HSV-2) on RC-37 cells. Interestingly, sandalwood oil only affected the virus before adsorption into the cells by some non-specific inhibition of interaction between the virus and host cells.[42]

Sandalwood oil constituents, and santalols, their mixtures and derivatives had been implicated in treatment of warts in human, especially HPV and DNA pox virus that causes *Molluscum contagiosum* and speculated to be a cure against HIV and other RNA viruses, as well as dryness, flakiness and dryness associated with seborrheaic dermatitis, psoriasis and allergic or eczematous rashes of the skin as well as in the treatment of acne lesions of the face and the body and in the eradication of pustular acne lesions caused by staphylococcal acne and streptococcal bacterial infections. Additionally, sandalwood oil and santalol derivatives claimed for use in treating cold sores and herpes.[43]

Recently, single cell and somatic embryo suspension cultures of Indian sandalwood tree was demonstrated as the alternative and renewable resource of shikimic acid, the precursor for industrial-scale synthesis of Tamiflu, the sole commercially available neuraminidase inhibitor drug against Influenza A virus.[44]

Antioxidant efficacy

The phytochemical and pharmacological investigations proved the presence of antioxidant principles that justify their traditional medicinal values.[45] *S. album* and other Indian medicinal plants were tested *in-vitro* for their possible regulatory effect on nitric oxide (NO) levels using sodium nitroprusside as NO donor. Most of the plant extracts demonstrated significant direct dose dependant scavenging activity on NO.[46] It has been reported to have nitrous oxide scavenging activity and DPPH antioxidant activity.[46,47] *Santalum album* can protect cardiac tissue from oxidative stress induced cell injury and lipid peroxidation and also interferes with DOX-induced inflammatory and apoptotic induction in cardiac tissue.[48]

Recently, an anthocyanic pigment cyanidin-3-glucoside from *S. album* was shown to be antioxidant and nutritionally important.[49] Additionally, in a comparative study it was shown that *in vitro* grown callus cells demonstrated comparable antioxidant activities with sandalwood oil, using nine *in vitro* antioxidant tests.[50] Sandalwood oil increased glutathione S-transferase (GST) activity and acid soluble sulfhydryl (SH) levels in the liver of adult male Swiss albino mice.[51] Enhanced GST activity and acid-soluble SH levels were suggestive of a possible chemopreventive action of sandalwood oil on carcinogenesis through a blocking mechanism.

Similarly, methanolic extracts of sandalwood demonstrated acetyl cholinesterase inhibitory and DPPH and super oxide free radical scavenging activities in albino mice, there by indicating potential to tackle dementia and memory loss, associated with Alzheimer's disease. Recently, anti-hyperglycemic and antioxidant potential of sandalwood oil and its major constituent santalol in alloxan and D-galactose mediated oxidative stress induced diabetic male Swiss albino mice models had been demonstrated in an *in vivo* study.[52]

Haemolytic activity

Haemolytic activity of any compounds was an indicator of general cytotoxicity towards normal healthy cells.[53] In a study, it was found that leaf extract of the plant produced the lyses of RBC. However, this haemolytic activity only takes place with parenteral administration. The study revealed that the leaves of the plant contained saponins responsible for haemolytic activities against blood.[54]

Anticancer activity

Investigations had shown the chemo-preventive effects and molecular mechanisms of santalol on skin cancer development in both animal models and skin cancer cell lines.[55] Anticancer effects of oil had been reported in chemically-induced skin carcinogenesis in CD-1 and SENCAR mice, ultraviolet-B-induced skin carcinogenesis in SKH-1 mice and *in vitro* models of melanoma, non-melanoma, breast and prostate cancer and its ability to induce cell-cycle arrest and apoptosis in cancer cells had also been demonstrated.[56]

Sandalwood oil constituent, santalol delayed the papilloma development in both strains of mice.[57] Alpha-santalol at a concentration of 25-75 μM had been found to induce apoptotic death of human epidermal carcinoma A431 cells via caspase activation together with loss of mitochondrial potential and cytochrome release.[58] In a similar study, in female hairless mice strain SKH-1, topical application of santalol demonstrated chemopreventive effects as observed from reduced ornithine decarboxylase activity, tumour incidence, and multiplicity.[59]

Moreover, santalol was shown to delay skin tumour development, reduced tumour multiplicity, inhibited *in vitro* lipid peroxidation in skin and liver microsomes and hence prevented UVB-induced skin tumour development possibly by acting as an antiperoxidant.[60] Alpha-santalol reported to increase significantly apoptosis related proteins, caspases 3 and 8 levels and tumour suppressor protein p53, via an extrinsic pathway in UV B induced skin tumour development model in SKH-1 mice.[61]

In human prostate cancer cells, □-santalol induced apoptosis by causing caspases-3 activation.[62] About six novel sesquiterpenoids, two aromatic glycosides and several neolignans were identified from sandalwood heartwood chips, which were evaluated for *in vitro* Epstein-Barr virus early antigen (EBV-EA) activation in Raji cells, for assessing antitumor promoting activity.

Further, *in vivo* two-stage carcinogenesis assays demonstrated its potent inhibitory effect on EBV-EA activation strong suppressive effect on two-stage carcinogenesis on

mouse skin.[63] Moreover, derivatives of □-santalol demonstrated tumour-selective cytotoxicity in HL-60 human promyelocytic leukemia cells and TIG-3 normal human diploid fibroblasts.[64]

Two lignans obtained from the heartwood samples, demonstrated apoptosis induced tumour cell cytotoxicity against HL-60 human promyelocytic leukemia cells and A549 human lung adenocarcinoma cells.[65] α-Santalol, an active component of sandalwood essential oil had been studied for skin cancer preventive efficacy in murine models of skin carcinogenesis.[66]

Antipyretic activity

The sandalwood oil at a dose of 200 mg/kg showed highly significant antipyretic effect against yeast induced pyrexia in albino rats.[67]

Antiinflammatory activity

Santalols had been reported to possess a significant anti-inflammatory property, in several experimental models.[68]

500 mg/kg *Santalum album* possessed anti-inflammatory and antiulcer activities as evidenced by its significant inhibition in the carrageenan induced paw edema, cotton pellet induced granuloma, as well as pylorus ligation induced ulcer. These findings could substantiate the inclusion of this plant in the effective management of inflammatory disorders like ulcer in traditional system of medicine. The *in vitro* antioxidant and *in vivo* analgesic and antiinflammatory activities in mice were established for methanolic extracts of heartwood.[69]

Antihyperglycemic and antihyperlipidemic effect

Studies on antihyperglycemic and antihyperlipidemic effect of long-term oral administration of petroleum ether fraction of sandalwood in streptozotocin induced diabetic rats showed reduction in blood glucose level. Metformin treated group also showed a decrease in blood glucose as against an increase in diabetic control group. Further, total cholesterol (TC), low density lipoprotein (LDL) and triglyceride (TG) levels were decreased in treated diabetic rats whereas, cardioprotective, high density lipoprotein (HDL) were increased. Significant improvement in atherogenic index was observed that led to the conclusion that *S. album* has potential antihyperglycemic and antihyperlipidemic activities.[70]

Cardioprotective activity

Aqueous extract of sandalwood reported to inhibit significantly the cardiac tissue damage by reducing lipid peroxidation on doxorubicin induced cardiotoxicity in rat model and significant protective effect against ISO induced myocardial infarction in albino Wistar rats in dose dependant manner.[71]

Physiological effects

Sandalwood oil and its major constituent, santalol were found to affect several physiological functions and sensory stimulation. The oil reported to elevate pulse rate, skin conductance level and systolic blood pressure where as santalol elicited higher ratings of attentiveness and mood than the oil.[72] Inhalation of sandalwood oil reported to improve audibility.[73] Recently, sandalwood tea was demonstrated to increase significantly the myocardial contractility and heart rate of the isolated and failed frog heart, while it showed good effect as anti-fatigue in contracting the smooth muscle of isolated rabbit aortic strips.[74] Sandalwood oil did not demonstrate any phototoxic effects though occasional cases of irritation or sensitization reactions in humans are reported.[75]

Metabolic effects

Sandalwood oil reported to demonstrate changes in neonatal hepatic xenobiotic metabolizing enzymes in suckling mouse pups on trans-mammary exposure. It is further observed that sandalwood oil and its constituents passed through milk and modified the hepatic xenobiotic metabolizing enzymes such as increased hepatic glutathione-S-transferase, glutathione reductase and glutathione peroxidase activities, with concomitant increase in hepatic cytochrome b5 and acid soluble sulfhydryl contents and lowering of hepatic cytochrome P 450 content.[76]

Genotoxicity effects

The DNA damaging activity of sandalwood oil in *Bacillus subtilis* was studied and was found to be non-genotoxic.[77] Similarly, sandalwood oil-induced inhibition of *B. subtilis* showed it to be non-genotoxic.[78]

Genitourinary system effects

Genitourinary tract infections such as cystitis and gonorrhea had been treated by sandalwood oil for years owing to the astringent properties of the oil and its effect on the mucus membranes of genitourinary tract; helps remove mucous congestion, restore mucous membrane and minimize the risk of infections such as herpes virus.[79] These

traditional uses make sandalwood oil suitable for anti-ageing skin care, for toning effects and to prevent skin from ugly scars in modern cosmeceutical applications.

Insecticidal activities

Sandalwood oil acts as a repellent against *Varroa jacobsoni* in honey bee colonies thus used as an acaricide.[80] A modest activity against *Lycoriella mali* (the mushroom fly) is also reported.[81] The oil was also found to be impenetrable to termites.[82] Owing to its acaricidal and oviposition deterring effects, santalol was found to be active against spider mites *Tetranychus urticae*.[83,84,85]

3.5. KORAI KIZHANGU – Cyperus rotundus, Linn.

Taxonomic Classification

Kingdom : Plantae – Plants

Subkingdom : Tracheobionta – Vascular plants

Superdivision : Spermatophyta – Seed plants

Division : Magnoliophyta – Flowering plants

Class : Liliopsida – Monocotyledons

Subclass : Commelinidae

Order : Cyperales

Family : Cyperaceae – Sedge family

Genus : Cyperus L. – flatsedge

Species : Cyperus rotundus L. – nutgrass

Vernacular Name:

Sans. : Musta

Eng. : Nut Grass

Hind. : Korehi jhar

Tam. : Korai kizhangu

Mal. : Karimuttan, Korakizanna

Tel. : Tungamusti

Can. : Tangahullu

Habitat:

It is a plentiful species occurring throughout the plants of India, especially South India.

Parts Used:

Tuber or bulbous root

Constituents:

Fat, Sugar, Gum, Carbohydrate, Essential oil, Albuminous matter, Starch, Fiber and Ash. There are traces of an alkaloids.

Action:

Stimulant

Tonic

Demulcent

Diuretic

Anthelmintic

Stomachic,

Carminative

Diaphoretic

Astringent

Emmenagogue

Vermifuge

Uses:

Tubers are useful in infusion of as soup in fever, diarrhoea, dysentery, dyspepsia, vomiting, cholera etc.

Bulbous roots are scraped and pounded with green ginger, mixed with honey and given in cases of dysentery, gastric acid intestinal disorders, in doses of about a scruple.

The Romans used it as emmenagogue in uterine complaints. In larger doses it is used as an anthelmintic to get rid of worms. Fresh tubers are applied to the breast in the form of paste or warm plaster as a galactagogue. Paste is applied to scorpion stings and when dried, to spreading ulcers.

The *Shadanga Paneeya* decoction is recommended for use in fever. This decoction is given as a drink for appeasing thirst and relieving heat of the body in fever.

Phyto-chemical constituents in *Cyperus rotundus* L.

Table 4 Phytochemical constituents in *Cyperus rotundus* L.

Constituents	**Class**	**Bioactivity**	**References**
Cyperene	Sesquiterpene	Apoptotic, anti-oxidant and anti-bacterial	Ahn, *et al.*, 2015 Essien , *et al.*, 2018
β-selinene	Hydrocarbon	Anti-microbial and anti-oxidant	Chandra, *et al.*, 2017
Cyperenone	Sesquiterpene	Antiulcer	Berger, 2007
α-cyperone	Sesquiterpene	Selective cytotoxic, anti-inflammatory and nueroprotective	Al-snafi, 2016
4α-5α,oxidoeudesm-11-en-3α-ol	Sesquiterpenic oxido alcohol	Anti-hepatitis B virus	Hikino, *et al.*, 1976
Copadiene	Sesquiterpene	Anti-malarial	Khoi, 1999
Epoxyguaiene	Essential oil	Anti-oxidant, Anti-malarial and Anti-diabetic	Khoi, 1999
Rotundone	Sesquiterpene	Anti-mutagenic	Kilani, *et al.*, 2005
Cyperenol	Sesquiterpene	Hypotensive and anti-microbial	Sahu, *et al.*, 2010
Eugenol	Ether-alcohol	Antiseptic	Didry, *et al.*,

			1994
Cyperol	Sesquiterpene	Insecticidal	Pubchem
Isocyperol	Sesquiterpene	Anti-inflammatory	Seo, *et al.*, 2016
α-and β-rotunol	Sesquiterpene	Fungitoxic	Hiking, *et al.*, 1971
Kobusone	Sesquiterpene	Anti-inflammatory and Analgesic	Ross, 2003
Isokobusone	Sesquiterpene	Anti-inflammatory	Kittayaruksakul, *et al.*,2013

Pharmacological Activity

Antidepressant, Anxiolytic, Anticonvulsant And Hypnotic And Muscle Relaxant Activities:

Haja Sherief Sheik et al., was reported the antidepressant, anxiolytic, anticonvulsant and hypnotic and muscle relaxant activities in two different animal models to find out its scientific values. Oral administration of *Cyperus rotundus* Ethanolic Extract (EECR) at doses of 200 and 400 mg kg G1 on various behavioural models such as tail suspension, hole-board, elevated-plus-maze, locomotor, strychnine, maximal electroshock induced seizure, pentylenetetrazole, rotarod, climbing an inclined screen in mice and forced swim light-dark box models in rats was utilized. In the open field test, EECR (200 and 400 mg kgG1) ($p<0.05$, $p<0.01$) increased in numbers of rearing. However, the number of central motor and ambulation reduced. The number of entries and the time spent in the open arm were increased while the number of locomotion was decreased ($p<0.01$) in elevated-plus-maze and actophotometer test, respectively. The EECR (200 and 400 mg kgG1) protected the mice against the pentylenetetrazole and strychnine induced convulsions; it causes significant ($p<0.05$ and $p<0.01$) dose dependent increase in latency of convulsion. Treatment with EECR decreased the duration of the tonic hind limb extension induced by electroshock. The EECR treatment also significantly increased the hypnotic's time and decreased motor co-ordination of experimental animals. These

findings were consistent with the hypothesis that *C. rotundus* treatment triggers immobility behavior, time spent in light, locomotor and climbing time in rat and mice model.[86]

Anti Inflammatory Activity

400 mg/kg of alcoholic extract of *C. rotundus* possessed anti inflammatory activity against carrageenan induced oedema and also found effective against formaldehyde induced arthritis in albino rats.[87]

In another study the petroleum ether extract of the rhizomes 200, 400 mg/kg showed anti-inflammatory activity against carrageenan induced oedema in albino rats. The triterpenoid obtained by chromatographic separation from petroleum ether extract revealed a high potent anti-inflammatory activity. This terpenoid was also found to possess significant antipyretic and analgesic effects similar to acetyl salicylic acid. *C.rotundus* had also reported as protective in inflammatory bowel disease.[87]

In addition, the 200 mg/kg extract suppressed the production of O_2 - by phorbol ester stimulated RAW 264.7 cells. Collectively, these results suggest that the methanol extract of rhizomes of *C. rotundus* could be developed as anti-inflammatory candidate for the treatment of inflammatory diseases mediated by overproduction of NO and O_2.[88]

Another study on alcoholic extract 400 mg /kg of *C. rotundus* showed highly significant ($P<0.001$) anti-inflammatory activity against the exudative and proliferative phases of inflammation in two animal models (carrageenan induced oedema and formaldehyde induced arthritis in rats). Its anti-inflammatory relative effect was higher than that of hydrocortisone (75.9% versus 47.3% in carrageenan-induced oedema model; 55.1% versus 35.6% in formaldehyde induced arthritis model.[89 - 92]

Antipyretic activity

The 200 mg/kg of 70 % alcoholic extract of *C. rotundus* showed highly significant ($P<0.001$) antipyretic activity against pyrexia produced in albino rats by the subcutaneous injection of suspension of dried Brewer's yeast in gum acacia in normal saline. A specific fraction obtained by chromatographic method from the petroleum ether extract was found to possess a significant anti-pyretic effect similar to acetyl salicylic acid when used on the same animal model.[93]

Analgesic activity

The petroleum ether extract and 500 mg/kg essential oil of *C.rotundus* were reported to possess analgesic activity.[93,94]

Tranqulizing activity

The 400 mg/kg ethanolic extract of *C. rotundus* showed potent tranquilizing activity in various tests: reduced the spontaneous motor activity, potentiated the pentobarbital narcosis and deranged the motor coordination, abolished the conditioned avoidance response in animals.[90]

Anticonvulsant activity

Pretreatment with 80 mg/kg ethanolic extract of *C.rotundus* caused significant protection against strychnine and leptazol-induced convulsions in mice.[95]

The ethanol extract of rhizomes (100mg/kg, p.o.) reduced hind limb extension and duration of convulsion significantly, ($p<0.001$) which was comparable to standard drug Phenytoin (25mg/kg, i.p.) and Diazepam (4mg/kg, i.p.), respectively. These results suggested that the ethanol extract of its rhizomes is worthwhile to develop the potent phytoconstituent for treatment of epilepsy and the flavonoids present in ethanol extract could be attributed for aiticonvulsant activity.[96]

Anti-emetic activity

The ethanolic extract of *C. rotundus* in the dose of 128.1± 11.6 mg/kg was found to protect 50% dogs against apomorphine induced vomiting.[90]

Antispatic activity

500 mg/kg of Ethanolic extract of *C. rotundus* produced relaxation of rabbit ileum and spasmolytic effect against contractions induced by acetylcholine, barium chloride and 5-hydroxitriptamine, showed a direct relaxant action on the smooth muscle.[90]

Inhibition of gastric motility activity

The rhizome of *C.rotundus* Linn. was assessed for its cytoprotective effects against ethanol induced gastric damage. Decoctions of Rhizoma Cyperi were given orally to rats 30 min. before ethanol was administered. The findings in this study suggested that the protective action of *C. rotundus* Linn. is related to its inhibition of gastric motility and endogeneous prostaglandins may play an important role.[97]

Gastroprotective activity

C.rotundus extract protected against gastric mucosal injury induced by ischemia and reperfusion in rats. The mean ulcer index of rats treated with 200 and 100 mg/kg *C. rotundus* were significantly lower than that of control. The activities of glutathione-peroxidase and malondialdehyde were significantly affected by treatment of *C. rotundus.*[98]

Cytoprotective effects of *C.rotundus* had been mentioned also in case of ethanol induced gastric damage in rats. Decoctions of Rhizoma Cyperi were given orally (1.25, 2.5, 4.0 g crude drug/kg) to rats 30 min before ethanol showed an ulcer inhibitory effect in a dose dependent manner. Pretreatment of rats with indomethacin (5 mg/kg) significantly reduced the gastric protective action of *C. rotundus*. The authors suggested that the gastroprotective action of *C.rotundus* was related to its inhibition of gastric motility and endogeneous prostaglandins.[97]

Antidiarrhoeal Activity

The methanol extract of *C. rotundus* rhizome, given orally at the doses of 250 and 500 mg/kg showed significant antidiarrhoeal activity in castor oil induced diarrhoea in mice. Among the fractions, tested at 250 mg/kg, the petroleum ether fraction and residual methanol fraction were found to retain the activity, the latter being more active as compared to the control. The ethyl acetate fraction did not show any antidiarrhoeal activity.[99]

Hypolipidaemic Activity

Administration of 200 - 400 mg/kg/day *C. rotundus* extract restored the age associated change in serum lipids (total cholesterol, LDL cholesterol, DL cholesterol, triglycerides and VLDL triglyceride level) to the level of young control rats. In young rats, treatment of *C. rotundus* significantly increased HDL cholesterol level.[100]

Hepatoprotective activity

Ethyl acetate extract and two crude fractions, solvent ether and ethyl acetate, of the rhizomes of *C. rotundus* (Cyperaceae) were evaluated for hepatoprotective activity in rats by inducing liver damage by carbon tetrachloride. The ethyl acetate extract at an oral dose of 100 mg/kg exhibited a significant protective effect by lowering serum levels of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase

and total bilirubin. These biochemical observations were supplemented by histopathological examination of liver sections. Silymarin was used as positive control.[101]

Inhibitory activity on Brain Na +/K+-ATP-ase

100 mg/kg of Extract of *C. rotundus* showed high potent inhibitory activity on crude enzyme Na+/K+-ATP-ase from rat brain.[102]

Anti-obesity activity

220 mg/kg of *C. rotundus* preparation (powder in fine suspension, aqueous and alcoholic extracts) exhibited a lipolytic action and mobilized fat from the adipose tissues in rats, thus helping to reduce the obesity.[103]

A pilot study carried out on 30 obese people who were administered the powdered tuber 400 mg/kg of *C. rotundus* for 90 days, showed reduction in weight along with a decrease in serum cholesterol and triglycerides.[104]

Antiarthritic activity

A double blind trial of crude powder of *C. rotundus*, *Withania somnifera* and their combination (1:1) was carried out in 200 patients suffering from rheumatoid arthritis. Out of the 200 patients selected for the study 196 completed the trial of 3 months. Each group (including placebo group) consisted of 50 patients. Each patient received 500 mg capsule three times a day for three months. During this period biweekly general assessment based on global criteria (duration of morning stiffness, grip strength, articular index, consumption of escape analgesic, erythrocyte sedimentation rate, haemoglobin, rheumatoid factor titre, x-ray findings) was made. *C. rotundus* was more effective than W. somnifera, and when both drugs were combined, the response was better than the response of single drug. Also the patients' preference (against escape analgesic) was highest in the case of combined herbs.[105]

Wound healing activity

An alcoholic extract of tuber parts of *C.rotundus* was examined for wound healing activity in the form of ointment in three types of wound models on rats: the excision, the incision and dead space wound model. The extract ointments showed considerable

difference in response in all the above said wound models as comparable to those of a standard drug nitrofurazone ointment (0.2 % w/w NFZ) in terms of wound contracting ability, wound closure time and tensile strength.[106]

Anticancer activity

Anticancer *C. rotundus* ethanolic extract was found to have only weak to moderate anticancer activity (LC50=2.528-4.939 mg/ml calculated from dose-dependent cell death) in a study which used neuro-2a cells for screening of plants with tumoricidal effects.[107]Another study showed that *C.rotundus* essential oil was very effective against L1210 leukaemia cells line. This result correlated with significantly increased apoptotic DNA fragmentation.[108]

Antidiabetic Activity

Oral daily administration of 500 mg/kg of the extract (once a day for seven consecutive days) significantly lowered the blood glucose levels in rats with alloxan induced diabetes. The scientists concluded that this antihyperglycemic activity can be attributed to its antioxidant activity as C. rotundus showed a strong 1,1-diphenyl-2-picryihydrazyl (DPPH) radical scavenging action *in vitro*. These results were convergent with C. rotundus potential to suppress AGE formation and protein oxidation in a model of fructose-mediated protein glycoxidation. Scientists concluded that, since non-enzymatic glycation had been shown to correlate with severity of diabetes and its complications, C. rotundus could be a candidate for targeting diabetic complications.[109, 110]

Antimicrobial activity

In-vitro antimicrobial activity by agar disc diffusion and agar well diffusion method was evaluated for aqueous and ethanolic extracts. The 0.50 mL of ethanolic extract was active against all the investigated bacterial strains, while aqueous extract was inactive. In another study acetone and ethanol extracts showed significant broad spectrum antibacterial activity in disc diffusion method.[111]

Antimicrobial activity tests were carried out on human pathogens bacteria (gram negative and gm positive) and fungi viz. *C.albicans* and *A. niger*. The highest percentage of inhibition was observed against *K.pneumoniae* (133.33%). Amoxicillin 20µg/ml and ethanol (as fungicide) 70% were used as positive control. Moderate inhibition was

observed in case of *A. niger* and *S. aureus* (90 and 70% respectively). No zone of inhibition was observed in *Acinto bacter* and *Candida.*[112]

Antimalarial Activity

Activity guided investigation of sesquiterpenes *C.rotundus* rhizomes showed *in-vitro* antimalarial activity against Plasmodium falciparum.[113]

Some Tanzanian medicinal plants were extracted and tested for in vitro antimalarial activity, using the multidrug resistant K1 strain of Plasmodium falciparum. Of the forty-nine plants investigated, extracts of three plants were found to have an IC50 between 5-10 mg/ml; extracts of 18 other plants showed an IC50 between 10 and 50 mg/ml, all others were less active. The three most active extracts were obtained from the tubers of *C. rotundus* Linn. the root bark of *Hoslundia opposita* Vahl. and the root bark of *Lantana camara* L.[114]

The underground parts of several weedy species contain essential oils, about 0.5-1% in the case of the fresh tubers of *C. rotundus*, mainly consisting of terpenoids or sesquiterpenoids (e.g. cyperone, cyperol, cyperolone, cyperene, copadiene, epoxy-guiaene, rotundone, rotundol, patchoulenone (cyperotundon), kobusone, sugeonolacetate, sugetriol, oxido-eudesmenol, C. rotunduskone and 'BETA'-selinene).When Tanzanian medicinal plants were screened, *C. rotundus* showed activity in a test for in vitro antimalarial activity.[115]

Cytoprotective effects

The rhizome of *C. rotundus* was assessed for its cytoprotective effects against ethanol induced gastric damage. Decoctions of Rhizoma Cyperi were given orally (1.25, 2.5, 4.0 g crude drug/kg) to rats 30 min before ethanol (40% v/v, 10mL/kg) was administered. The decoction showed an ulcer inhibitory effect in a dose dependent manner. Moreover, the activity was also observed when the decoction was given subcutaneously (0.3-0.6 g/kg), suggesting that the herb possessed systemic effects on protecting the stomach. Compared with controls, gastric motility of the ethanol-treated rats was delayed significantly by either oral (2.5-4.0 g/kg) or subcutaneous (0.3g/kg) administration of the decoction. Pretreatment of rats with indomethacin (5 mg/kg) significantly reduced the gastric protective action of *C. rotundus*.[116]

Antigenotoxic activity

The activation of microsomal enzymes leads to the generation of various embolic intermediates and ROS involved in DNA breakage. Natural phenolic compounds inhibited other chemical activities such as the antioxidant, antimutagenic, and anti-inflammatory effects (Jahan *et al.,* 2012; Lydia and Sundarsanam, 2012; El-Karеam, 2012).

Flavonoids and tannins detected in *C. rotundus* extracts synergistically exhibited antigenotoxic activity (Lee *et al.,* 2003; Baratto *et al.,* 2006).

The TOF and ethyl acetate extracts were found to be effective in reducing the production of thiobarbituric acid-reactive substance (TBARS) and protecting against H2O2/UV-induced DNA damage (Kilani-Jaziri *et al.,* 2009).

In addition, the TOF extract exerts an antioxidant effect on the growth of K562 cells through the induction of apoptosis. Luteolin was found to be an active ingredient in reducing TBARS production and K562 cell proliferation. The antigenotoxic potential of different *C. rotundus* extracts was evaluated against 10 μg/assay of nifuroxazide and AFB1-induced genotoxicity (Kilani-Jaziri *et al.,* 2011).

At concentrations of 200 and 500μg/assay, the TOF and ethyl acetate extracts were found to significantly reduce the influence of AFB1-induced genotoxicity to 87.16% and 73.68%, respectively.

Hemanth-Kumar *et al.* (2013) observed the inhibition of nifuroxazide- and AFBI-induced genotoxicity by different *C. rotundus* extracts. In contrast to the aqueous and methanolic extracts, at 200μg/assay, the TOF and ethyl extracts, significantly reduced the genotoxic effect via the necessary metabolic stimulations. These metabolic stimulations inhibited the activation of microsomal enzymes to 87.16% and 73.68%, respectively; further, they protect DNA strands from electrophilic metabolites of the mutagens.

Anti-Uropathogenic activity:

On investigating the anti-uropathogenic property of *C. rotundus,* Sharma *et al.* (2014) suggested that the crude extract of *C. rotundus* possesses significant anti-uropathogenic activity at 2.5–10 mg/ml against multidrug-resistant uropathogens with a significant inhibition zone of 8–30 mm, justifying its use in the treatment of urinary tract infections.

Lactogenic activity

Oral administration of 300–600 mg of the aqueous extract of *C. rotundus* significantly improved the milk yield (23–40%) and pup b.w. (8.82–35.78 g/pup per day). The aqueous extract stimulated prolactin synthesis and increased the weights of the pups and the mother, as well as the protein and carbohydrate content in the mammary glands. In addition, all the treated experimental groups showed the development and differentiation of the lobulo-alveolar system with milk secretion. However, as the ingredients responsible for the lactogenic activity of *C. rotundus* were still unknown, studies on the isolation, purification, and characterization of these ingredients were necessary for developing herbal medicine for lactating women.

Nootropic activity

Soman *et al.* (2013) confirmed this effect by inducing acute memory loss with a 2-mg/kg dose of midazolam in rats specifically targeting the taste aversion memory. The transfer latency in rats was measured at the time of acquisition, consolidation, and retrieval using an elevated plus maze apparatus.

Oral administration of 100–200 mg/kg of the *C. rotundus* ethanolic extract resulted in significantly increased memory at retrieval compared with the control. Although the TOF of *C. rotundus* was assumed to amend these neurological alterations, more research on the responsible chemical constituents and their mode of action is warranted.

3.6. PARPADAGAM – Hedyotis carymbose

Taxonomic Classification:

Kingdom	**:**	Plantae
Division	**:**	Embryophytasiphongama
Subdivision	**:**	Angiospermae
Class	**:**	Dicotyledonae
Order	**:**	Gentianales
Family	**:**	Rubiaceae
Genus	**:**	Oldenlandia
Species	**:**	Corymbosa

Vernacular Name:

Sans. **:** Parpata, parpataka, Kshetraparpata

Eng. **:** Flat top mille grains, Old World Diamond flower, Five leaved fumitory

Hind. **:** Daman pappar, pitpapra

Tam. **:** Parpatagam, kattucayaver, pappanpuntu

Mal. **:** Parpatakapullu

Tel. **:** Verrinella- vemu

Can. **:** Parpatahullu, Kallasabatrasige

Pharmacological Activity:

Anticancer Activity:

The ethanolic extract of leaves of *Hedyotis corymbosa* had shown significant anticancer activity on k 562 human leukemia cell lines. The cell viability was measured by SRB (sulforhodamine B) assay. The cell lines were grown under RPMI1640 medium containing 2 mml - glutamine, 10 % fetal bovine serum. The result was recorded on ELISA plate reader at 540 nm to 690 nm wavelength. The non toxic dose of *H.corymbosa* showed anticancer activity as compared to the standard drug adriamycin.[117]

The anticarcinogenic property of methanolic extract of the whole plant was studied by Microculture tetrazolium salt (MTT) assay on the MCF-7 human breast carcinoma dependent hormone cell lines. The highest anticancer activity on MCF-7 cell

line observed with IC 50 value of 22.67µg/ml. The anticancer activity of the plant extract was mainly due to its antioxidant activity.[118]

Hepatoprotective Activity

Hedyotis corymbosa showed significant hepatoprotective activity against Perchloroethylene, Carbon tetrachlorideand D-Galactosamine induced liver damage in experimental animals.

Hepatoprotective action of ethanolic extract of *Hedyotis corymbosa* on perchloroethylene induced hepatic damage was studied in wistar albino female rats. The extract was administered orally at the dose of 400 mg/kg of bodyweight for ten days, showed significant reduction in liver marker enzymes (AST, ALT, LDH), lipid peroxidation and with significant increase in antioxidant enzyme levels. The results indicates *H.corymbosa* had potent hepatoprotective activity upon perchloroethylene induced hepatic damage in rats and also had antilipidperoxidative and free radical scavenging activities.[119]

The hepatoprotective activity of ether, ethanol, butanol,butanone, petroleum ether and ethyl acetate extract fraction of *H.corymbosa* against CCl4 induced hepatic damage was evaluated in albino rats (200-250g). Acute toxicity study was carried out in albino mice of either sex for determining LD 50 values for different extracts. The petroleum ether and ethyl acetate extract did not show any significant hepatoprotective activity. The elevated levels of SGPT and SGOT, were significantly decreased in ether and butanol extracts at $P < 0.001$ and in butanone and ethanol at $p < 0.005$. The enzymatic levels and histopathological studies showed that ether, butanol, ethanol, butanone extracts of *H.corymbosa* had hepatoprotective activity in CCl4 induced hepatic damage.[120]

Antihepatotoxic potential of methanolic extracts of *H.corymbosa* against D-Galactosamine induced hepatotoxicity in wistar rats was studied. Increased levels of marker enzymes with D-galactosamine (AST, ALT, ALP, γ-glutamyltransferase) were significantly reduced by *H.corymbosa* extract. The significant reduction in lipid peroxidation was observed at the dose of 200 mg/kg.[121]

Antiulcer

The alcoholic and aqueous extract of whole plant of *Hedyotis corymbosa* had shown significant antiulcer activity against aspirin in rats. The alcoholic and the aqueous

extract were administered in two doses 200 mg/kg and 400 mg/kg by oral route 45 minutes prior to the administration of aspirin. The standard drug lansoprazole 8 mg / kg was used for the comparison. Both the extract showed significant decrease in ulcer compared to control group. Antiulcer effect was characterized by reduction in ulcer index, gastric volume, free acidity, total acidity and pH. The protection percentage in alcoholic and aqueous extract at 200 mg/kg, 400mg/kg showed 65.7%, 33% respectively in comparison with standard lansoprazole 88.89%.[122]

Antioxidant

The antioxidant activity of methanolic extract of aerial parts of *H.corymbosa* was determined by different invitro methods such as; 1,1 diphenyl-2-picryl hydroxyl (DPPH) assay, 2,2'-azinobis-3 ethylbenzothiozoline-6-sulfonic acid (ABTS) cation decolorization test, ferric reducing power(FRP), scavenging capacity towards hydroxyl ion (OH.) radicals and nitric oxide (NO) radical inhibition assay. The methanolic extract of aerial part showed high antioxidantactivity against DPPH, ABTS, Nitric oxide and hydroxylradical at 82, 130, 150, 170 µg/ml respectively. The study showed that *H.corymbosa* extract effectively attenuated the oxidative stress via antioxidant property.[123]

Analgesic

Oral dose of 250 and 500 mg/kg of ethanolic extract of *H.corymbosa* showed significant analagesic activity in mice using three different models; hot plate reactiontime, acetic acid writhing test and formalin induced pain method, with ketorolac as standard drug. Formalin test procedure revealed the involvement of both peripheral and central mechanism. The acetic acid writhing test involved the peripheral mechanism and the hot plate method involves the central mechanism. The ethanolic extract of *H.corymbosa* showed significant antinociceptive effect in 250 and 500 mg/kg, but more significant effect was observed at 500 mg/kg.[124]

Anti malarial

Antimalarial activity of the methanolic extract of *H.corymbosa* was studied by both invitro and invivo methods. The plant extract showed significant antimalarial activity on chloroquine sensitive (MRC-pf20) and chloroquine sensitive (MRC-pf.303) stains of plasmodium falciparum. In-vivo antimalarial activity of the plant was studied

using mice. Drug treatment was initiated 1 day (24 hr) prior to the parasite treatment starting from 4thday of post infection. Every alternate day, the blood was collected from tail to check the level of parasitaemia. The combination of plant extract with curcumin showed more effective antimalarial activity.[125]

Antibacterial

Methanolic extract of *H.corymbosa* was evaluated for its antibacterial activity by disc diffusion method against gram positive and gram positive bacteria (*Bacillus, Klebisella, Escherichia coli, proteus, staphylococcus aureus and pseudomonas*). The plant extract was observed to inhibit the growth of both gram positive and gram negative bacteria significantly and had broad spectrum of anti bacterial activity. The order of inhibition was found to be *Proteus* (22mm) *Pseudomonas* (26mm) *Bacillus* (27mm) *Staphylococcus aureus* (28mm) *Escherichiacoli* (32mm) *Klebsiella* (33mm).[126]

Antifungal

The whole plant extract showed significant antifungal activities against *Candida albicans* and *Aspergillus nigar*. The maximum antifungal activity was found in *Candida albicans*. The Antifungal activity was due to the presence of the constituents like, steroids and glycosides.[126]

Uterine Contraction

The ethanolic extract of *Hedyotis corymbosa* showed significant effect on uterine contraction, this was observed in the isolated uterine horn preparation of virgin female sprague Dawley rat. The extracts were tested in different concentration 0.014, 0.14, 0.44 and1.40 mg/ml. De. Jalon solution was used as the physiological solution and the response was compared against the standard (acetylcholine) and blank (ethanol).[127]

3.7. PEIPUDAL – Tricosanthes cucumerina,Linn.(Dioica)Roxb.

Taxonomic Classification

Kingdom : Plantae – Plants

Subkingdom : Tracheobionta – Vascular plants

Superdivision : Spermatophyta – Seed plants

Division : Magnoliophyta – Flowering plants

Class : Magnoliopsida – Dicotyledons

Subclass : Dilleniidae

Order : Violales

Family : Cucurbitaceae – Cucumber family

Genus : Trichosanthes L. – trichosanthes

Vernacular Name:

Sans. : Patola

Eng. : Wild snakegourd

Hind. : Palwal, Parvar

Tam. : Peipudal

Mal. : Kaattu potolalm

Tel. : Adavipatola

Can. : Kahipadavala

Habitat:

Common in Bengal and cultivated in Northern India, the Punjab and Baroda.

Parts Used:

Stems, Roots

Action:

Fruit is febrifuge, laxative and antbilious

Juice of leaves and the fruit is a cholagogue and aperient.

Root is a drastic purgative [128]

Unripe fruit is eaten and is especially suited for convalescents

Leaves are tonic and febrifuge, are used as diet in subacute case of enlarged liver and spleen, Pitha variety Arsa (Piles) and fistula in ano, when there is no fever as it checks pitta.

Decoction is a reputed expectorant. Chakkaradatta recommends a decoction "*PatoladiKvatha"* in fevers. Another compound decoction useful as a valuable alterative, tonic and febrifuge given in boils and other skin diseases, the old physicians placed much confidence in it in the treatment of leprosy.

A compound poder known as*patoladyachooranam*is used as a drastic purgative in jaundice, anasarca and the ascites. Dose I drachm with cow urine. After the use of this medicine light food only such as gruel should be taken.

Fruit of the bitter variety is used in scorpion sting.

Phytochemical constituents in *Trichosanthes cucumerina* L.

Table 5 Phytochemical constituents in *Trichosanthes cucumerina* L.

Constituents	**Class**	**Bioactivity**	**References**
Bryonolic acid	Triterpenoid	Neurotoxic activity, anti-inflammatory activity	Que, *et al.*, 2016 Gatbonton-Schwager, *et al.*, 2012
Cucurbitacin B	Triterpenoid	Inhibition of carcinoma cells	Piao, *et al.*, 2018

Cucurbitacin E	Triterpenoid	Anti-cancer and immunomodulatory actions	Attard, *et al.*, 2015
Isocucurbitacin B	Triterpenoid	Cytotoxic	Bean, *et al.*, 1985
β-sitosterol	Phytosterol	Anti-cancer and anti-atherogenic	Mahaddalkar, *et al.*, 2015, Zhao, *et al.*, 1990
Stigmasterol	Phytosterol	Anti-cancer and anti-trypanosomal	Aminu, *et al.*, 2017
23, 24-dihydrocucurbitacin	D Triterpenoid	Anti-inflammatory activity	Park, *et al.*, 2004

Pharmacological Activity:

Antiinflammatory Activity

Devendra *et al*. reported anti-inflammatory activity of tricosanthes cucumerina in carrageenan induced paw oedema in rats. The extracts (chloroform and ethanol) were administered orally 30 min prior to carrageenan administration. The paw volume was measured at intervals of 60, 120, 180 and 240 min by the mercury displacement method using a plethysmograph. The percentage inhibition of paw volume in drug treated group was compared with the carrageenan control group (Group- I). Diclofenac sodium (5 mg/kg/p.o.) was used as reference drug. Significant effect on drug treated group in the dose of ethanol extracts of *T. cucumerina L. var. cucumerina* seed at the dose level of 200 and 400 mg/kg was reported.[129]

Antifertility Activity

Ethanol extract of whole plant of *Trichosanthes cucumerina* L. var. *cucumerina* was evaluated for antiovulatory activity in adult rats. The ethanol extract at the doses 200 and 400mg/kg body weight (orally) affected the normal estrous cycle showing a

significant increase in estrus and metestrus phases and decrease in diestrus and proestrus phases. The extract also significantly reduced the number of healthy follicles (Class I-Class VI) and corpora lutea and increased the number of regressing follicles (Stage IA, Stage IB,Stage IIA, and Stage IIB). The protein and glycogen content in the ovaries were significantly reduced in treated rats. The cholesterol level was significantly increased, whereas, the enzyme activities like 3b-HSD and 17b-HSD were significantly inhibited in the ovary of treated rats. Serum FSH and LH levels were significantly reduced in the treated groups were measured by RIA.[130]

3.8. SUKKU – Zingiber officinale

Taxonomic Classification

Kingdom : Plantae – Plants

Subkingdom : Tracheobionta – Vascular plants

Superdivision : Spermatophyta – Seed plants

Division : Magnoliophyta – Flowering plants

Class : Liliopsida – Monocotyledons

Subclass : Zingiberidae

Order : Zingiberales

Family : Zingiberaceae – Ginger family

Genus : Zingiber Mill. – ginger

Species : Zingiber officinale Roscoe – garden ginger

Vernacular Name:

Sans. : Srangavera, Sringaberam (dried) – Sunta, Nagara, Nagaram

Eng. : Ginger

Hind. : Duk

Tam. : Chukku (dried)

Mal. : Chukka

Tel. : Sonti (dried)

Can. : Vona shunti (dried)

Habitat:

Ginger is cultivated in many parts of India. On large scale in the warm, moist regions, chiefly in Madras, Cochin and Tranvacore, and to a somewhat less extent in Bengal and the Punjab.

Parts Used:

Scraped and dried rhizomes as well as the green ones.

Actions:

Aromatic

Carminative

Stimulant to gastro intestinal tract

Stomachic

Sialagogue and Digestive

Externally,

A local stimulat and rubefacient.

Uses:

Ginger is extremely valuable in dyspepsia, flatulence, colic, vomiting, spasms and other painful affections of the stomach and the bowels unattended by fever.

For cold, cough, asthma, dyspepsia and indigestion is highly recommended a preparation called “Allaepauk” of “Ginger jam or Conserve, it consist of ginger juice water and sugar in sufficient quantities, boiled down to the consistence of a syrup, and to which are added saffrom, cardamoms, nutmeg and cloves all in powder, and preserved in a well Stoppard bottle, chinaware or earthenware

Phytochemical constituents in *Zingiber officinale* Roscoe

Table 6 Phytochemical constituents in *Zingiber officinale* Roscoe

Constituents	**Class**	**Bioactivity**	**References**
6-Shogaol	Phenol	Anti-inflammatory, anti-cancer and anti-oxidant	Li, *et al.*, 2012, Zhu, *et al.*, 2013, Bak, *et al.*,2012
6-Gingerol	Phenol	Anti-cancer, anti-inflammatory and anti-oxidant activity	Weng, *et al.*, 2014
Zingiberol	Sesquiterpene alcohol	Anti-cancer activity	Ezebuo, *et al.*, 2016
β-phellandrene	Monoterpene	Anti-bacterial activity	Utegenova, *et al.*, 2018
α-zingiberene	Sesquiterpene	Anti-cancer activity	Aras, *et al.*, 2014
Ar-curcumine	Sesquiterpene	Anti-oxidant and anti-microbial activity	El-Baroty, *et al.*, 2010
β-bisabolene	Sesquiterpene	Cytotoxicity against breast cancer cells	Yeo, *et al.*, 2015
Gingerenones A, B & C	Diarylheptenones	Anti-fangal activity	Endo, *et al.*, 1990
Isogingerenone B	Diarylheptenones	Anti-fangal activity	Endo, *et al.*, 1990
Hexahydrocurcumin	Diarylheptenones	Anti-inflammatory and antioxidant	Li, *et al.*, 2012
Gingerdiols	Phenol	Anti-microbial	Pubchem

		activity	
6-gingesulphonic acid	Methoxy phenols	Anti-ulcer property	Yoshikawa, *et al.*, 1994
Gingerglycolipids A, B & C	Glycerol	Anti-ulcer property	Yoshikawa, *et al.*, 1994
Paradols	Ketone	Anti-oxidative and anti-cancerb	Pubchem
Farnesol	Alcohol	Apoptotic activity	Rioja, *et al.*, 2000
Geraniol glycosides	Terpene glycoside		
α-santalol	Sesquiterpene	Chemoprevention and antifungal Activity	Kim, *et al.*, 2017
β-eudesmol	Sesquiterpene	Anti-inflammatory activity	Kim, *et al.*, 2017
Nerolidol	Sesquiterpene	Anti-inflammatory activity	Pubchem
Elemol	Sesquiterpene	Insecticidal activity	Pubchem
1,8 cineole	Phenol	Repellent	Tripathy, *et al.*, 2001
α-pinene	Monoterpene	Anti-inflammatory and anti-microbial Activity	Pubchem, Silva, *et al.*,2012
β-pinene	Monoterpene	Anti-microbial activity	Silva, *et al.*, 2012
Camphene	Monoterpene	Anti-microbial	Pubchem

		activity	
Sabinene	Monoterpene	Anti-oxidant and repellent	Jeramillo, *et al.*, 2012
Limonene	Cyclohexene	Anti-crcinogenic	Elson, *et al.*, 1988
Myrcene	Monoterpene	Analgesic activity	Lorenzetti, *et al.*, 1991

Pharmacological Activity

Antiplatelet activity

5, 10, 20, 30 and 40 μL aqueous extract of ginger inhibited platelet aggregation induced by ADP, epinephrine, collagen and arachidonic acid *in vitro*. Ginger acted by inhibiting thromboxane synthesis. It also inhibited prostacyclin synthesis in rat aorta.[131]

Gingerol concentration-dependently (0.5-20 microM) inhibited the aggregation and release reaction of rabbit washed platelets induced by arachidonic acid and collagen, but not those induced by platelet-activating factor (PAF), U46619 (9,11-dideoxy-9 alpha,11 alpha-methano-epoxy-PGF2 alpha) and thrombin. Gingerol also concentration-dependently (0.5-10 microM) inhibited thromboxane B2 and prostaglandin D2 formation caused by arachidonic acid, and completely abolished phosphoinositide breakdown induced by arachidonic acid but had no effect on that of collagen, PAF or thrombin even at concentrations as high as 300 microM. In human platelet-rich plasma, gingerol and indomethacin prevented the secondary aggregation and blocked ATP release from platelets induced by adenosine 5'-diphosphate (ADP, 5 microM) and adrenaline (5 microM) but had no influence on the primary aggregation. The maximal antiplatelet effect was obtained when platelets were incubated with gingerol for 30 min and this inhibition was reversible. It is concluded that the antiplatelet action of gingerol is mainly due to the inhibition of thromboxane formation.[132]

Anti-cancer effects:

The anticancer effects of ginger were thought to be attributed to various constituents including vallinoids, viz. 20, 100 μM of (6)-gingerol and (6)-paradol, shogaols, zingerone, and Galanals A and B.[133 - 135]

10 and 50 μM of Galanals A and B had been found to be potent apoptosis inducers of human T lymphoma Jurkat cells.[134]

Antiemetic Activity:

Recent animal models and *in vitro* studies had demonstrated that ginger extract of 16.3 and 26.2 μg/ml possesses antiserotoninergic and 5- HT3 receptor antagonism effects, which play an important role in the etiology of postoperative nausea and vomiting.[136 – 138]

Cardiovascular Effects

In vitro research indicated that gingerols and the related shogaols exhibit cardio depressant activity at low doses and cardiotonic properties at higher doses.[139]

Both (6)-shogaol and (6)- gingerol, and the gingerdiones, were reportedly potent enzymatic inhibitors of prostaglandin, thromboxane, and leukotriene biosynthesis.

Anti-inflammatory and Antithrombotic agent.

The effect of an aqueous extract of ginger (Zingiber officinale) on serum cholesterol and triglyceride levels as well as platelet thromboxane-B(2) and prostaglandin-E(2) production was examined. A raw aqueous extract of ginger was administered daily for a period of 4 weeks, either orally or intraperitoneally (IP) to rats. Fasting blood serum was investigated for thromboxane-B(2), prostaglandin-E(2), cholesterol and triglycerides. A low dose of ginger (50 mg/kg) administered either orally or IP did not produce any significant reduction in the serum thromboxane-B(2) levels when compared to saline-treated animals. However, ginger administered orally caused significant changes in the serum PGE(2) at this dose. High doses of ginger (500 mg/kg) were significantly effective in lowering serum PGE(2) when given either orally or IP. However, TXB(2) levels were significantly lower in rats given 500 mg/kg ginger orally but not IP. A significant reduction in serum cholesterol was observed when a higher dose of ginger (500 mg/kg) was administered. At a low dose of ginger (50 mg/kg), a significant reduction in the serum cholesterol was observed only when ginger was administered IP. No significant changes in serum triglyceride levels were observed upon administration of either the low or high dose of ginger. These results suggest that ginger could be used as an cholesterol-lowering, antithrombotic and anti-inflammatory agent.[140]

There was evidence that ginger rhizome (root) 1 - 3 g increases stomach acid production. If so, it may interfere with antacids, sucralfate (Carafate), H2 antagonists, or

proton pump inhibitors. In contrast, other *in vitro* and animal studies had revealed gastro protective properties.[141]

In addition, 20, 100 μM of (6) shogaol, generally more potent than (6)-gingerol, had inhibited intestinal motility in intravenous preparations and facilitated gastrointestinal motility in oral preparations. Ginger extract had also been reported to inhibit the growth of *Helicobacter pylori in vitro*.[142]

A significant increase in the exfoliation of gastric surface epithelial cells following the consumption of 6g or more of ginger (after examining gastric aspirates in 10 healthy volunteers).[143]

Antitussive Effects

Intravenous (i.v.) administration of (6)-gingerol (at 1.75-3.5 mg/kg) or (6)-shogaol (at 1.75-3.5 mg/kg) and oral administration of them (at 70-140 mg/kg) produced an inhibition of spontaneous motor activity, an antipyretic and analgesic effects, prolonged hexobarbital-induced sleeping time, and these effects of (6)-shogaol were mostly more intensive than that of (6)-gingerol. (6)-Shogaol showed an intense antitussive effect in comparison with dihydrocodeine phosphate. In the electro-encephalogram of cortex, the low amplitude fast wave pattern was observed for 5 min after i.v. administration of (6)-shogaol, and then changed to the drowsy pattern, which was restored after 60 min. In the gastro-intestinal system, (6)-shogaol intensively inhibited the traverse of charcoal meal through the intestine in contrast with (6)-gingerol after i.v. administration of 3.5 mg/kg, but (6)-shogaol facilitated such an intestinal function after oral administration of 35 mg/kg. Both (6)-shogaol and (6)-gingerol suppressed gastric contraction in situ, and the suppression by the former was more intensive than that by the latter. In the cardiovascular system, both (6)-shogaol and (6)-gingerol produced depressor response at lower doses on the blood pressure. At high doses, both drugs produced three phase pattern. (6)-shogaol, generally more potent than (6)- gingerol, had exhibited antitussive effects.[144]

Antibacterial Effects

two highly alkylated gingerols, [10]-gingerol and [12]-gingerol effectively inhibited the growth of these oral pathogens at a minimum inhibitory concentration (MIC) range of 6-30 microg/mL. These ginger compounds also killed the oral pathogens at a minimum bactericidal concentration (MBC) range of 4-20 microg/mL, but not the other

ginger compounds 5-acetoxy-[6]-gingerol, 3,5-diacetoxy-[6]-gingerdiol and galanolactone.[145]

Lipid Effects

Oral ingestion of ginger extract had hypocholesterolemic, hypolipidemic, and antiatherosclerotic effects in cholesterol-fed rabbits in the dosage of 200 mg/kg[146] and 50 mg/kg in rats[147]. Inhibition of LDL oxidation and attenuated development of atherosclerosis had also been observed in apolipoprotein E-deficient mice in the dosage of 250 microg of ginger extract/day.[148, 149]

Antiarthritic Effect

A study investigated the antiarthritic effects of ginger and its bioactive constituents. A well characterized crude ginger extract 26 mg gingerols/kg/day was compared with a fraction containing [6] - gingerol and their derivatives to inhibit joint swelling in an animal model of rheumatoid arthritis, streptococcal cell wall-induced arthritis. Both extracts demonstrated anti-inflammatory activity. The crude dichloromethane extract, containing essential oils and more polar compounds, was more efficacious, when normalized to [6] - gingerol content, in preventing, both joint inflammation and destruction. Non-gingerol components enhanced the antiarthritic effects of the more widely studied [6] - gingerol.[150]

Antimicrobial Activities

Ingenol and [6]–shogaol, isolated from ginger rhizome, demonstrated antiviral activity in the dosage of 8 mg/kg.[151]

[10]-gingerol had been reported as active inhibitor of *M. avium* and *M. tuberculosis in vitro.* Gingerol and related compounds had been investigated for antimicrobial activities. [6]- gingerol and [12]-gingerol, isolated from ginger rhizome, demonstrated antibacterial activity against periodontal bacteria. These ginger compounds 27 mg/bw also killed the oral pathogens at a minimum bactericidal concentration (MBC) range of 4-20 microg/mL[152]

Antiviral activity

Among the different viruses which cause the common cold, *Rhinovirus* is one. In plaque reduction test, the dried rhizome of ginger had been investigated for anti-rhino-viral activity. Fractionation by solvent extraction 97 mg/kg, solvent partition and repeated chromatography guided by bioassay, allowed the isolation of several sesquiterpenes with anti-rhino-viral actiuvity. The most effective activity of these was β - sesquiphellandrene.[153]

Anthelmintic activity

The rhizome of *Z. officinale* aqueous extracts showed antihelmintic activity against the earthworm *Pheretima posthuma*. The result showed that the test extract (10 mg/ml) possess significant antihelmintic activity.[154]

Methanol extracts of *Z. officinale* 10 mg/ml was screened for their in vitro anthelmintic activity. Results revealed that *Zingiber officinale* killed all the test worms (*Haemonchus contortus*) within two hours post exposure being 100% effective.[155]

Antipyretic activity

Yeast induced fever in rats was reduced when soxhlet extract of ginger in 80% ethanol was administered at 38% (100mg/kg). Antipyretic effect of acetylsalicylic acid was same as at same dose of extract of ginger. The ginger extract did not affect the temperature of normothermic rats. This antipyretic activity may be mediated by COX inhibition.[156]

Anti-atherosclerotic activity

Rabbits with experimentally induced atherosclerosis for 75 days, when fed air dried ginger powder (100 mg/kg orally daily) inhibited atherosclerotic changes in the aorta and coronary arteries by about 50%.[157]

Ginger treatment did not cause any significant lowering of serum lipids in the study but fibrinolytic activity increased and lipid peroxidation was decreased.

Anti-neoplastic activity

Ginger was considered as a powerful neoplastic agent. Extracts of ginger 200 mg/kg/b.wt orally 10 days along with Gentamicin 80 mg/kg 7 days shows the suppress of cell proliferation and act against resistance of cancerous cells, found in several studies.[158]

Effect on female reproductivity

Labania, (2005) and Abu Baker (2013) confirmed the ability of ginger to improve the functional efficiency of the uterus and ovary. And also dosage of 200 mg/kg/b.wt ginger extract along with 162 mg/kg/b.wt gabapentin it is recommended to take ginger in parallel with GBP during pregnancy for better fetal brain development.[159,160]

40 female adult rats 6-8 weeks old weighing (260 -300 gm.) was used for the study. Animals was divided into four (10 rats for each groups) groups: Animals in Groups I served as control and were given distilled water. Group II was given cadmium chloride at a dose of 0.008 mg/kg B.w once daily for two months by oral gavage tube. Group III were given cadmium chloride at a dose of 0.008 mg/kg B.w once daily along with ginger extract 200 mg/kg B.w. through oral gavage tube once daily for two months. Group IV were given ginger extract 200 mg/kg B.w. through oral gavage tube once daily for two months. At the end of the experiment, blood samples were taken via cardiac puncture for follicular stimulating hormone (FSH), luteinizing hormone (LH) analysis. After dissection of animals, uterui and ovaries were excised for histopathological examination. Results showed that cadmium chloride cause a significant decrease in the levels of sex hormones , and induce toxicopathologicalchanges in genital organsthese changes were ameliorated with extract of ginger. The results of the present study concluded that cadmium chloride - induce toxicopathological changes in uterui and ovaries of rats, which in turn many affected on thereproductive efficiency of animals, thesechanges were improved after co-giving ginger extract which provide a strong evidence for the beneficial role of antioxidants plants in improving the effect of cadmium chloride toxicity in rats female.[161]

Effect on male reproductivity

Doses of 500 mg/kg and 1000 mg/kg and found that extract of *Zingiber officinale* possesses pro-fertility properties.[162]

Ginger administration significantly increased serum testosterone levels if given at 100 mg/kg b.wt, in rats (Khaki *et al.*, 2009).[163]

Doses of 50 mg/kg/mouse and 100 mg/kg/mouse are found that Protective effect of ginger against cisplatin-induced reproductive toxicity and resulted that therapeutic efficacy of ginger increased the activities of testicular antioxidant enzymes and restored sperm motility of cisplatin-treated in rats.[164]

3.9. MILAGU – Piper nigrum,Linn.

Taxonomic Classification

Kingdom	**:**	Plantae – Plants
Subkingdom	**:**	Tracheobionta – Vascular plants
Superdivision	**:**	Spermatophyta – Seed plants
Division	**:**	Magnoliophyta – Flowering plants
Class	**:**	Magnoliopsida – Dicotyledons
Subclass	**:**	Magnoliidae
Order	**:**	Piperales
Family	**:**	Piperaceae – Pepper family
Genus	**:**	Piper L. – pepper
Species	**:**	Piper nigrum L. – black pepper

Vernacular Name:

Sans.	**:** Maricham, Maricha, Krishnam
Eng.	**:** Black pepper, decorticated peper
Hind.	**:** Gulmirch, Kalimirich
Tam.	**:** Milagu
Mal.	**:** Kurumulaka, Kurumilagu
Tel.	**:** Miriyalu
Can.	**:** Volloymenasu

Habitat:

This perennial climbing shrub is indigenous to Malabar and Travancore coasts, i.e. Western coast of India.

Parts Used:

Dried unripe fruit – Black papper.

Constituents:

A volatile alkaloid Pipernie or pipirine pc a balsamic volati5 to 9 pc. Piperidine or piperidin 5 essential oil 1 to 2 pc fat 7 pc. Masocarp contains chavicin a balasamic volatile oil, starch, lignin, gum, fat 1 pc proteids 7 pc and ash containing organic matter 5 pc. Chavicin is a soluble pungent concrete resin, it contains very little piperine and no volatile oil. Piperine crystallizes in flat, four sided glassy prisms insoluble in water.

Actions:

Black pepper is acrid, pungent, hot, carminatiove also used as antiperiodic.

Externally,

It is rubefacient and stimulant to the skin and resolvent. On the mucous membrane of the urethra it acts like cubebs, Piperine is a mild antipyretic and antiperiodic.

Phytochemical Constituents in *Piper nigrum* L.

Table 7 Phytochemical Constituents in *Piper nigrum* L.

Constituents	**Class**	**Bioactivity**	**References**
Piperine	Alkaloid	Anti-inflammation, anti-nociceptive, anti-arthritic, anti-cancer and immunomodulatory	Bang, *et al.*, 2009 Rodgers, *et al.*, 2009
Piperonal	Aldehyde	Antiobesity	Meriga, *et al.*, 2017

Piperoleine B	Organic compound	Hepatoprotective	Pubchem
Pipercide	Alkaloid	Hepatoprotective	Pubchem
Sabinene	Monoterpene	Anti-oxidant and repellent	Jeramillo, *et al.*, 2012
D-limonene	Monoterpene	Chemoprevention	Sun, 2007
β-caryophyllene	Sesquiterpenoid	Anti-inflammatory, analgesic, antipyretic, and platelet-inhibitory actions	Pubchem
α-pinene	Terpene	Anti-inflammatory	Kim, *et al.*, 2015
β-ocimene	Monoterpene	Insecticidal	Pubchem
δ-cadinol	Alcohol	Antioxidant	Zeng, *et al.*, 2011
Guaiacol	Phenolic compound	Expectorant and antiseptic	Pubchem
N-trans-feruloylpiperidine	Phenolic compound	Antioxidant	Abdulazeez, *et al.*, 2016
1,8 cineole	Phenol	Repellent	Tripathy *et al.*, 2001
p-cymene	Monoterpene	Antioxidant and vasorelaxant	Silva *et al.*, 2015
N-trans-feruloyl tyramine	Phenol	Anti-inflammatory	Pubchem
Guineensine	Alkene	Anti-plasmodial	Pubchem
Feruperine	Alkaloid	Antioxidant	Nakatani, *et al.*,1986

Trachyone	Pyrollidine Alkamide	Antibacterial	Reddy, *et al.*, 2004
Isopiperolein B	Pyrollidine Alkamide	Antibacterial	Reddy, *et al.*, 2004
Pergumidiene	Pyrollidine Alkamide	Antibacterial	Reddy, *et al.*, 2004
Pellitorine	Pyrollidine Alkamide	Antibacterial	Reddy, *et al.*, 2004
Pipnoohine	Amide	Insecticidal	Siddiqui, *et al.*,2004
Pipyahyine	Amide	Insecticidal	Siddiqui, *et al.*,2004
N-isobutyl-2E,4E-octadec adienamide	Amide	Hepatoprotective	Pubchem
N-isobutyl-2E,4E,8Z-eico satrienamide	Amide	Antibacterial	Reddy, *et al.*, 2004
Piperchabamide D	Amide	Insecticidal	Hwang, *et al.*, 2017
Retrofractamide A	Amide	Adipogenetic	Mourad, *et al.*, 2013
Dehydroretrofractamide	Amide	Enzyme inhibition	Rho, *et al.*, 2004

Pharmacological Activity

Antimicrobial Activity

The spicy tang of pepper is due to the presence of piperamides which are the pungent bioactive alkaloids accumulate in the skin and seeds of the fruit. Among them piperine is the major chemical constituent responsible for the bitter taste of the black pepper. In the present study piperine was evaluated for its antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Alternaria alternata, Aspergillus niger, Aspergillus flavus* and *Fusarium oxysporum.* The antibacterial activity was measured by agar well diffusion method in the dosage of 25,50,100,150 μg/ml. and antifungal activity by poisoned food technique in the dosage of 100, 500, 1000 μg/ml. Piperine showed antimicrobial activity against all tested bacteria with zone of inhibition ranged from 8-18mm. maximum zone of inhibition was against Gram positive bacteria *Staphylococcus aureus* (18mm) and minimum against Gram negative bacteria *Escherichia coli* (8mm). Piperine showed maximum antifungal activity towards *Fusarium oxysporum* (14mm) and very least effect against *Aspergillus niger* (38mm).[165]

Antimicrobial activity of black pepper

Khan and Siddiqui in 2007 evaluated the antibacterial potential of aqueous decoction of *Piper nigrum* L. (black pepper), *Laurus nobilis* L. (bay leaf), *Pimpinella anisum* L. (aniseed), and *Coriandum sativum* L. (coriander) against different bacterial isolates from oral cavity of two hundred individual volunteers. Black pepper (aqueous decoction) showed strongest antibacterial activity comparable to aqueous decoction of *Laurus nobilis* and *Pimpinella anisum* at the concentration of 10μL/disc.[166]

In a recent study, silver nanoparticles were synthesized by using the leaf and stem extract of Piper nigrum. The synthesized nanoparticle was characterized by UV-vis spectroscopy, X-ray diffraction (XRD), scanning electron microscope (SEM), transmission electron microscope (TEM), energy dispersive X-ray analysis (EDAX), and Fourier Transform Infrared Spectroscopy (FTIR). The observation of the peak at 460 nm in the UV-vis spectra for leaf- and stem-synthesized silver nanoparticles reveals the reduction of silver metal ions into silver nanoparticles. Further, XRD analysis has been carried out to confirm the crystalline nature of the synthesized silver nanoparticles. The TEM images show that the leaf- and stem-synthesized silver nanoparticles were within

the size of about 7-50 nm and 9-30 nm, respectively. The FTIR analysis was performed to identify the possible functional groups involved in the synthesis of silver nanoparticles. Further, the antibacterial activity of the green-synthesized silver nanoparticles was examined against agricultural plant pathogens. The antibacterial property of silver nanoparticles is a beneficial application in the field of agricultural nanotechnology.[167]

Anti-cancer activity of black pepper:

Effects of orally supplemented piperine on lung tumour initiation by B(a)p, its effects on ATPase enzymes were first evaluated. Lung cancer bearing mice showed an increase in erythrocyte membrane and tissues ATPase enzymes (Na(+)/K(+)-ATPases, Mg(2+)-ATPases and Ca(2+)-ATPases). Na(+) K-ATPase and Mg-ATPase enzyme activities were decreased and calcium ATPase increased ($P < 0.05$) in erythrocyte membrane and tissues of lung cancer bearing animals compared with control groups. The elevation of these enzyme activities in membrane and tissues were indicative of the persistent deteriorating effect of B(a)p in cancer bearing animals. These enzyme activities were reversed to near normal control values in animals treated with piperine (50 mg/kg body weight). It is apparent that the beneficial effect of piperine is primarily exerted on the during initiation phase and post-initiation stage of B(a)p induced lung carcinogenesis. Overall, these data indicative that piperine has chemopreventive effects when administered orally on lung cancer bearing animals.[168,169]

Anti-inflammatory activity of black pepper:

The piperine was evaluated for the anti-inflammatory, analgesic, and anti-arthritic activities. The *in vitro* anti-inflammatory activities were evaluated on interleukin 1β stimulated fibroblast like synoviocytes obtained from rheumatoid arthritis, while anti-arthritic including analgesic activities were evaluated on carrageen an induced acute paw model of pain and arthritis in rats. The prostaglandin E2, cyclooxygenase 2, interleukin 6 and matrix metallo-proteinase levels were evaluated by ELISA and RT-PCR methods of analysis. Piperine treated groups were found to reduce the synthesis of prostaglandin E2 in a dose dependant comportment at the concentrations of 10-100 μg/mL. It significantly inhibited the synthesis of prostaglandin E2 even at 10 μg/ mL. The expression of interleukin 6 and matrix metallo-proteinase 13 were also inhibited. The migration of activator protein1into the nucleus in interleukin 1β treated synoviocytes was inhibited by piperine while migration of nuclear factor κB was not affected by piperine. The pain and

arthritic symptoms in rats were significantly reduced by piperine. It was concluded that piperine showed anti-inflammatory, analgesics and anti-arthritic activities in arthritis model of rats.[170]

Hepatoprotective activity of black pepper:

It was found that piperine inhibited the increased level of serum GPT and GOT in dose-dependent manner in a hepato-toxicity model of mice caused by D-galactosamine. The hepatoprotective activity of methanolic extract of *Piper nigrum* fruits was evaluated in ethanol CCl4 induced hepatic damage in Wistar rats. Ethanol-CCl4 was used to induce hepatotoxicity in the rats. Prophylactic treatment with methanolic extract of *Piper nigrum* at a dose of 100 and 200 mg/kg body weight, p.o. and pre-treatment with piperine at a dose of 50 mg/kg body weight, p.o. for 15 days with Ethanol-CCl4 treatment rats showed significant liver protection as evidenced from the triglycerides levels, Alanine transaminase, Aspartate transaminase, alkaline phosphatase, bilirubin and superoxide dismutase, Catalase, Glutathione reductase and Lipid peroxidation levels to assess the liver functions. In this study, administration of Ethanol-CCl4 exhibited significant boost in triglycerides, Alanine transaminase, Aspartate transaminase, alkaline phosphatase, and bilirubin levels while there was significant decrease in the superoxide dismutase, catalase, and glutathione reductase levels which were restored to normal level after pre-treatment of methanolic extract of *Piper nigrum* and Piperine. Lipid peroxidations were also significantly decreased after pretreatment with methanolic extract of *Piper nigrum* and Piperine at given doses. The results were similar to that of reference standard-Liv52 at a dose of 1 mL/kg, p.o. for 15 days. The Morphological and histopathological studies of liver were also supportive of the biochemical parameters. Thus it is concluded that *Piper nigrum* possesses potential hepato-protective activity due to the presence of piperine alkaloids and have great therapeutic potential in treatment of liver ailments.[171]

Anti-diarrheal activity of black pepper:

Aqueous back pepper extract (ABPE) at a dose of 75, 150, 300 mg/ kg, po was evaluated for anti-diarrheal, anti-motility and anti-secretory activity in mice. The castor oil and magnesium sulphate were used to induce diarrhea for the evaluation of anti-diarrheal activity and gastrointestinal motility was assessed by charcoal meal, while castor oil was used for the evaluation of anti-motility and anti-secretory activities. ABPE showed a significant and dose dependent anti-diarrheal, antimotility and anti-secretary

effect. Anti-motility and anti-secretory activities of *Piper nigrum* might be due to the presence of carbohydrates and alkaloids, and anti-diarrheal activity of ABPE may be due to its anti-motility and anti-secretory activities.[172]

Immuno-modulatory activity

Immuno-modulatory and antitumor activity of piperine was evaluated. Piperine (250 µg/mL)was reported to be cytotoxic to Ehrlich ascites carcinoma cells and Dalton's lymphoma ascites. Piperine at a concentration of 50 µg/mL showed cytotoxicity to L929 cells in culture. Piperine administration also causes an increase in the total WBC counts in Bal b/c mice. Administrations of piperine were also increase the bone marrow cellularity and alpha-esterase positive cells.[173]

In vitro immunomodulatory activity of piperine was evaluated to enhance the efficacy of rifampicin in a murine model of *Mycobacterium tuberculosis* infection. Mouse splenocytes were used to evaluate *in-vitro* immunomodulation of piperine for cytokine production, macrophage activation and lymphocyte proliferation. Piperine treated mouse splenocytes demonstrated an increase in the secretion of Th-1 cytokines (IFN-γ and IL-2), increased macrophage activation and proliferation of T and B cell. Protective efficacy of piperine and rifampicin (1 mg/kg) combination against *Mycobacterium tuberculosis* was reported due to immuno-modulatory activity.[174]

Anticonvulsant activity

The Anticonvulsant activity of piperine in maximal electroshock (MES) and pentylenetetrazol (PTZ) models of convulsions in mice was examined and further participation of transient receptor potential cation channel subfamily V member 1 (TRPV1) receptor was acknowledged in the inhibition of convulsion caused by pentylenetetrazol and maximal electroshock models. A significant delay in the onset of myoclonic jerks and generalized clonic seizures was observed after administration of Piperine at doses of 40 and 80 mg/kg and Piperine also diminish the seizure stage and mortality as compare to the animals treated with vehicle. A significant reduction was also observed in the incidence of MES-induced tonic hind limb extension (THE) and PTZ-induced Fos immune reactivity in the dentate gyrus after of piperine administration. Capsazepine (TRPV1- selective antagonist) blocked the anti-seizure effects of piperine. These data reveals the anti-convulsant activity of piperine.[175]

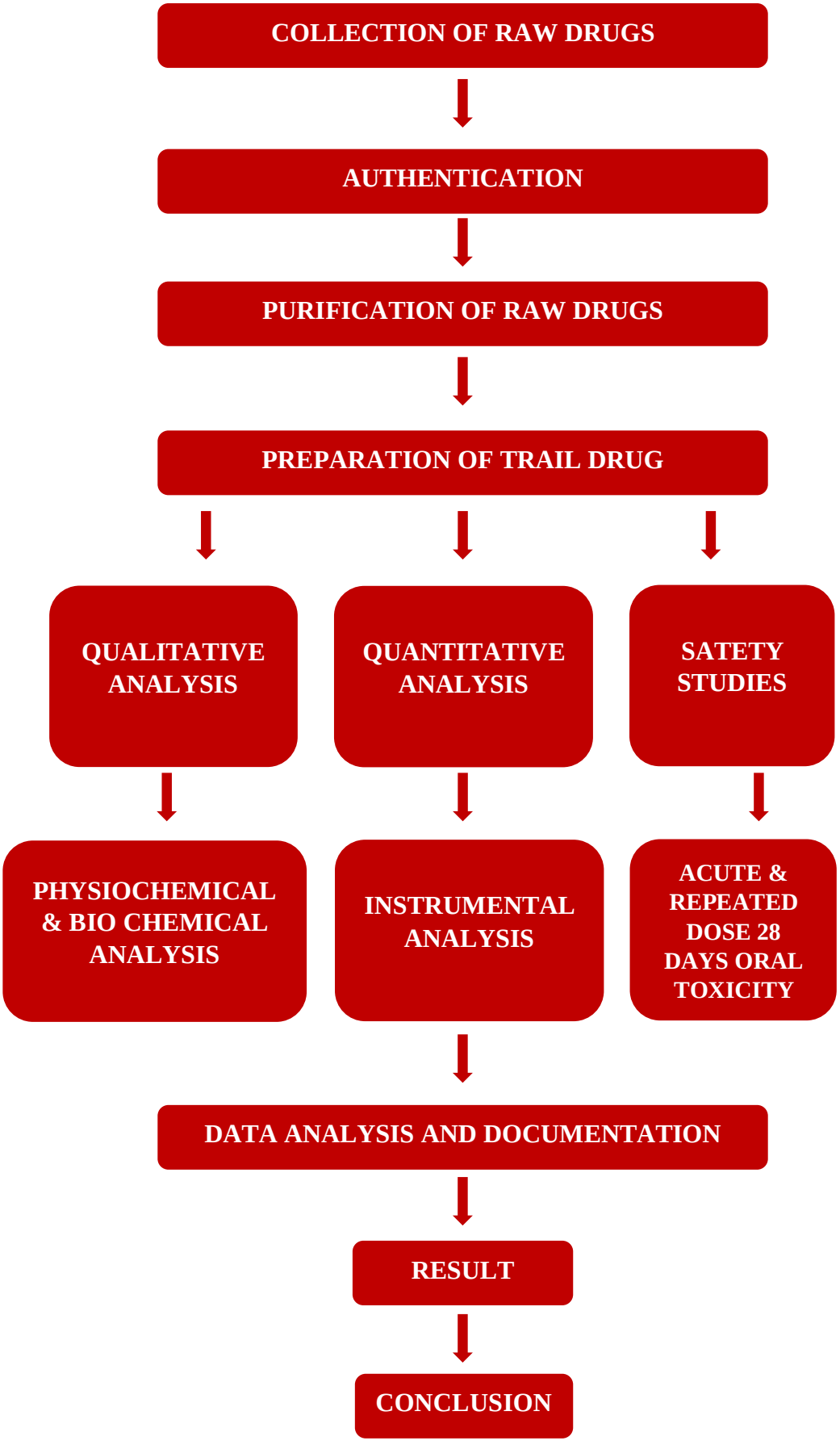
In another study, *in vivo* anticonvulsant activity of piperine was evaluated in pentylenetetrazole (PTZ) and picrotoxin (PIC)-induced seizures models of epilepsy in mice. A significant ($P<0.01$) delayedin the onset of PTZ-and PIC-induced seizures was observed after intra-peritonial injection of piperine at a dose of 30, 50 and 70 mg/kg (i.p.), valproic acid at a dose of 200 mg/kg, Carbamazepine at a dose of 30 mg/kg and diazepam at a dose of 1 mg/kg in mice. These results revealed the anticonvulsant effects of piperine which possibly mediated via GABA ergic pathways.[176]

Anti-platelet Activity

Piperine also possess anti-platelet activity. Park et al. observed the toxic effect of piperine on aggression of platelet in experimental rabbit induced by different factors which activate platelets, by collagen and thrombin.[177]

WORK PLAN

WORK PLAN


COLLECTION OF RAW DRUGS
AUTHENTICATION
PURIFICATION OF RAW DRUGS
PREPARATION OF TRAIL DRUG
QUALITATIVE ANALYSIS
QUANTITATIVE ANALYSIS
SATETY STUDIES
PHYSIOCHEMICAL & BIO CHEMICAL ANALYSIS
INSTRUMENTAL ANALYSIS
ACUTE & REPEATED DOSE 28 DAYS ORAL TOXICITY
DATA ANALYSIS AND DOCUMENTATION
RESULT
CONCLUSION

MATERIALS AND METHODS

4. MATERIALS AND METHODS

4.1. DRUG PROFILE:

The study drug *"**Nilavembu Kudineer**"* has been selected from the classical Siddha literature – ***"Siddha Vaithiya Thirattu".***[178]

Ingredients of Nilavembu Kudineer:

1. Nilavembu *(Andrographis paniculata)*
2. Vettiver *(Vertiveria zizanioides)*
3. Vilamichuver *(Plectranthus vettiveroides)*
4. Santhana siraai *(Santalum album)*
5. Pei pudal *(Tricosanthes cucuerino)*
6. Parppadagam *(Hedyotis corymbosa)*
7. Koraikkizhangu *(Cyperus rotundus)*
8. Sukku *(Zingiber officinale)*
9. Milaku *(Piper nigrum)*

Dosage:

60 - 120 ml (¼ to ½ Aazhakku)

Medicinal use:

Suram (Fever)

4.2. PREPARATION OF NILAVEMBU KUDINEER CHOORANUM

Procurement of raw drugs:

Nilavembu (Andrographis paniculata) was collected from National Institute of Siddha, Herbal Garden, Chennai – 47. And remaining raw materials were obtained from K. Ramasamy Chetty country drug shop, No : 177, Rasappa Chetty Street, Park Town, Chennai - 600003

Figure 1 Ingredients of Nilavembu Kudineer

Nilavembu *(Andrographis paniculata)*

Vettiver *(Vertiveria zieanioides)*

Vilamichuver *(Plectranthus vettiveroides)*

Santhana sirai *(Santalum album)*

Pei pudal *(Tricosanthes cucuerino)*

Parppadagam *(Hedyotis corymbosa)*

Koraikkizhangu *(Cyperus rotundus)*

Sukku *(Zingiber officinale)*

Milaku *(Piper nigrum)*

Nilavembu Kudineer Chooranam

Identification and Authentication:

All the raw drugs was identified and authenticated by the Dr. D. Aravind MD(S)., M.Sc., Assistant Professor, Department of Medicinal Botany, National Institute of Siddha, Chennai – 47 and Certificate Number : NISMB3892019

Purification of raw drugs:

All the Raw drugs are washed with RO water and then dried under shade until become dry except Sukku and Milaku. Sukku was purified by peeled the skin and Milaku was purified by soaked in buttermilk for three days and dried under shade.

Preparation procedure of Nilavembu Kudineer Chooranam:

All the purified raw drugs are coarsely powdered and mixed well. Finally the Nilavembu kudineer chooranum was stored in closed air tight container.

4.3. STANDARDIZATION OF NILAVEMBU KUDINEER:

The standardization of Nilavembu Kudineer chooranam is essential to exhibit the purity and quality of drug. This is basically done by Physiochemical analysis as per WHO Guidelines, and Phytochemical, Biochemical, Instrumental analysis as per Protocol for Testing of Ayurvedha, Siddha, and Unani Drugs.

The Physiochemical analysis, Phytochemical analysis had been done at The TN Dr. M G R Medical University, Guindy, Chennai-32. Biochemical analysis was done at National Institute of Siddha Chennai – 47. Microbial contamination were done at VS Clinical research and hospital Pvt.Ltd. CSIR road, Taramani, Chennai - 13. And Aflatoxin, Instrumental analysis were done at Noble Research Solutions, ISO 9001-2015 certified company, Chennai - 47

4.3.1. ORGANOLEPTIC CHARECTERS: [179]

The Organoleptic characters of the Nilavembu Kudineer was analysed as per standard procedure of 1 gm of the Nilavembu Kudineer chooranam was taken and the colour, texture, particle size and other morphology were viewed by naked eye under sunlight. Then the result is noted.

4.3.2. PHYSICO - CHEMICAL ANALYSIS

Physico - chemical studies of the trial drug have been done according to the WHO guidelines.

Determination of Ash Values:

Total Ash:

2 g is accurately weighed and incinerated in a crucible dish at a temperature not exceed 600^{o}C until free from carbon. It is then cooled and weighed. The % w/w of ash with reference to the air-dried powder is calculated.

Water Soluble Ash:

The total ash is obtained as the above method for preparation of total ash. The ash is boiled for 5 minutes with 25 ml water. The insoluble ashes is collected using filter paper and washed with hot water and then transferred to the silica crucible then ignite for 15minutes at temperature not exceeding 450^{o}C. The silica crucible and residue are weighed until constant weight is attained for determination of weight of insoluble ash. The weight of the water soluble ash is determined by subtracting the weight of insoluble ash from the weight of total ash.

Acid insoluble Ash:

The total ash is obtained as the above method for preparation of total ash. The ash is boiled for 5minutes with 25ml 10% Hcl. The insoluble ashes is collected using filter paper and washed with hot water and then transferred to the silica crucible then ignite for 15minutes at temperature not exceeding 450^{o}C. The silica crucible and residue are weighed until constant weight is attained.

Determination of Extractive Value:

Water soluble Extractive value:

5 gm of Nilavembu Kudineer chooranam powder is weighed and macerated with distilled water, respectively, at 80^{o}C for 24 hrs. The resulting solution is shaken continuously for 6 hours and allowed to stand and soak for 24 hrs then filtered. The solution from both chloroform and water respectively is filtered and evaporated of the filtrate in a flat bottomed shallow dish and dried at 105^{o}C then cooled and weighed.

Alcohol Soluble Extractive Value:

2.5 gm of Nilavembu Kudineer chooranam powder is weighed and macerated with 100ml of ethanol in a closed container for 24 hours. The resulting solution is shaken continuously for 6 hours and allowed to stand and soak for 18 hours. The solution is

filtered and evaporated of the filtrate in a flat bottomed shallow dish and dried at 105ºC then cooled and weighed.

Loss on Drying:

2 gm of powdered drug is dried in the oven at 100- 105°C to constant weight. The result was noted.

P^H:

1 % of the substance was prepared using distilled water and stored properly. Prior to P^H measurement, P^H electrode was calibrated using buffers of P^H 4, 7 and 10. after calibration, measurement was taken with a solution of known P^H such that the reading should not differ by more than 0.02 from the original value. If the difference is greater than 0.05, the set of measurement was repeated. Later the P^H dipped into the drug to be tested and kept as such until a constant reading was obtained.

4.3.3. TEST FOR HEAVY / TOXIC METALS [179]

Standard:

Hg, As, Pb and Cd

Methodology:

Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environmental samples. The total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) model AA 240 Series. In order to determination the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test item.

Sample Digestion:

Test sample was digested with 1 mol / L Hcl for determination of arsenic and mercury. Similarly, for the determination of lead and cadmiun the sample were digested with 1 mol / L HNO_3.

Standard Preparation:

As and Hg - 100 ppm sample in 1 mol / L Hcl

Cd and Pb - 100 ppm sample in 1 mol / L HNO_3

4.3.4. MICROBIAL CONTAMINATION [179]

Objective:

The liquefied casein soyabean digest agar and Sabouraud doxtrose agar with antibiotics 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).

Determination of Microbial Contamination:

10 g of the preparation being examined was dissolved properly in a buffered sodium chloride peptone solution, P^H 7.0 with no antimicrobial activity. Later the volume was made up to 100 ml using the same media chosen, maintaining the P^H

For determining bacterial and fungal count petri dishes 9 to 10 cm in diameter was plated with 15 ml of liquefied casein soyabean digest agar and Sabouraud dextrose agar with antibiotics at not more than 45^0 along with 1 ml of the pretreated preparation. Alternatively, it was also spread plated with the pretreated preparation on the surface of solidified medium in a Petri dish of the same diameter. The drug of preparation was diluted so that a colony count of not more than 300 could be expected. Two petri pates were plated at same dilution and incubated at 30^0 to 35^0 C for 5 days for bacteria and 20^0 to 25^0 C for 5 days for fungus until a more rliable count was obtained in a shorter time. After incubation the results were chosen from the plate with greatest number of colonies but not more than 300 colonies.

4.3.5. TEST FOR SPECIFIC PATHOGEN [179]

10 g of the preparation being examined was dissolved properly in a buffered sodium chloride peptone solution, P^H 7.0 with no antimicrobial activity. Later the volume was made up to 100 ml using the same media chosen, maintaining the P^H.

Escherichia coli:

10 ml of pretreated drug preparation mixed with 50 ml of nutrient broth, placed in a screw capped container shaken properly and allowed to stand for 1 hour. Later the cap was loosened and incubated at 37^0 C for 18 to 24 hours. As a primary test for E. Coli 5 ml

of Mac Conkey broth mixed well with 1 ml of enrichment culture in a centrifuge tube, incubated in water bath at 36 - 38^0 C for about 2 days. As the acid and gas formation was not found it indicates that the drug to be tested was free from E. Coli.

Salmonella Species:

10 ml of pre-treated drug preparation along with 1 g of the product was added with 100 ml of nutrient broth in a sterile screw capped jar, shaken properly and allowed to stand for 4 hours. Later the cap was loosened and incubated at 35^0 to 37^0 C for 24 hours. As a primary test 10 ml of enrichment culture was added to 2 tubes containing 10 ml of selenite Froth and tetrathionate bile brilliant green broth, incubated at 36^0 to 38^0 for 48 hours. From each of these two cultures sub - culturing was done by two of the following four agar media like bismuth sulphate agar, brilliant green agar, desoxycholatecitrate agar and xylose - lysine - desoxycholate agar then incubated at 36 to 38^0 C for 18 to 24 hours. As the colonies were not found it indicates that the drug to be tested was free from Salonella Spp

Psudomonas aeruginosa:

The pre-treated preparation was inoculated in 100 ml of fluid soyabean - casein digest mediam alone with 1 g of preparation being examined, mixed well and incubated at 35 to 37^0 C for 24 to 48 hours. On examination of media if growth was found streaked a portion of the medium on the surface of cetrimide agar medium, each plated on petri dishes, covered and incubated at 35 to 37^0 C for 18 to 24 hours. Streaked the colonies from the agar surface of cetrimide agar on the surfaces of pseudomonas agar medium for detection of fluoresce in and pseudomonas agar medium for detection of pyocyanin contained in petri dishes, covered, inverted and incubated at 33 to 37^0 C for not less than 3 days. The streaked srufaces were examined under ultra - violet light, placed 2 to 3 drops dihydrochloride on filter paper and prepared the smear. As pink color formation was not found it indicates that the drug to be tested was free from pseudomonas aeruginosa.

Staphylococcus aureus:

The pretreated preparation was inoculated in 100 ml of fluid soyabean - casein digest medium alone with 1 g of preparation being examined, mixed well and incubated at 35 to 37^0 C for 24 to 48 hours. If growth occurs, coagulation test was conducted by transferring the colonies from the agar surface of media to individual tubes each

containing 0.5 ml of mammalian preferably rabbit plasma with or without additives incubated in water bath at 37^0 C and examined at 3 hours and subsequently at suitable intervals up to 24 hours. As coagulation was not found it indicates that the drug to be tested was free from staphylococcus aureus.

4.3.6. AFLATOXINS [179]

The Aflatoxin analysis were carried out as per standard procedure at Noble Research Solutions, ISO 9001 - 2015 Certified Company. Chennai.

Standard:

Aflatoxin B_1

Aflatoxin B_2

Aflatoxin G_1

Aflatoxin G_2

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 B_1 and aflatoxin G_1 and 0.1 µg per ml each of aflatoxin B_2 and alfatoxin G_2.

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 2.5µL, 5µL, 7.5µL and 10µL. Similarly, the test sample was placed and allow the spots to dry and develop the chromatograme in an unsaturaed chamber containing a solvent system consisting of a mixture of chloroform, acetone and isoporpyl 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 from and allow the plate to air - dry. Locate the spots on the plate by examination under UV light at 365 nm.

4.3.7. DETERMINATION OF PESTICIDE RESIDUE [179]

For analysis reagents without any external components were chosen and the samples were analyzed using Gas Chromatographic methods. Later the amount of different components such as Organophophorus, Organochlorine and Pyrethroids contents was recorded.

4.3.8. PHYTO - CHEMICAL ANALYSIS

Phyto - chemical Screening of the Nilavembu Kudineer chooranam have been done using standard procedures

1. **Detection of alkaloids:**

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Mayer's Test:

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

b) Wagner's Test:

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c) Dragendroff's Test:

Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

d) Hager's Test:

Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow colored precipitate.

2. Detection of carbohydrates:

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) **Molisch's Test:**:

To 2 ml of plant sample extract, two drops of alcoholic solution of α- naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid is

added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

b) **Benedict's Test:**

Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

3. Detection of Glycosides:

Extracts were hydrolyzed with dil. HCl, and then subjected to test for glycosides.

a) **Modified Borntrager's Test:**

Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides.

b) **Cardiac Glycoside (Keller-Killiani test):**

Extract was shaken with distilled water (5 mL). To this, glacial acetic acid (2 m L) containing a few drops of ferric chloride was added, followed by H_2SO_4 (1 m L) along the side of the test tube. The formation of brown ring at the interface gives positive indication for cardiac glycoside and a violet ring may appear below the brown ring.

4. Detection of Saponins:

Froth Test:

Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test:

0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phytosterols

Salkowski's Test:

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes.

6. Detection of phenols Ferric Chloride Test:

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

7. Detection of tannins - Gelatin Test:

The extract is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

8. Detection of flavonoids

Alkaline Reagent Test:

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test:

Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

9. Detection of proteins and aminoacids

Xanthoproteic Test:

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins.

10. Detection of diterpenes Copper Acetate Test:

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes

11. Gum and Mucilage:

To 1ml of extract add 2.5ml of absolute alcohol and stirring constantly. Then the precipitate was dried in air and examine for its swelling properties. Swelling was observed that will indicate presence of gum and mucilage.

12. Test for Fixed oils and Fats

Spot test:

A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

13. Test for Quinones

Extract was treated with sodium hydroxide blue or red precipitate indicates the presence of Quinones.

4.3.9. BIO CHEMICAL ANALYSIS:

Preliminary Basic and Acidic radical:

Preparation of the extract:

5 g of the Nilavembu Kudineer chooranam is weighed accurately and placed in a 250 ml clean beaker. Then 50 ml of distilled water is added and dissolved well. Then it is boiled well for about 10 minutes. It is cooled and filtered in a 100 ml volume metric flask and then it is made up to 100ml with distilled water. This preparation is used for the qualitative analysis of acidic / basic radicals and biochemical constituents in it.

Qualitative analysis for basic radicals:

Table 8 Qualitative analysis for basic radicals

S. NO	EXPERIMENT	OBSERVATION
1.	Appearance of sample	Dark Yellow in Color.
2.	**Solubility** a. Little of the sample was shaken well and mixed with distilled water.	Insoluble / Sparingly soluble
	b. A little of the sample is shaken well with	Completely Soluble

	Con.HCL / Con.H_2So_4	
3.	**Action of heat:** A small amount (500mg) of the sample was taken in a dry test tube and heated gently at first and then strong.	White fumes evolved.
4.	**Flame test :** A small amount of sample was made into paste with con.HCL in a watch class and introduced into non luminous part of the Bunsen flame.	No bluish green flame appeared.
5.	**Ash test:** A filter paper was soaked into a mixture of sample and cobalt nitrate solution introduced into the Bunsen flame and ignited.	No Yellow coloured flame appeared

TEST FOR ACID RADICLES

Table 9 Test for Acid Radicles

S. NO	EXPERIMENT	OBSERVATION
1.	**Test for Sulphate :** a. 2 ml of above prepared extract was taken in the test tube to this added 2 ml of 4% ammonium oxalate solution.	Cloudy appearance Present
	b. 2 ml of the above prepared extract is added with 2 ml of diluted HCL is added until the effervescence cases off. Then 2 ml of Barium	A white precipitate insoluble in concentrated HCL is observed.

	chloride solution is added.	
2.	**Test for Chloride :** 2 ml of the above preparedsolution was added with diluted HNO_3 till the Effervesce science ceases. Then 2 ml of Silver nitrate solution was added.	Cloudy appearance Present / Not Present
3.	**Test for Phosphate:** 2 ml of the extract was treated with 2 ml of Ammonium molybdate Solution and 2 ml of Con. HNO_3	Cloudy yellow appearance Present
4.	**Test for Carbonate:** 2 ml of the extract was treated with 2 ml of Magnesium sulphate Solution.	Cloudy appearance Present
5.	**Test for Nitrate :** 1 drop of the substance was heated with Copper tunics and concentrated H_2SO_4 and viewed the test tube vertically down.	Yes / No characteristic changes of formed
6.	**Test for Sulphide:** Substance was treated with 2 ml ofCon.HCL	Rotten egg smelling gas evolved
7.	**Test for Fluride and Oxalate:** 2 ml of the extract was added with 2 ml of diluted Acetic acid and 2 ml Calcium chloride solution and heated.	Cloudy appearance Present / Not Present
8.	**Test for Nitrite:** 3drops of the extract was placed on the filter paper on that 2	Yes / No characteristic changes observed

	drops of Acetic acid and 2 drops of Benzidine solution is placed.	
9.	**Test of Borate:** 2 pinches of the substances was made into paste by sulphuric acid alcohol (95%) and introduce into blue flame	Bluish yellow coloured flame Appeared / Not Appeared

TEST FOR BASIC RADICLES:

Table 10 Test for Basic Radicles

S. NO	EXPERIMENT	OBSERVATION
1.	**Test for Lead:** 2 ml of extract was added with 2 ml of Potassium iodide solution.	No Yellow coloured precipitate was Obtained.
2.	**Test for Copper:** a. One pinch of substance was made into paste with Con.HCL in a watch glass and introduced into the non-luminous part of the flame.	Yes / No blue coloured flame appeared
	b. 2 ml of extract is added with excess of ammonia solution	
3.	**Test for Aluminum:** To the 2 ml of the extract Sodium hydroxide was added in drops to excess.	No Characteristic changes observed

4.	**Test for Iron:** To the 2 ml of extract add 2ml of ammonium thiocynate solution. To the 2 ml of extract add 2ml ammonium thiocynate solution and 2 ml of con HNO3 is added.	Mild red colour appeared
5.	**Test for Zinc:** To 2ml of the extract sodium hydroxide solution was added in dropsto excess.	White precipitate was Not Appeared.
6.	**Test for Calcium :** 2 ml of the extract was added with 2 ml of 4% ammonium oxalate Solution	Cloudy appearance and white precipitate is obtained
7.	**Test for Magnesium:** To 2ml of extract sodium hydroxide solution is added in drops to excess.	White precipitate was Not appeared.
8.	**Test for Ammonium :** To 2ml of extract few ml of Nessler's reagent and excess of sodium hydroxide solution areadded.	Brown colour was appeared
9.	**Test for Potassium:** 1ml of substance was treated a pinch with 2ml of sodium and then treated with 2ml of cobalt nitrate in 30% glacial acetic acid.	Yellowish precipitate was obtained / Not obtained
10.	**Test for Sodium:** 2 pinches of the substance was	Yellow colour flame

	made into paste by using HCL and introduced into the blue flame of Bunsen burner.	Appeared / Not Appeared
11.	**Test for Mercury:** 2ml of the extract was treated with 2ml of sodium hydroxide Solution	Yellowish precipitate was Not obtained
12.	**Test for Arsenic:** 2 ml of the extract was treated with 2 ml of sodium hydroxide solution.	Brownish red precipitate was Not obtained

MISCELLANEOUS

Table 11 Miscellaneous

S. NO	EXPERIMENT	OBSERVATION
1.	**Test for Starch:** 2ml of the extract was treated with weak iodine solution.	Blue colour developed / Not developed
2.	**Test for reducing sugar:** 5ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for two minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes. The coloris noted.	Brick red colour Not developed
3.	**Test for alkaloids :** a. 2 ml of the extract is treated with 2 ml of Potassium Iodide solution	Red colour was not developed.
	b. 2 ml of extract was	Yellow Color was

	treated with 2 ml of picric acid.	developed.
	c. 2 ml of the extract is treated with 2 ml of Phosphotungstic acid.	No white color was developed.
4.	**Test for Tannic acid:** 2 ml of extract was treated with 2 ml of ferric chloride solution.	Block colour precipitate is appeared
5.	**Test for Unsaturated compounds:** To the 2ml of extract 2ml of potassium permanganate solution was added.	Potassium permanganate is decolored / Not decolored.
6.	**Test for Aminoacids:** 2 drops of the extract was placed on a filter paper and dried well.	Violet colour developed / Not developed
7.	**Test for type of compound:** 2ml of the extract is treated 2ml of ferric chloride solution.	Green / Red / Violet / Blue colour developed.

4.4. QUANTITATIVE ANALYSIS

4.4.1. TLC Analysis [180]

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 different solvent system Chloroform: n-Butanol: Methanol: Water: Acetic Acid (4:1:1:0.5:0.5). 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

High Performance Thin Layer Chromatography Analysis:[181]

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. In addition it is a reliable method for the quantitation of nano grams level of samples. 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 medicinal plant raw materials.

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 analysed. 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 phytoconstituents present in each extract and Rf values were tabulated.

4.5. TOXICOLOGICAL STUDIES

Preclinical toxicity studies of **Nilavembu Kudineer** was conducted on wistar albino rats as per OECD guideline 423 and 407 after getting IAEC approval. (NIS / IAEC - VII / 28082018 / 18)

4.5.1. ACUTE ORAL TOXICITY

Experimental Details;

Species	: Wister Albino Rat
Age / Weight	: 6 to 8 weeks / 140-160 g
Gender	: Female
Acclimatization Period	: 7 days prior to dosing
Housing	: Polypropylene cages
Husbandry	: 12-h light/12-h dark cycle
Temperature	: Room temperature 22°C (±3°)
Humidity	: 30 –70%
Feed and water	: Rodent Pelleted feed
	RO purified water ad libitum

Identification:

Animals were kept in individually marked with picric acid i.e Head, Body and Tail.

Animal selection and identification:

The animals are randomly selected for acute toxicity control and treatment group contains 3 female animals. They were marked in head, body, and tail with picric acid solution the group number cage number, sex of the animals was mentioned in the front of each cage.

Experimental procedure:

Acute toxicity study was conducted as per OECD guideline 423. Control group animal was given RO water and 5000 mg / Kg b.wt of Nilavembu Kudineer was given to test animal group. Each group contains 3 females. Before drug administration animals kept under fasting for 12 hours without food not water. After drug administration all the

animals will be observed continuously for first ½ hour then 1 hour once upto 24 hours for any behavioural changes and mortality. The animals will be observed for further 14 days. At the end of the study all the animals will be weighed and sacrificed excessive Anesthesia (Thiopental sodium 1mg/100g) to access any gross pathology. If any gross pathology will be noted histopathology of organs will be done.

Cage side observation:

The animals were monitored for Abnormal Gait Aggressiveness, Akinesia, Analgesia, Catalepsy, Convulsion, Defecation, Excitation, Exopthalmos, Head twiches, Lacrimation, Lethality, Loss of corneal reflex, Loss of righting reflex, Loss of traction, Piloerction, Ptosis, Reactivity to touch, Respiration, Sedation, Stereotypis (Chewing), Sterotypis (Head movements), Sterotypis (Sniffing), Straub, Tremor, Writhes.

4.5.2. SUB ACUTE TOXICITY STUDY:

28 days repeated oral toxicity study was conducted as per OECD 407.

Experimental Details:

Species	: Wister Albino Rat
Age / Weight	: 6 to 8 weeks / 140-160 g
Gender	: Male and Female
Acclimatization Period	: 7 days prior to dosing
Housing	: Polypropylene cages
Husbandry	: 12-h light/12-h dark cycle
Temperature	: Room temperature 22°C (±3°)
Humidity	: 30–70%
Feed and water	: Rodent Pelleted feed
	RO purified water ad libitum

Identification:

Animals were kept in individually marked with picric acid i.e Head, Body, Tail, Head Body, and Body Tail in each group.

Animals selection and identification:

5 group of animals was used in this study animals are randomly selected for each group. Each group contains 5 male and 5 female animals. They were marked in Head,

Body, Tail, Head Body and Body Tail with picric acid solution. The Group number, Cage number, Sec of the animal was mentioned on front of each cage.

Table 12 Treatment Groups of 28 days repeated oral toxicity study

Treatment Groups	**Animal**
Control	5 Male + 5 Female
Low Dose	5 Male + 5 Female
Mid Dose	5 Male + 5 Female
High Dose	5 Male + 5 Female
Satellite Group	5 Male + 5 Female

Experimental Procedure:

Totally five groups was used to this study. Each group contains 10 animals (5 F + 5 M). Group I set as control and received water. Group II, III, IV, & V was received Nilavembu Kudineer 6 ml, 12 ml, 48 ml, 48 ml / kg b. wt/ p.o respectively by oral cavage for 28 days. Animal drug dosage was calculated from Human therapeutic dose as per Paget and Burnas 1964. Animals was fasted over night before drug administration with free access of water. After drug administration all the animals was observed daily for any mortality and morbitity. At the end of the study (28^{th} day) all the animals was fasted overnight, weighed and sacrificed under excessive Anesthesia (Thiopental sodium 1mg/100g). Blood was collected from the anesthetized animals through abdominal aorta. The following investigations like Haematology, Biochemical analysis study will be done. Histopathology was done in all the vital organs of control and high dose treated groups. Satellite groups were observed for further 14 days without drug administration. After 14 days of observations all the animals was fasted overnight, weighed and sacrificed under excessive Anesthesia (Thiopental sodium 1mg/100g). Blood was collected from the anesthetized animals through abdominal aorta. The following investigations like Haematology, Biochemical analysis study was done. Histopathology was done in all the vital organs of test animals in satellite group.

Statistical Analysis:

All data were expressed as mean ± standard deviation (SD). The treatment groups were compared with control for testing significance by one way analysis of variance (ANOVA), Dunnett's multiple range test was applied for post hoc analysis by using GraphPad Instat 3.0 version (GraphPad, San Diego, CA). Values of $p < 0.05$ will be considered to be statistically significant.

RESULTS

5. RESULTS

Many studies have been carried out to bring the efficacy and potency of the drug ***Nilavambu Kudineer.*** The present study includes literary collections, Organoleptic character, Physicochemical and instrumental analysis and toxicological analysis of *Nilavambu Kudineer*. The drug *Nilavembu Kudineer* has been use full for treating Fever (Suram) in reference with the text "Siddha Vaithiaya Thirattu".

5.1. RESULTS OF LITERATURE REVIEW

Literature collections about the drug from various text books were done. Siddha literature's related to the drug bring the evidence and importance of its utility in treating the Fever.

Botanical aspect explains the identification, description, active principle and medicinal uses of the plants ingredients of *Nilavambu Kudineer.*

Modern and Siddha aspect of the drug was also reviewed.

5.2. RESULTS OF STANDARDIZATION:

Standardization of the drug is more essential to derive the efficacy and potency of the drug which was analyzed by the various methods. The results of Organoleptic characters (Table 13), Physicochemical analysis (Table 14), Phytochemical analysis (Table 19) Bio - Chemical analysis (Table 20) and Instrumental analysis (Table 21) of *Nilavembu Kudineer* is tabulated below.

5.2.1. RESULTS OF ORGANOLEPTIC CHARACTERS:

The following characters have been noted in ***Nilavambu Kudineer***

Table 13 Organoleptic Characters of Nilavembu Kudineer Chooranam and Nilavmebu Kudineer

Parameters	**Nilalvembu Kudineer Chooranam Results**	**Nilalvembu Kudineer Results**
State	Solid - Crude Raw Material	Decoction - Water Extraction
Appearance	Brown	Dark Brown

Odour	Aromatic	Mild Characteristic
Nature	Hard Fiber / Woody	Slightly viscous

5.2.2. RESULTS OF PHYSICO - CHEMICAL PROPERTIES:

Table 14 Physico - Chemical properties of Nilavembu Kudineer Chooranam

PARAMETER	RESULT
Loss on drying	**3.53 %**
Total Ash content	**5.37 %**
Acid insoluble Ash	**1.49 %**
Water soluble Ash	**1.43 %**
Water soluble Extraction	**13.61 %**
Alcohol soluble Extraction	**20.15 %**
P^H	**4.05 %**

Results of Physico - chemical properties of *Nilavembu Kudineer chooranam* is suggest to

Loss on drying

The total of volatile content and moisture present in the drug was established in loss on drying. Moisture content of the drug reveals the stability and its shelf-life. High moisture content can adversely affect the active ingredient of the drug. Thus low moisture content could get maximum stability and better shelf life. Loss on drying of *Nilavembu Kudineer chooranam* is **3.53 %**. This result is suggesting the stability better shelf life of *Nilavembu Kudineer chooranam*.

Total Ash

Total Ash value of plant material indicated the amount of minerals and earthy materials present in the plant material. The total inorganic content (ammonium,

potassium, calcium, chloride, iron, etc.,) present in the drug is measured through the Total Ash value and it is of **5.37 %**

Acid insoluble Ash

The acid insoluble Ash value of the drug denotes the amount of siliceous matter present in the plant. The quality of the drug is better if the acid insoluble value is low. In *Nilavembu Kudineer chooranam* acid insoluble Ash is **1.49 %**. It denotes the quality of *Nilavembu Kudinner chooranam.*

Water soluble Ash

Water-soluble Ash is the part of the total Ash content, which is soluble in water. It is **1.43 %** in *Nilavembu Kudineer chooranam.*

Water soluble extraction

Water soluble extractive value is **13.61 %** in *Nilavembu Kudineer chooranam.*

Alcohol soluble Ash

Alcohol-soluble extractive value is **20.15 %** in *Nilavembu Kudineer chooranam*.

The percentage of soluble matters present in the drug is determined by the values of water extractive and ethanol extractive. Based on the extractive value suitable solvent can be selected. It also gives the percentage of drug which will correlate with the metabolism reactions. Water-soluble extractive value plays an important role in evaluation of crude drugs. The alcohol-soluble extractive value was also indicative for the same purpose as the water-soluble extractive value.

P^H

P^H of the Nilavembu Kudineer is **4.05 %** it denotes it is weekly acidic. So Nilavembu Kudineer is absorbed quickly in the stomach when it is administered orally. It revealed that Nilavembu Kudineer is suitable for oral administration.

5.2.3. RESULTS OF HEAVY METAL ANALYSIS:

Nilavembu Kudineer chooranam was analyzed by the by the Atomic Absorption Spectrometry (AAS) to detect the trace elements and other elements quantitatively. The result of AAS is given on the **Table 15**

Table 15 Heavy Metal Analysis of Nilavembu Kudineer chooranam

Elements	Max. Absorption	Result	Max. Limit
Arsenic (As)	193.7 nm	**BDL**	3 ppm
Mercury (Hg)	253.7 nm	**BDL**	1 ppm
Cadmium (Cd)	228.8 nm	**BDL**	0.3 ppm
Lead (Pb)	217 nm	**0.11**	10 ppm

BDL - Below Detection Limit

The results of the present investigations have clearly shows that the sample has no traces of heavy metals such as Arsenic, Mercury and Cadmium. Further the results show the presence of Lead 0.11 ppm level. The presence of Lead in *Nilavembu Kudineer chooranam* is very low when compared to the recommended limit.

5.2.4. RESULTS OF MICROBIAL CONTAMINATION TEST:

Table 16 Microbial Contamination Test of Nilavembu Kudineer Chooranam

S. No	Parameters	Result
1.	Total Bacterial Content	**Absent**
2.	Total Fungal Content	**18 CFU / g**
3.	E.Coli	**Absent**
4.	Salmonella Spp.	**Absent**
5.	Staphylococcus Aureus	**Absent**
6.	Pseudomonas aeruginosa	**Absent**

5.2.5. RESULTS OF AFLATOXIN ANALYSIS:

Table 17 Aflatoxin Analysis of Nilavembu Kudineer Chooranam

Afltoxin	Sample	AYUSH Specification Limit
B_1	Not Detected - Absent	**0.5 ppm**
B_2	Not Detected - Absent	**0.1 ppm**
G_1	Not Detected - Absent	**0.5 ppm**
G_2	Not Detected - Absent	**0.1 ppm**

The results shows that there was no spots were been identified in the test sample loaded on TLC plates when compare to the standard, which indicates that the sample were free from Aflatoxin B_1, Aflatoxin B_2, Aflatoxin G_1 and Aflatoxin G_2.

5.2.6. RESULTS OF PESTICIDE RESIDUE ANALYSIS:

Table 18 Pesticide Residue Analysis of Nilavembu Kudineer Chooranam

S.No	Parameters	Units	Results
1.	Aldrin	mg/Kg	BDL (DL:0.1)
2.	Dicofol	mg/Kg	BDL (DL:0.1)
3.	Dieldrin	mg/Kg	BDL (DL:0.1)
4.	Endosulfan 1	mg/Kg	BDL (DL:0.1)
5.	Endosulfan 2	mg/Kg	BDL (DL:0.1)
6.	Endosulfan sulphate	mg/Kg	BDL (DL:0.1)
7.	Heptachlor (sum, ofitsepoxides)	mg/Kg	BDL (DL:0.1)
8.	Hexacholarobenzene (HCB)	mg/Kg	BDL (DL:0.1)
9.	Methoxychlor	mg/Kg	BDL (DL:0.1)
10.	O,p – DDT	mg/Kg	BDL (DL:0.1)

11.	P,P – DDD	mg/Kg	BDL (DL:0.1)
12.	P,P – DDE	mg/Kg	BDL (DL:0.1)
13.	P,P – DDT	mg/Kg	BDL (DL:0.1)
14.	Alpha HCH	mg/Kg	BDL (DL:0.1)
15.	Beta HCH	mg/Kg	BDL (DL:0.1)
16.	Gamma HCH	mg/Kg	BDL (DL:0.1)
17.	Delta HCH	mg/Kg	BDL (DL:0.1)
18.	Endrin (sum, ofitaldehyde and ketone)	mg/Kg	BDL (DL:0.1)
19.	Chlordane (cisandgamma)	mg/Kg	BDL (DL:0.1)
20.	Butachlor	mg/Kg	BDL (DL:0.1)
21.	Captan	mg/Kg	BDL (DL:0.1)
22.	Chlorpyriphos	mg/Kg	BDL (DL:0.1)
23.	Monocrotophos	mg/Kg	BDL (DL:0.1)
24.	Propetamphos	mg/Kg	BDL (DL:0.1)
25.	Chlorfenvinphos	mg/Kg	BDL (DL:0.1)
26.	Dimethoate	mg/Kg	BDL (DL:0.1)
27.	Fenitrothion	mg/Kg	BDL (DL:0.1)
28.	Ethion	mg/Kg	BDL (DL:0.1)
29.	Malathion	mg/Kg	BDL (DL:0.1)
30.	Monocrotophos	mg/Kg	BDL (DL:0.1)
31.	Parathion - methyl	mg/Kg	BDL (DL:0.1)
32.	Parathion - ethyl	mg/Kg	BDL (DL:0.1)
33.	Phorate	mg/Kg	BDL (DL:0.1)

34.	Phospphomidon	mg/Kg	BDL (DL:0.1)
35.	Profenophos	mg/Kg	BDL (DL:0.1)
36.	Quinalphos	mg/Kg	BDL (DL:0.1)
37.	Triazophos	mg/Kg	BDL (DL:0.1)
38.	Disculfoton	mg/Kg	BDL (DL:0.1)
39.	Deltamethrin	mg/Kg	BDL (DL:0.1)
40.	Fenpropethrin	mg/Kg	BDL (DL:0.1)
41.	Fenvalerate	mg/Kg	BDL (DL:0.1)
42.	Alpha cypermethrin	mg/Kg	BDL (DL:0.1)
43.	Beta cyfluthrin	mg/Kg	BDL (DL:0.1)
44.	Lampda cyhalothrin	mg/Kg	BDL (DL:0.1)

BDL - Below Detection limit

DL - Detection limit

These results suggest *Nilavembu Kudineer chooranam* is free from pesticide residue.

5.2.7. RESULTS OF PHYTOCHEMICAL ANALYSIS:

The Preliminary phytochemical studies of ***Nilavembu Kudineer*** were done using standard procedures. The results were presented in below tables.

5.2.8. RESULT OF PHYTOCHEMICAL SCREENING - QUALITATIVE

Table 19 Results of Phyto - Chemical Analysis of Nilavembu Kudineer

S. No	Phyto-Chemicals	Test Name	H_2O Ext.
1.	Alkaloids	Mayer's Test	**Present**
		Wagner's Test	Absent
		Dragendroff's Test	Absent
		Hager's Test	Absent

2.	Carbohydrates	Molisch's Test:	Absent
		Benedict's Test	**Present**
3.	Glycoside	Modified Borntrager's Test	**Present**
		Cardiac Glycoside- Keller-Killiani test)	Absent
4.	Saponin	Froth Test	Absent
		Foam Test	**Present**
5.	Phytosterol	Salkowski's Test	**Present**
6.	Phenols	Ferric Chloride Test	Absent
7.	Tannins	Gelatin Test	**Positive**
8.	Flavonoids	Alkaline Reagent Test	Absent
		Lead acetate Test	Absent
9.	Proteins and amino acids	Xanthoproteic Test	Absent
10.	Diterpenes	Copper Acetate Test	**Positive**
11.	Gum and Mucilage	Extract + alcohol	**Positive**
12.	Fixed oils and Fats	Spot test	Absent
13.	Quinones	NAOH + Extract	Absent

Preliminary phytochemical screening revealed the presence of various bioactive compounds like **alkaloids, carbohydrates, diterpenes, glycoside, phytosetrol, saponins, tannins** and **mucilage** in ***Nilavembu Kudineer***.

5.2.9. RESULTS OF BIO CHEMICAL ANALYSIS:

RESULT OF BASIC AND ACIDIC RADICAL'S STUDIES

Table 20 Results of Bio - Chemical Analysis of Nilavembu Kudineer

PARAMETER	RESULTS
Test for Acid Radicles	
Test for Sulphate	**Present**
Test for Phosphate	**Present**
Test for Carbonate	**Present**
Test for Basic Radicles	
Test for Lead	Absent
Test for Aluminum	Absent
Test for Iron	**Present**
Test for Zinc	Absent
Test for Calcium	**Present**
Test for Magnesium	Absent
Test for Ammonium	**Present**
Test for Mercury	Absent
Test for Arsenic	Absent
Miscellaneous	
Test for Reducing Sugar	Absent
Test for Alkaloids	**Present**
Test for Tanni Acid	**Present**

From the basic and acidic radical studies Sulphate, Phosphate, Carbonate, Iron, Calcium, Ammonium, Alkaloids and Tannic Acid compounds are present in *Nilavembu Kudineer*.

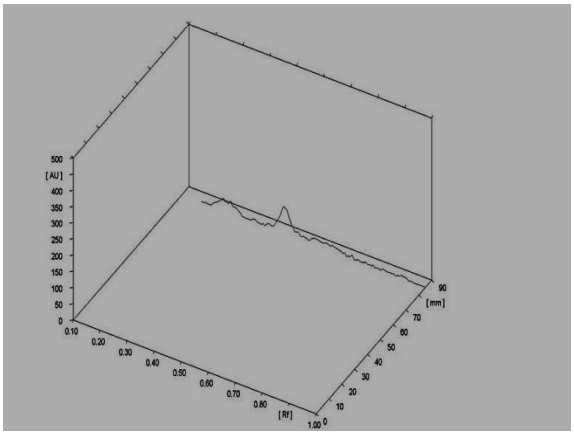
5.2.10. RESULTS OF HIGH PERFORMANCE THIN LAYER CHROMOTOGRAPHY (HPTLC) ANALYSIS:

Figure 2 High Performance Thin Layer Chromotography (Hptlc) Analysis Of Nilavembu Kudineer:

TLC Visualization of

Nilavembu Kudineer

3 D View of Chromatogram



HPTLC finger printing of Nilavembu Kudineer

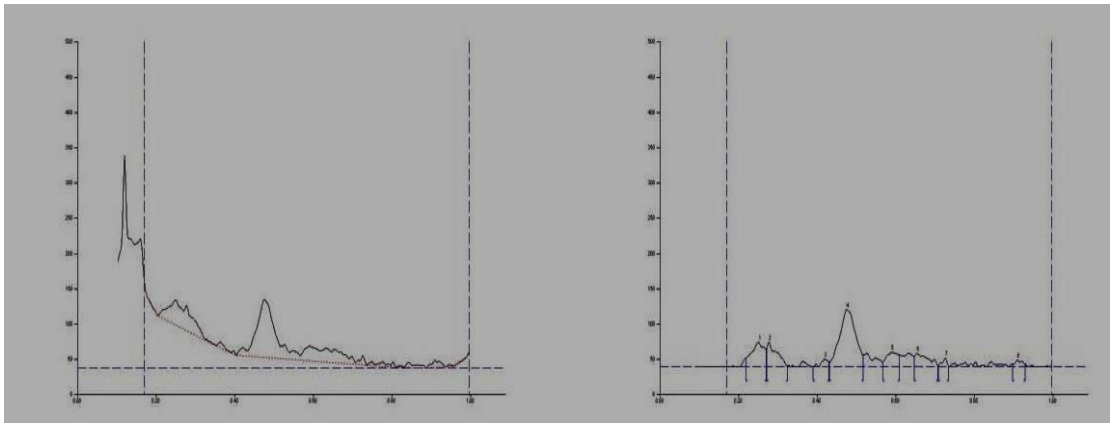


Table 21 High Performance Thin Layer Chromotography (Hptlc) Analysis Of Nilavembu Kudineer:

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.22	12.8	0.25	35.2	15.52	0.27	25.1	722.5	15.77
2	0.27	26.6	0.28	35.6	15.71	0.33	1.0	557.0	12.16
3	0.39	0.3	0.42	11.3	4.97	0.43	7.9	125.5	2.74
4	0.43	7.3	0.48	81.5	35.95	0.52	17.2	2127.1	46.43
5	0.57	6.5	0.59	20.8	9.18	0.61	17.4	396.6	8.66
6	0.65	14.8	0.66	19.1	8.41	0.71	2.2	423.6	9.25
7	0.71	2.7	0.73	13.0	5.74	0.74	1.6	103.3	2.26
8	0.90	4.3	0.91	10.2	4.52	0.93	4.1	125.6	2.74

The HPTLC analysis of the Nilavembu Kudineer showed that presence of eight prominent peaks corresponds to presence of eight versatile phyto - components present within it. Rf value of the peak ranges from 0.06 to 0.88. Further the peak four and two occupies the major percentage of are of 35.95 and 15.71 % which denotes the abundant existence of phyto - chemical compounds.

5.3. RESULTS OF TOXICITY STUDIES:

5.3.1. RESULTS OF ACUTE TOXICITY STUDY:

Acute toxicity study carried out as per OECD guideline 423; there was no treatment-related death or signs of toxicity developed in Wistar albino rats at the dosage of 5000 mg/kg b.wt throughout the study period. Further, No gross pathological changes have been seen in the internal organs of both control and treated group.

RESULTS OF BEHAVIORAL CHANGES IN ACUTE TOXICITY OF *NILAVEMBU KUDINEER* AT 5000 mg / kg DOSAGE

Table 22 Results of Behavioural Changes in Acute Toxicity of *Nilavembu Kudineer a*t 5000 mg / Kg Dosage

No.	**Response in 5000 mg / Kg body weight dosage of *Nilavembu Kudineer in wistar albino rats***	**Dose (5000 mg / Kg). (Number of animals:3)**											
		1.Head				**2.Body**				**3.Tail**			
		½	**1**	**2**	**4**	**½**	**1**	**2**	**4**	**½**	**1**	**2**	**4**
1.	Abnormal Gait	--	--	--	--	--	--	--	--	--	--	--	--
2.	Aggressiveness	--	--	--	--	--	--	--	--	--	--	--	--
3.	Akinesia	P	P	P	A	P	P	P	A	P	P	P	A
4.	Analgesia	--	--	--	--	--	--	--	--	--	--	--	--
5.	Catalepsy	--	--	--	--	--	--	--	--	--	--	--	--
6.	Convulsion	--	--	--	--	--	--	--	--	--	--	--	--
7.	Defecation	N	N	N	N	N	N	N	N	N	N	N	N
8.	Excitation	--	--	--	--	--	--	--	--	--	--	--	--
9.	Exopthalmos	--	--	--	--	--	--	--	--	--	--	--	--
10.	Head twitches	--	--	--	--	--	--	--	--	--	--	--	--
11.	Lacrimation	--	--	--	--	--	--	--	--	--	--	--	--
12.	Lethality	N	N	N	N	N	N	N	N	N	N	N	N
13.	Loss of corneal reflex	--	--	--	--	--	--	--	--	--	--	--	--
14.	Loss of righting reflex	--	--	--	--	--	--	--	--	--	--	--	--
15.	Loss of traction	--	--	--	--	--	--	--	--	--	--	--	--

16.	Piloerction	N	N	N	N	N	N	N	N	N	N	N	N
17.	Ptosis	--	--	--	--	--	--	--	--	--	--	--	--
18.	Reactivity to touch	P	P	P	P	P	P	P	P	P	P	P	P
19.	Respiration	P	P	P	P	P	P	P	P	P	P	P	P
20.	Sedation	--	--	--	--	--	--	--	--	--	--	--	--
21.	Stereotypis (Chewing)	N	N	N	N	N	N	N	N	N	N	N	N
22.	Sterotypis (Head movements)	N	N	N	N	N	N	N	N	N	N	N	N
23.	Sterotypis (Sniffing)	--	--	--	--	--	--	--	--	--	--	--	--
24.	Straub	--	--	--	--	--	--	--	--	--	--	--	--
25.	Tremor	--	--	--	--	--	--	--	--	--	--	--	--
26.	Writhes	--	--	--	--	--	--	--	--	--	--	--	--

A - Absence of Activity

N - Normal Activity

P - Presence of Activity

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 5000 mg / Kg body weight of *Nilavembu Kudineer* administered orally during the study period. (Table 22) There was no mortality observed after dosing of ***Nilavembu Kudineer*** up to 5000 mg/kg body weight. This indicates that LD_{50} of ***Nilavembu Kudineer*** is more than 5000 mg/kg body weight.

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 14^{th} day, necropsy was performed and there was no abnormality seen in test groups as compared to control group during the examination.

5.3.2. RESULTS OF 28 DAYS REPEATED ORAL TOXICITY STUDY

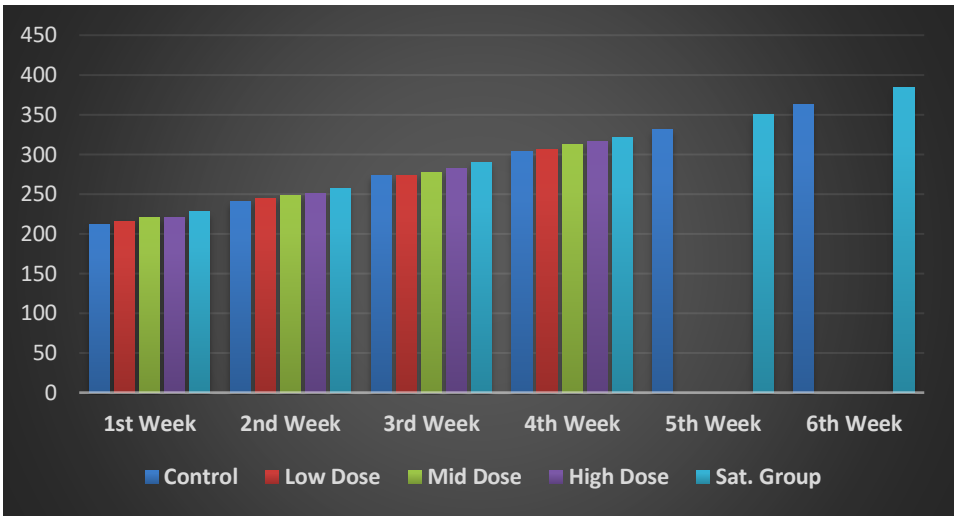
BODY WEIGHT CHANGES OF MALE WISTAR ALBINO RATS IN 28 DAYS REPEATED ORAL TOXICITY STUDY OF NILAVEMBU KUDINEER

Table 23 Body Weight Changes of Male Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer

GROUP	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	SATELLITE GROUP
1st Week	211.4±8.905	215.2±9.628	220.6±7.368	220.6±7.635	228±5.700
2nd Week	241±8.426	244.4±9.813	248.6±6.580	251±4.415	256.6±7.300
3rd Week	273.6±6.066	274 ± 9.380	277.4±8.905	281.6±10.597	290±12.145
4th Week	303±10.319	306.4±8.677	313±4.795	316.6±2.701	321.4±10.620
5th Week	331.4±15.192				349.6±6.426
6th Week	363.2±16.285				384.8±6.058

Values are expressed as mean SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (N = 5) ^{ns} $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ calculated by comparing treated groups with control groups.

Figure 3 Body Weight Changes Of Male Wistar Albino Rats In 28 Days Repeated Oral Toxicity Study Of Nilavembu Kudineer.



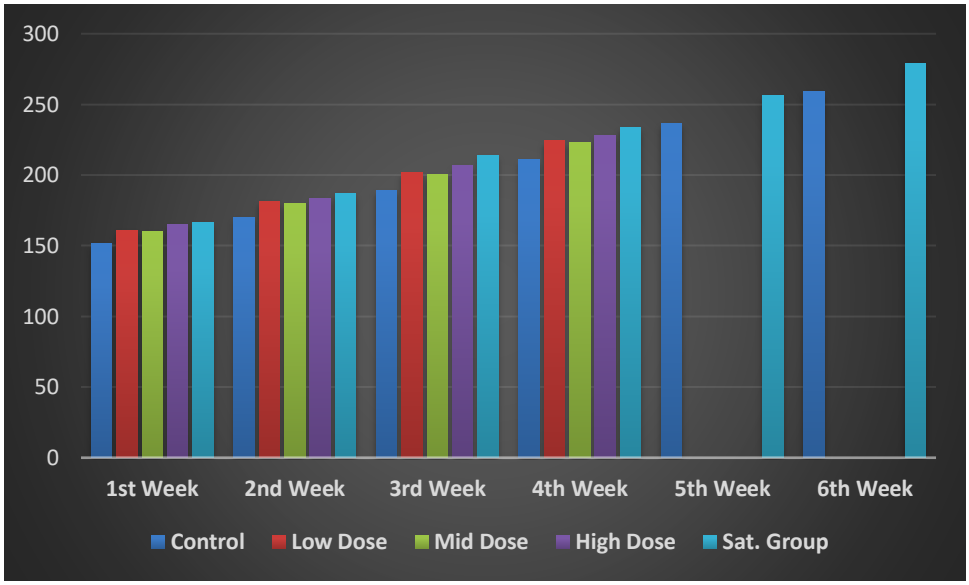
BODY WEIGHT CHANGES OF FEMALE WISTAR ALBINO RATS IN 28 DAYS REPEATED ORAL TOXICITY STUDY OF NILAVEMBU KUDINEER

Table 24 Body Weight Changes of Female Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer

FEMALE	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	SATELLITE GROUP
1st Week	151.6±8.142	161±4.898	160.2±3.563	165.2±4.764	166.2±9.859
2nd Week	170.2±9.679	181.4±6.066	179.6±5.983	183.4±8.08	187±9.433
3rd Week	189.4±9.607	201.8±7.362	200.2±8.136	207±10.271	214±11.937
4th Week	210.8±10.639	224.4±11.327	223.2±12.049	228.2±8.167	233.8±9.909
5th Week	236.4±10.212				256.2±11.966
6th Week	259±11.937				166.2±9.859

Values are expressed as mean SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (N = 5) ^{ns} $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ calculated by comparing treated groups with control groups.

Figure 4 Body Weight Changes of Female Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer



The results suggest body weight of the all the treatment animals male were gradually increased during the study period and there were no significant changes observed between control and test drug treated group (Low Dose, Mid Dose, High Dose & Satellite Group) (**Table No: 23.**) No Significant changes observed all the treatment animals' females (Low Dose, Mid Dose, High Dose & Satellite Group) when compared with control group (**Table No: 24.**)

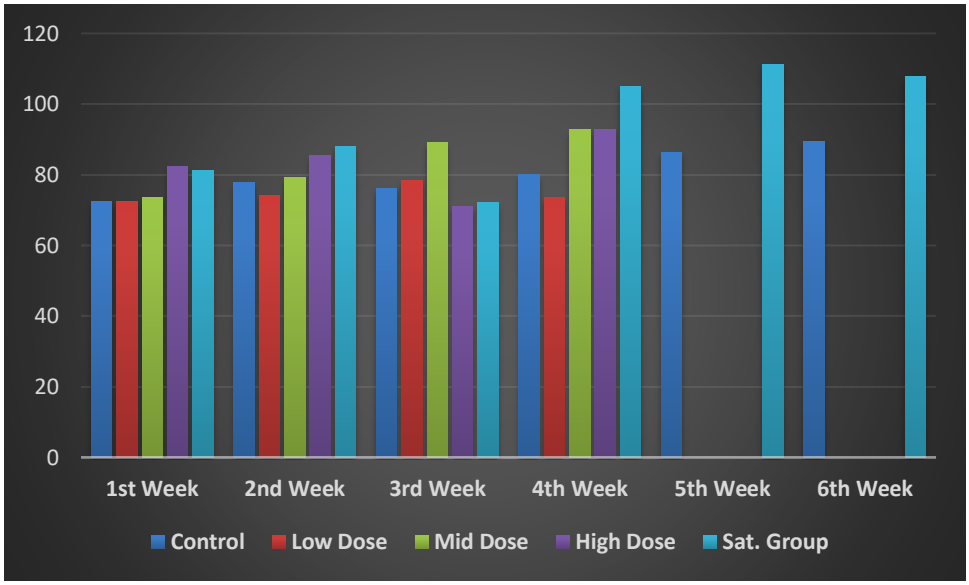
FEED INTAKE OF MALE WISTAR ALBINO RATS IN 28 DAYS REPEATED ORAL TOXICITY STUDY OF NILAVEMBU KUDINEER

Table 25 Feed Intake of Male Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer.

GROUP	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	SATELLITE GROUP
1st week	65.2±4.5	68.1±1	62.7±1.6	66.7±1.9	66.8±1.7
2nd week	71±2.8	71.7±1.1	64.7±1.1	71.2±1.1	67.1±1.9
3rd week	70.2±1.3	71.5±1.5	68.5±2.7	73.2±2.2	72.8±2.1
4th week	71.7±2	74.1±1.9	75.2±2.4	77±2	74.1±1.5
5th Week	71.7±2.4				77±2.1
6th week	73.7±3				77.4±4.6

Values are expressed as mean SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (N = 5) ^{ns} $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ calculated by comparing treated groups with control groups

Figure 5 Feed Intake of Male Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer



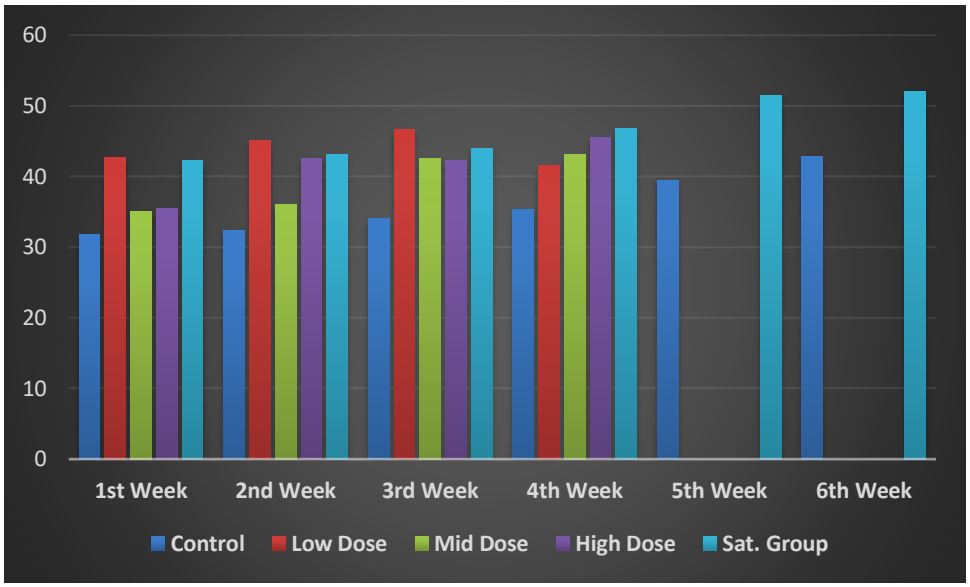
FEED INTAKE OF FEMALE WISTAR ALBINO RATS IN 28 DAYS REPEATED ORAL TOXICITY STUDY OF NILAVEMBU KUDINEER

Table 26 Feed Intake of Female Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer.

GROUP	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	SATELLITE GROUP
1st week	31.8±3.3	42.7±2	35±0.8	35.5±1.3	42.2±2.6
2nd week	32.4±2.5	45.1±2.1	36.1±1.7	42.5±2	43.1±2.1
3rd week	34.1±3.8	46.7±1.2	42.5±1.5	42.2±1.2	44±1.8
4th week	35.4±1.3	41.5±0.	43.1±2.9	45.5±1.9	46.8±1.4
5th Week	39.5±3.7				51.5±1.2
6th week	42.8±1.9				52.1±1

Values are expressed as mean SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (N = 5) ^{ns} $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ calculated by comparing treated groups with control groups

Figure 6 Feed Intake of Female Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer



Feed intake results suggest, the feed intake of all the test animals was gradually increased. But significantly feed intake was increased in Satellite group males when compared with control group males at **p < 0.01 (p = 0.0083) (**Table No: 25.)** And also significantly feed intake was increased low dose test drug treated female group at *p < 0.05 and satellite group females **p < 0.01 (p = 0.0029) when compared with control female. **(Table No: 26.)**

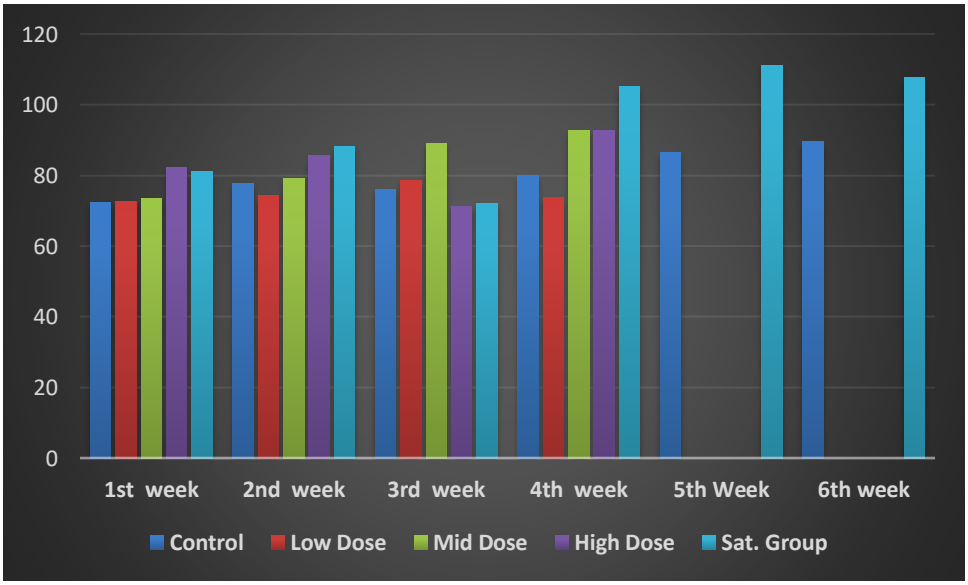
WATER INTAKE OF MALE WISTAR ALBINO RATS IN 28 DAYS REPEATED ORAL TOXICITY STUDY OF NILAVEMBU KUDINEER

Table 27 Water Intake of Male Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer

GROUP	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	SATELLITE GROUP
1st week	72.4±2	72.5±1.5	73.5±1.5	82.2±1.3	81.1±1.3
2nd week	77.7±4.8	74.2±1.2	79.1±4.7	85.5±1.2	88.1±1.8
3rd week	76.1±2.1	78.5±3.4	89±1.2	71.1±0.8	72.1±0.8
4th week	80±2.1	73.7±2	92.7±1.6	92.8±2.5	105.1±0.7
5th Week	86.4±3.7				111.2±2.8
6th week	89.5±0.7				107.8±4.6

Values are expressed as mean SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (N = 10) ^{ns} $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ calculated by comparing treated groups with control groups

Figure 7 Water intake of Male Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer



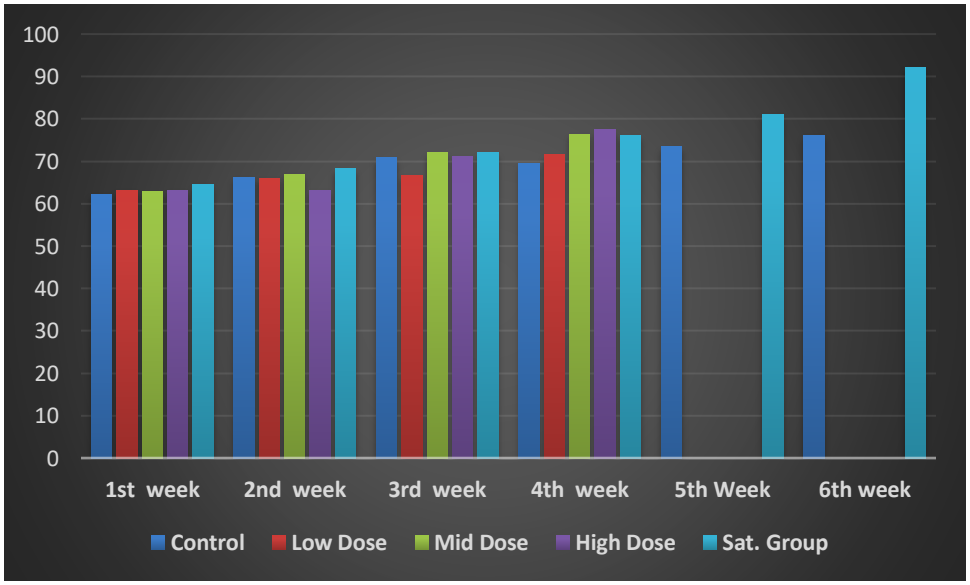
WATER INTAKE OF FEMALE WISTAR ALBINO RATS IN 28 DAYS REPEATED ORAL TOXICITY STUDY OF NILAVEMBU KUDINEER

Table 28 Water Intake of Female Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer

GROUP	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	SATELLITE GROUP
1st week	62.2±2.1	63.1±2.1	62.8±2.8	63.2±1.3	64.5±1.5
2nd week	66.2±1.6	66±1.6	66.8±2.1	63.1±5.2	68.2±0.7
3rd week	70.8±1.2	66.7±1.6	72.1±1.7	71.1±0.8	72.1±0.8
4th week	69.5±2.4	71.5±1.8	76.4±1.9	77.4±2.6	76±1.9
5th Week	73.4±2.3				81.1±1.8
6th week	76.1±2.1				92.2±4.1

Values are expressed as mean SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (N = 10) ^{ns} $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ calculated by comparing treated groups with control groups

Figure 8 Water Intake of Female Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer



Water intake results suggest, the water intake of all the male and female test animals was gradually increased when compared with control groups (p = 0.0758), (p = 0.3293) **(Table 27 and 28)**

EFFECT OF 28 DAYS REPEATED ORAL TOXICITY STUDY OF NILAVEMBU KUDINEER ON HEMATOLOGICAL PARAMETERS

Table 29 Effect of 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer on Hematological Parameters

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	SATELLITE
RBC ($\times 10^6$ µl)	6.4±0.9	6.18±1.21	6.45±1.3	6.37±1.01	6.77±1.3
WBC ($\times 10^6$ µl)	8.65±3.1	9.05±2.11	9.46±2.8	9.63±2.8	9.05±1.5
PLT ($\times 10^6$ µl)	707.2±48.7	510.82±110.8	703.6±144.1	632.6±264.8	755.8±105
HGB (g/dl)	11.6±0.7	11.59±1.7	12.58±1.66	11.16±2.3	11.7±1.9
MCH (pg)	17.94±1.7	26.21±17.1	34.94±50.9	19.78±2.31	19.9±1.9
MCV (fl)	60.97±5.4	56.55±4.25	61.65±6.09	61.75±6.1	59.3±5.4
N (fl)	2.35±0.52	2.16±0.71	2.28±0.5	2.53±0.84	2.2±0.5
E (%)	1.4±0.27	1.32±0.31	2.61±3.66	1.39±0.4	1.3±0.3
B (%)	0.1±0.31	0.2±0.42	0.3±0.4	0.4±0.51	0.2±0.4
L (%)	75.58±10.54	68.32±11.06	77.1±14.2	77.43±11.72	76.1±12.3
M (%)	2.98±1.4	3.15±1.19	2.71±1.23	3.74±1.06	3±1.2

Values are expressed as mean SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (N = 10) ^{ns} $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ calculated by comparing treated groups with control groups

Figure 10 Effect of 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer on Hematological Parameters

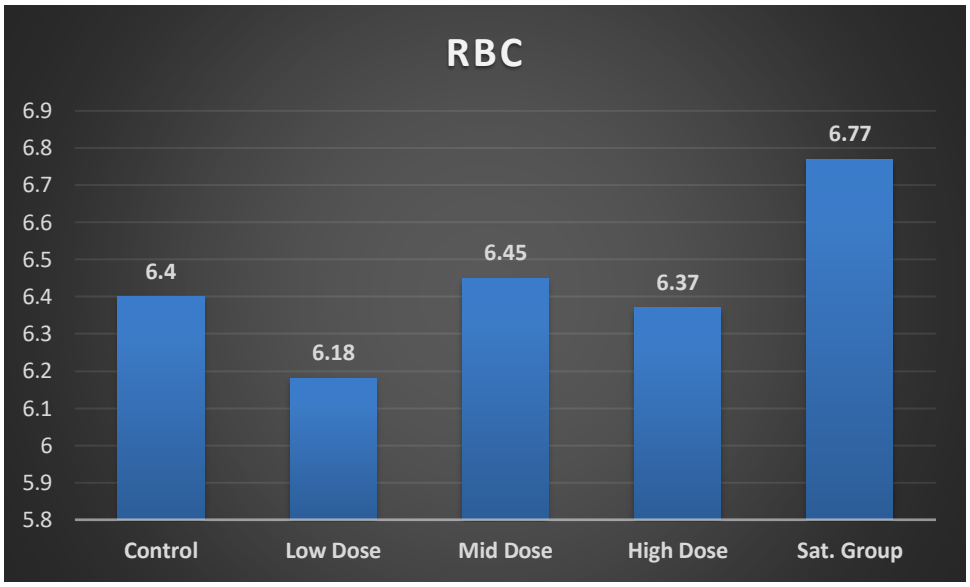


Figure 11 Effect of 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer on Hematological Parameters

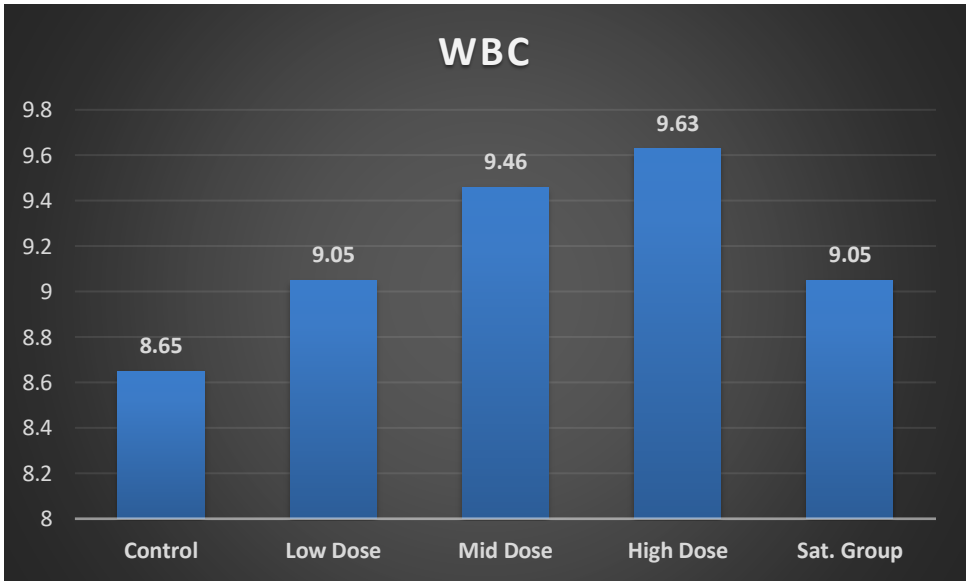


Figure 12 Effect of 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer on Hematological Parameters

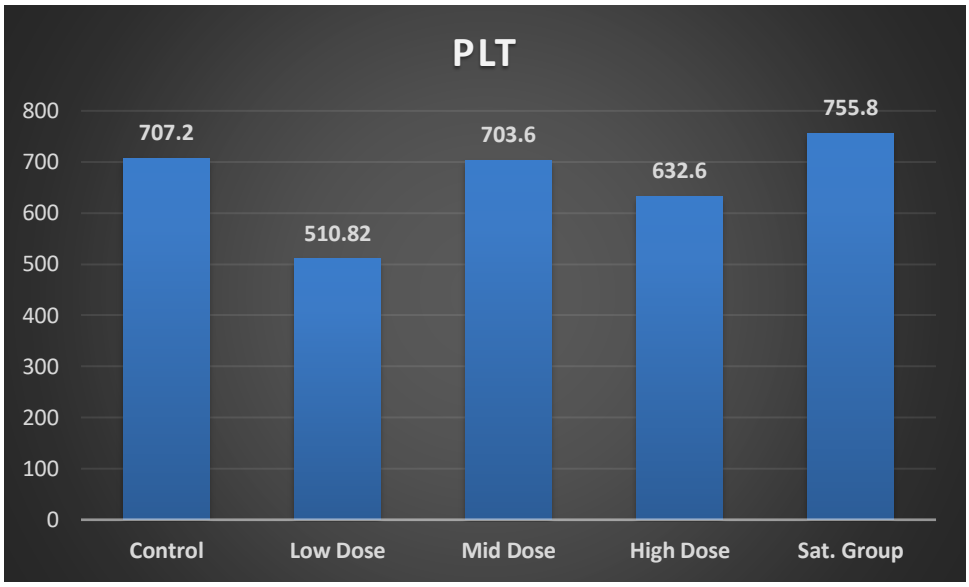


Figure 13 Effect of 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer on Hematological Parameters

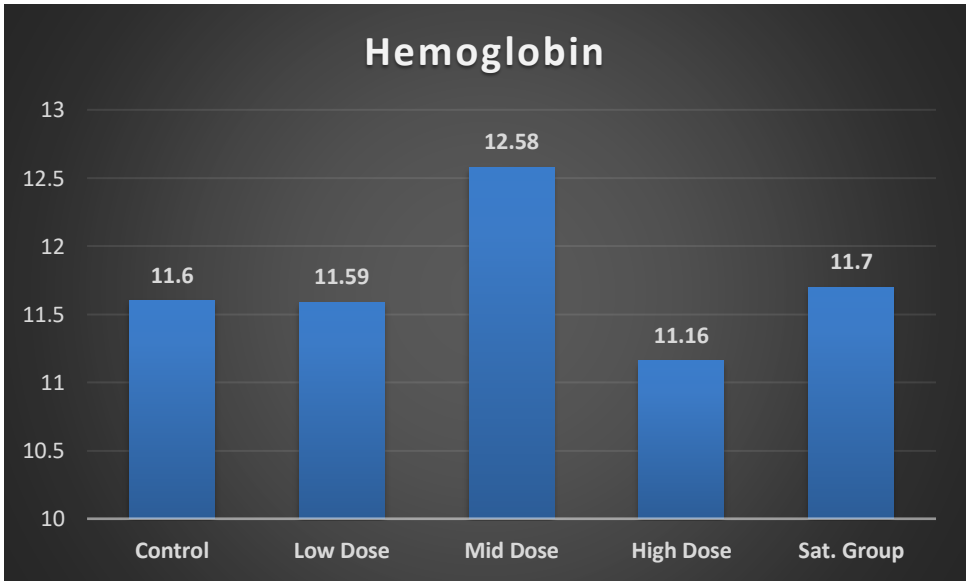


Figure 14 Effect of 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer on Hematological Parameters

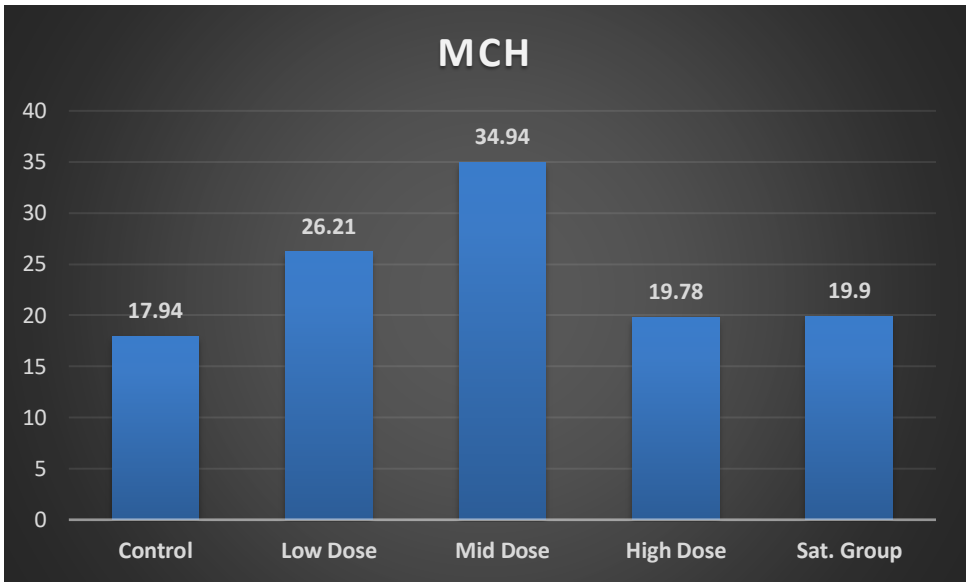


Figure 15 Effect of 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer on Hematological Parameters

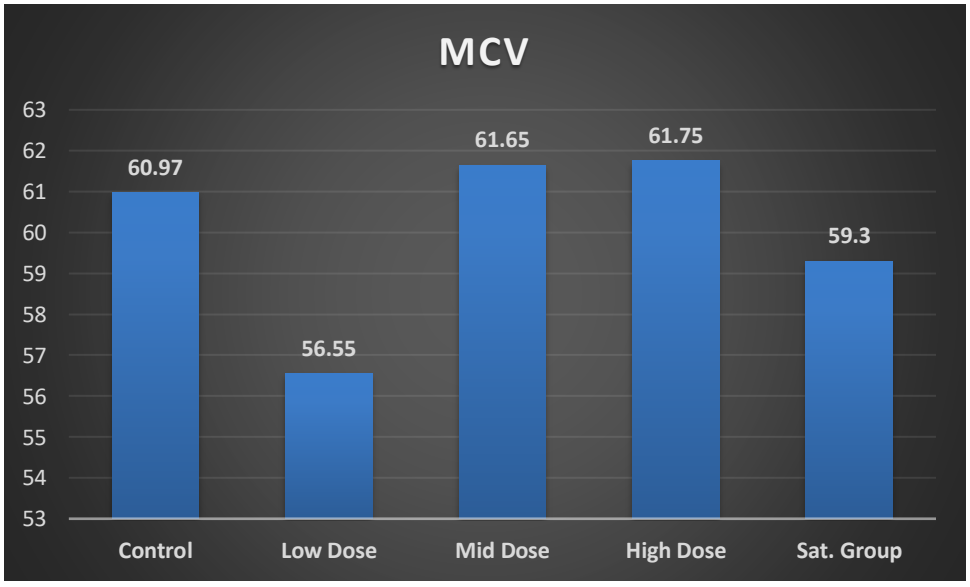
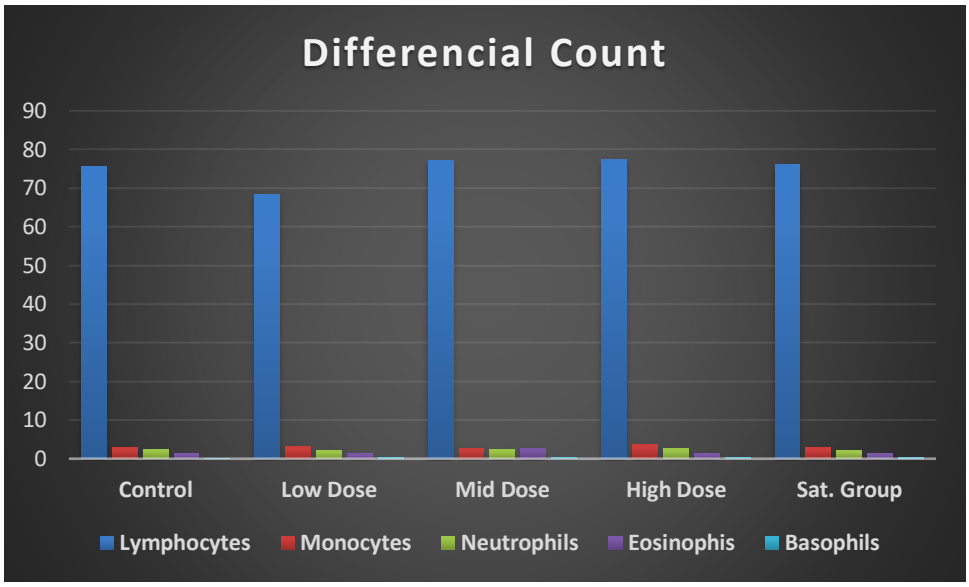


Figure 16 Effect of 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer on Haematological Parameters



Haematological parameters results suggest, the parameters of all the test animals were normal level when compared with control group. (p = 0.9989) **(Table 29)**

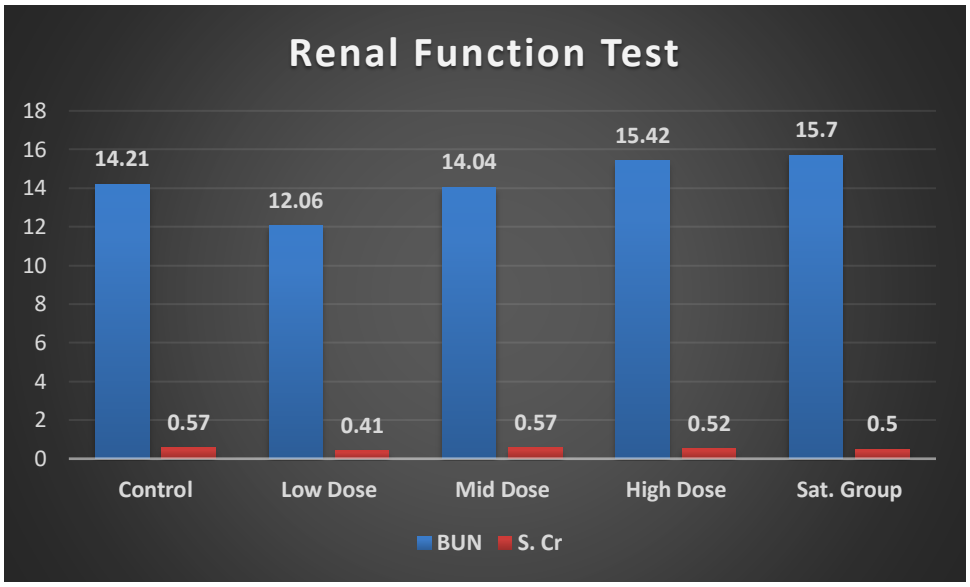
EFFECT OF 28 DAYS REPEATED ORAL TOXICITY STUDY OF NILAVEMBU KUDINEER ON SEROLOGICAL PARAMETER - RENAL FUNCTION TEST

Table 30 Effect of 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer on Serological Parameter - Renal Function Test

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	SATELLITE
BUN	14.21±2.6	12.06±1.09	14.04±1.97	15.42±2.4	15.7±1.5
S. Cr	0.57±0.19	0.41±0.2	0.57±0.25	0.52±0.19	0.5±0.1

Values are expressed as mean SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (N = 10) ^{ns} $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ calculated by comparing treated groups with control groups

Figure 17 Effect of 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer on Serological Parameter - Renal Function Test



Renal function test results suggest the parameters of all the tests animals was normal level compared with control group (p = 0.9996) **(Table 30)**

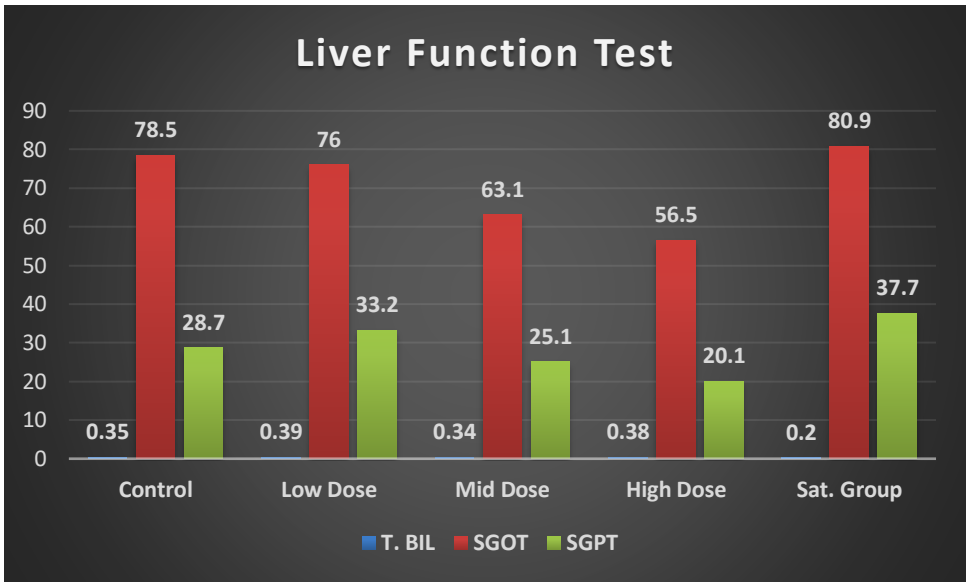
EFFECT OF 28 DAYS REPEATED ORAL TOXICITY STUDY OF NILAVEMBU KUDINEER ON SEROLOGICAL PARAMETER - LIVER FUNCTION TEST

Table 31 Effect of 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer on Serological Parameter - Liver Function Test

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	SATELLITE
T. BIL	0.35±0.18	0.39±0.16	0.34±0.08	0.38±0.19	0.2±0
SGOT	78.5±23.1	76±16.9	63.1±15.01	56.5±10.5	80.9±15
SGPT	28.7±9.9	33.2±6.2	25.1±9.02	20.1±3.9	37.7±4

Values are expressed as mean SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (N = 10) ^{ns} $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ calculated by comparing treated groups with control groups

Figure 18 Effect of 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer on Serological Parameter - Liver Function Test



Liver function test results suggest, the parameters of all the animals was normal level. (p = 0.9882)

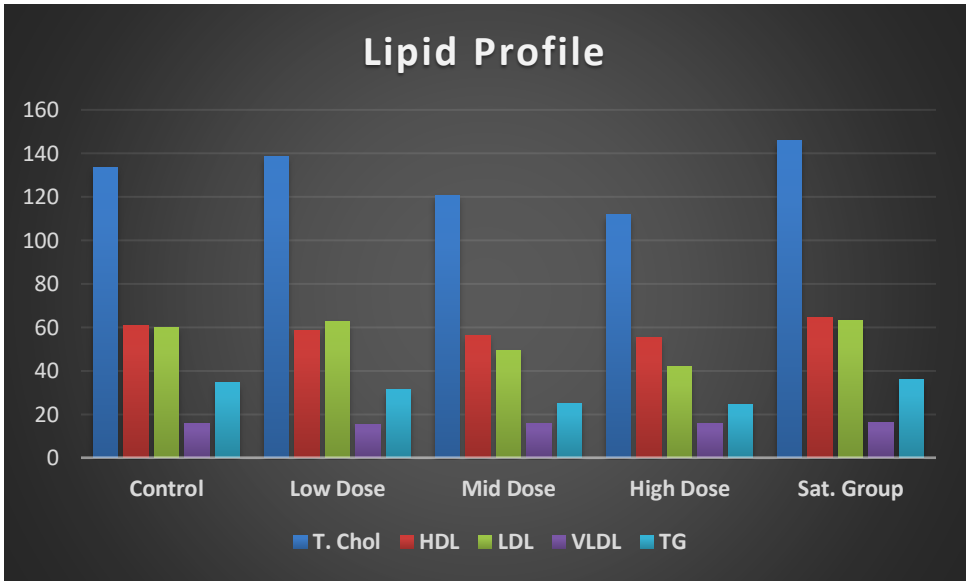
EFFECT OF 28 DAYS REPEATED ORAL TOXICITY STUDY OF NILAVEMBU KUDINEER ON SEROLOGICAL PARAMETER - LIPID PROFILE

Table 32 Effect of 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer on Serological Parameter - Lipid Profile

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	SATELLITE
T. Chol	133.37±9.01	138.66±9.09	120.8±14.7	111.69±7.9	145.8±7
HDL	61±9.9	58.7±7.4	56.3±8.1	55.5±9.9	64.5±5.7
LDL	60±10.9	62.7±16.09	49.5±16.9	41.8±9.02	63.3±5.7
VLDL	15.77±1.7	15.5±2.3	15.93±2.7	15.8±1.46	16.0±1.2
TG	34.4±6.9	31.3±3.05	24.9±7.4	24.5±3.02	35.8±8.5

Values are expressed as mean SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (N = 10) ^{ns} $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ calculated by comparing treated groups with control groups

Figure 19 Effect of 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer on Serological Parameter - Lipid Profile



In lipid profile results suggest, the parameters of all the test animals was normal level. p value is 0.9813 considered not significant.

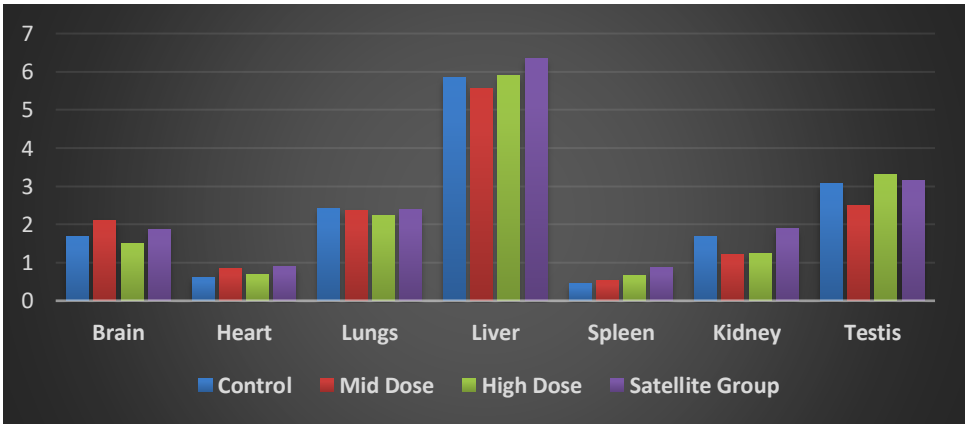
ORGAN WEIGHT CHANGES OF MALE WISTAR ALBINO RATS IN 28 DAYS REPEATED ORAL TOXICITY STUDY OF NILAVEMBU KUDINEER

Table 33 Organ Weight Changes of Male Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer

ORGANS	CONTROL	MID DOSE	HIGH DOSE	SATELLITE GROUP
Brain	1.68±0.148	2.1±0.316	1.5±0.223	1.88±0.148
Heart	0.62±0.148	0.84±0.114	0.7±0.148	0.9±0.212
Lungs	2.42±0.286	2.36±0.288	2.24±0.207	2.38±0.216
Liver	5.84±0.378	5.56±0.194	5.9±0.316	6.34±0.194
Spleen	0.44±0.114	0.52±0.13	0.66±0.207	0.86±0.144
Kidney	1.68±0.192	1.22±0.204	1.24±0.084	1.9±0.114
Testis	3.06±0.169	2.5±0.282	3.3±0.264	3.14±0.167

Values are expressed as mean SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (N = 10) ^{ns} $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ calculated by comparing treated groups with control groups

Figure 20 Organ Weight Changes of Male Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer



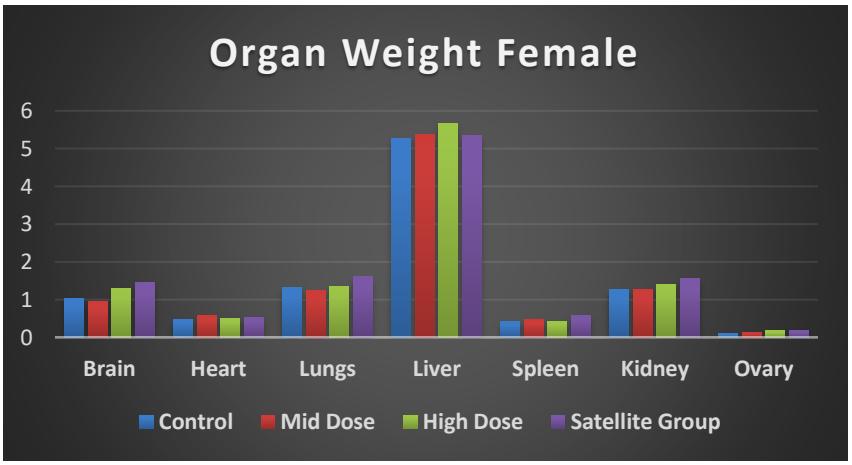
ORGAN WEIGHT CHANGES OF FEMALE WISTAR ALBINO RATS IN 28 DAYS REPEATED ORAL TOXICITY STUDY OF NILAVEMBU KUDINEER

Table No: 34 Organ Weight Changes of Female Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer

ORGANS	CONTROL	MID DOSE	HIGH DOSE	SATELLITE GROUP
Brain	1.02±0.083	0.94±0.116	1.29±0.162	1.45±0.166
Heart	0.48±0.083	0.57±0.043	0.51±0.073	0.53±0.057
Lungs	1.32±0.192	1.23±0.153	1.36±0.133	1.6±0.071
Liver	5.26±0.24	5.37±0.408	5.67±0.482	5.36±0.545
Spleen	0.41±0.135	0.47±0.033	0.42±0.065	0.58±0.165
Kidney	1.26±0.207	1.27±0.119	1.41±0.055	1.56±0.294
Ovary	0.1±0.02	0.13±0.03	0.17±0.02	0.18±0.03

Values are expressed as mean SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (N = 10) ^{ns} $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ calculated by comparing treated groups with control groups

Figure 21 Organ Weight Changes of Female Wistar Albino Rats in 28 Days Repeated Oral Toxicity Study of Nilavembu Kudineer

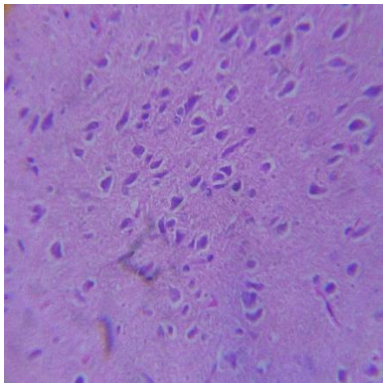


Organ weight of the all test male animals was normal in level. p = 0.9875, considered not significant. **(Table No: 33.)** Organ weight of all the female animals was normal. p = 0.9989, considered not significant.

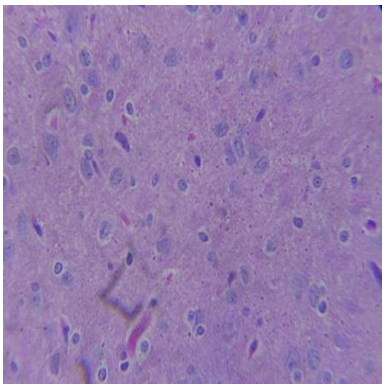
HISTOPATHOLOGICAL FINDINGS AND RESULTS OF 28 DAYS REPEATED ORAL TOXICITY STUDY OF *NILAVEMBU KUDINEER*

HISTOPATHOLOGY OF BRAIN

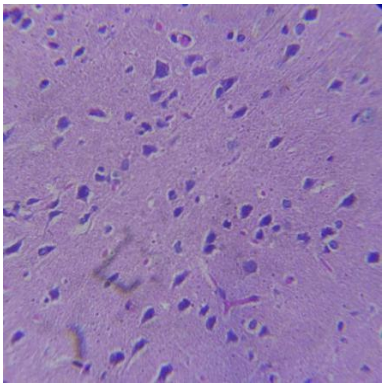
CONTROL MALE



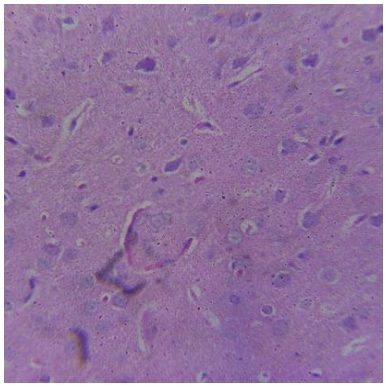
CONTROL FEMALE



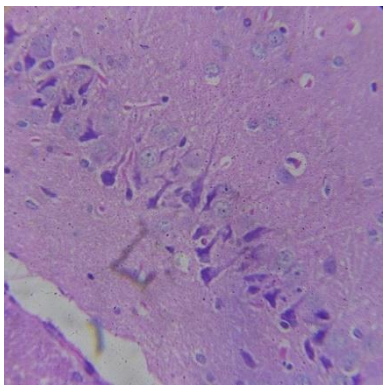
HIGH DOSE MALE



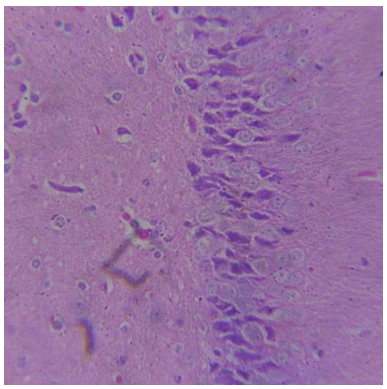
HIGH DOSE FEMALE



SATELLITE MALE



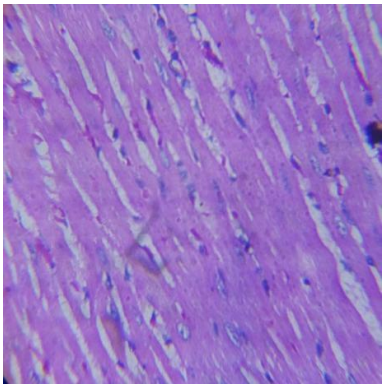
SATELLITE FEMALE



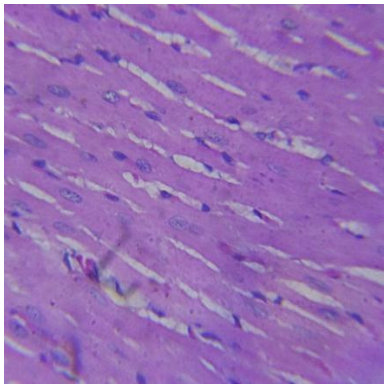
HISTOPATHOLOGICAL FINDINGS AND RESULTS OF 28 DAYS REPEATED ORAL TOXICITY STUDY OF *NILAVEMBU KUDINEER*

HISTOPATHOLOGY OF HEART

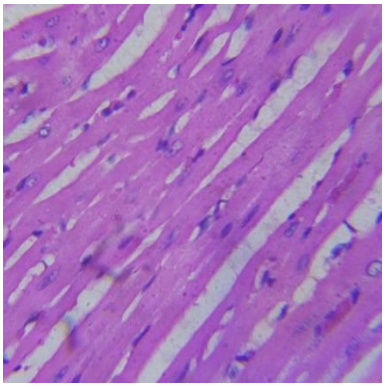
CONTROL MALE



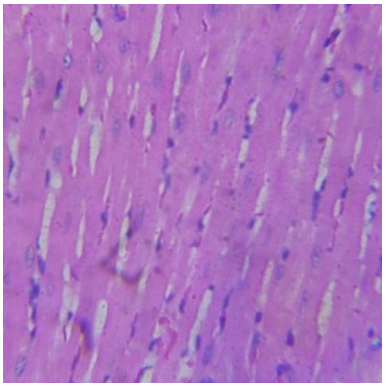
CONTROL FEMALE



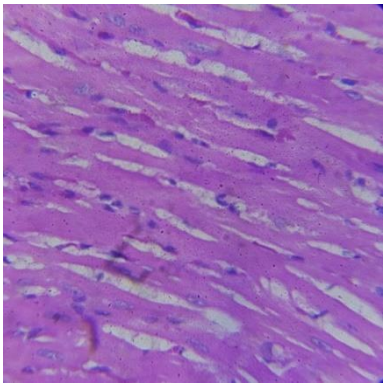
HIGHDOSE MALE



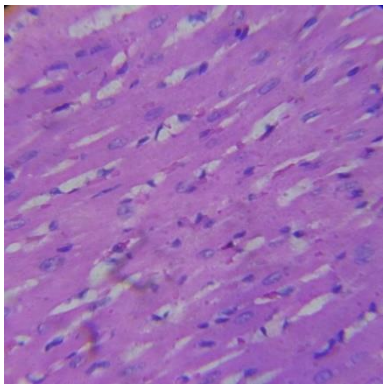
HIGH DOSE FEMALE



SATELLITE MALE



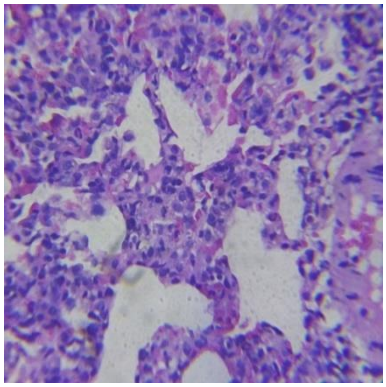
SATELLITE FEMALE



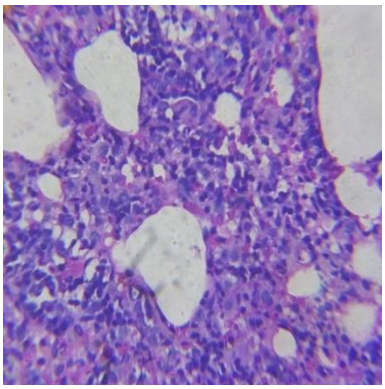
HISTOPATHOLOGICAL FINDINGS AND RESULTS OF 28 DAYS REPEATED ORAL TOXICITY STUDY OF *NILAVEMBU KUDINEER*

HISTOPATHOLOGY OF LUNGS

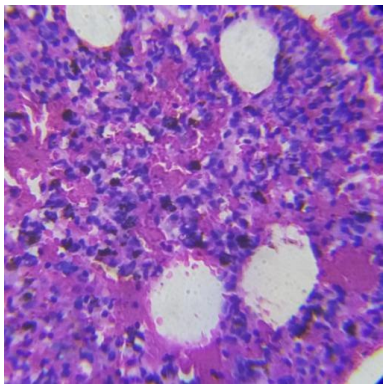
CONTROL MALE



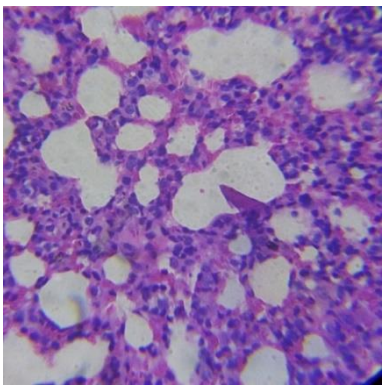
CONTROL FEMALE



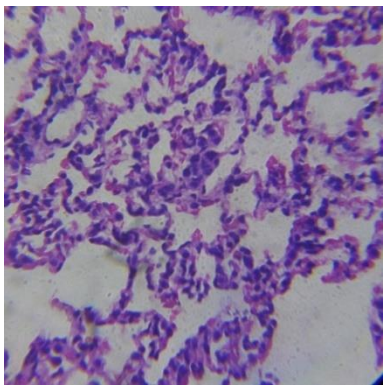
HIGH DOSE MALE



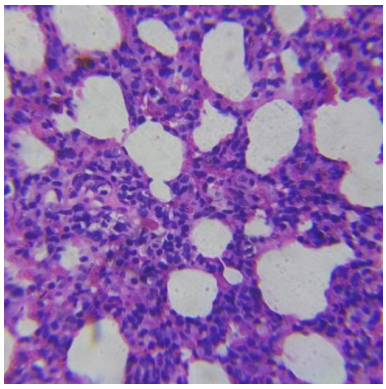
HIGH DOSE FEMALE



SATELLITE MALE



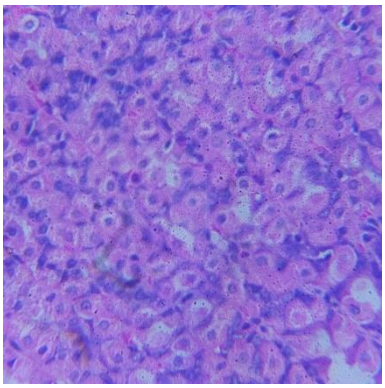
SATELLITE FEMALE



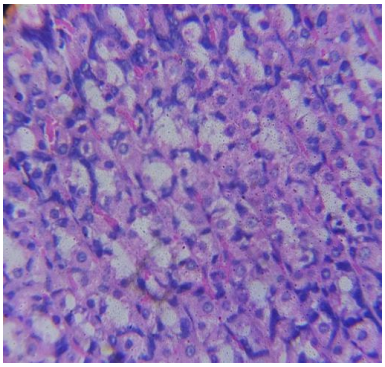
HISTOPATHOLOGICAL FINDINGS AND RESULTS OF 28 DAYS REPEATED ORAL TOXICITY STUDY OF *NILAVEMBU KUDINEER*

HISTOPATHOLOGY OF STOMACH

CONTROL MALE



CONTROL FEMALE



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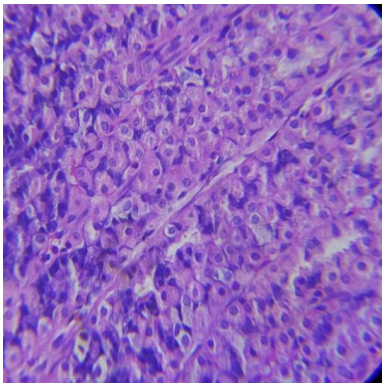

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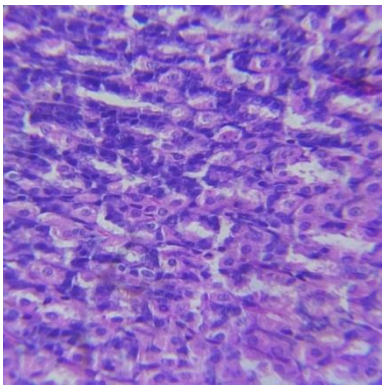
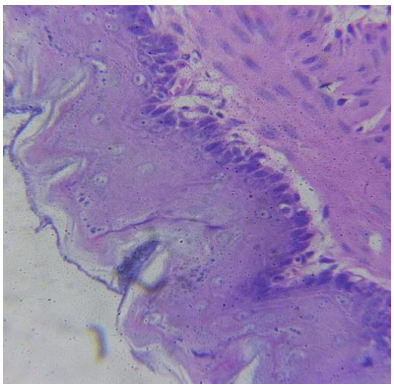


SATELLITE MALE



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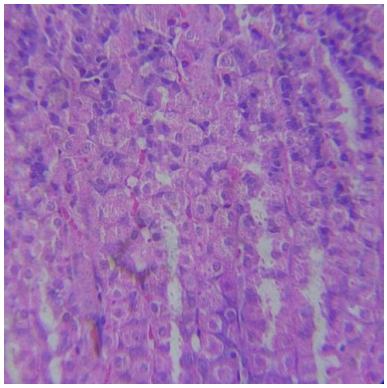
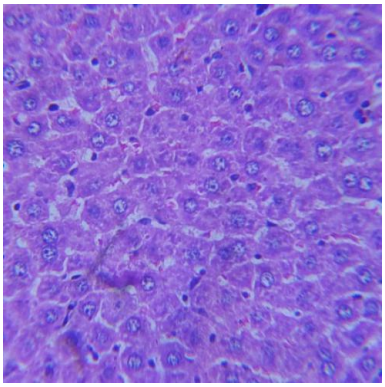


HISTOPATHOLOGICAL FINDINGS AND RESULTS OF 28 DAYS REPEATED ORAL TOXICITY STUDY OF *NILAVEMBU KUDINEER*

HISTOPATHOLOGY OF LIVER

CONTROL MALE



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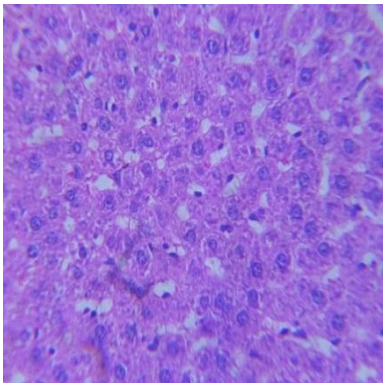

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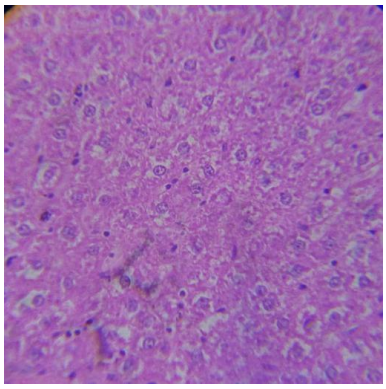

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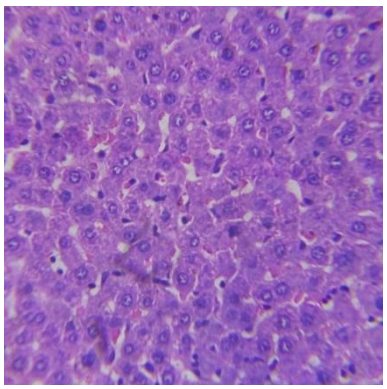

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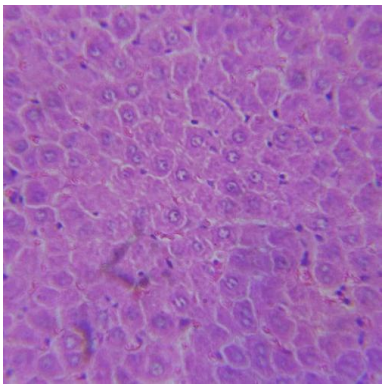

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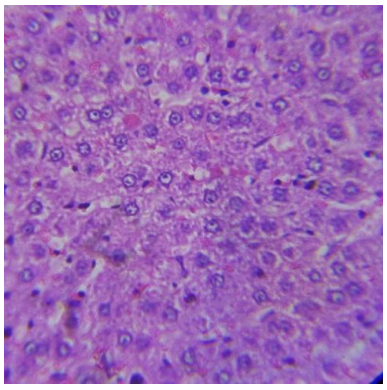
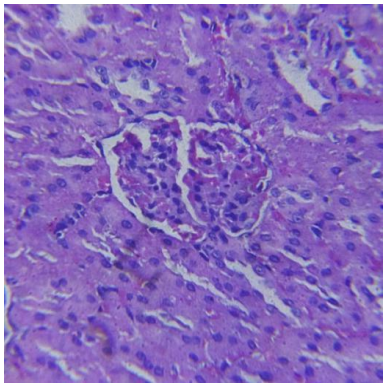


HISTOPATHOLOGICAL FINDINGS AND RESULTS OF 28 DAYS REPEATED ORAL TOXICITY STUDY OF *NILAVEMBU KUDINEER*

HISTOPATHOLOGY OF KIDNEY

CONTROL MALE



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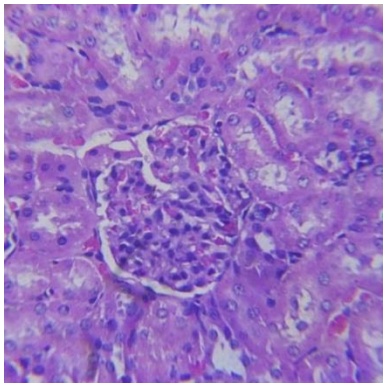

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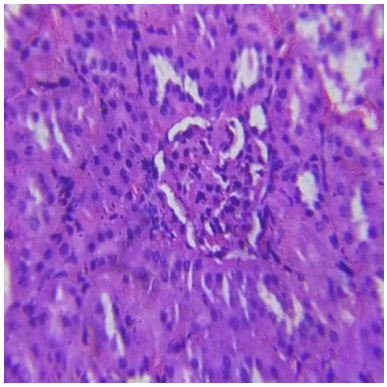

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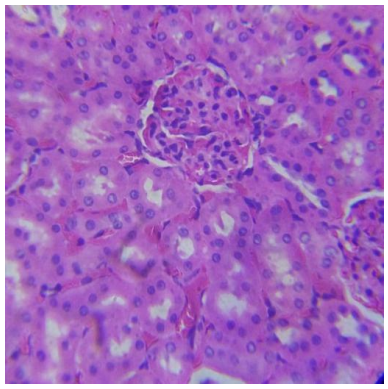

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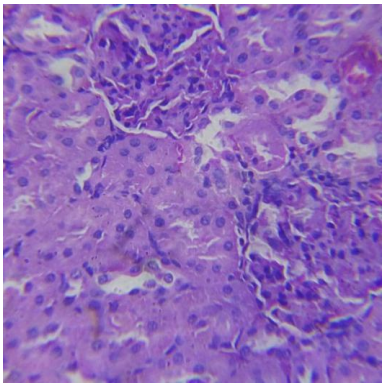

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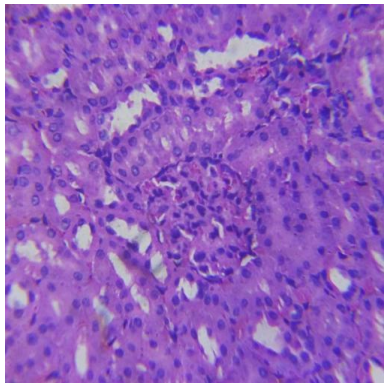
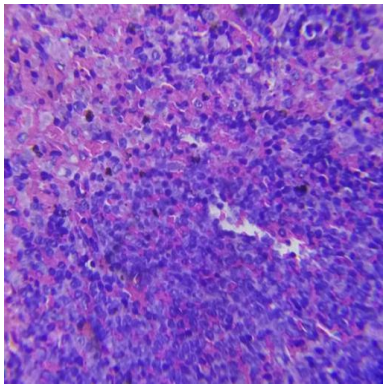


HISTOPATHOLOGICAL FINDINGS AND RESULTS OF 28 DAYS REPEATED ORAL TOXICITY STUDY OF *NILAVEMBU KUDINEER*

HISTOPATHOLOGY OF SPLEEN

CONTROL MALE



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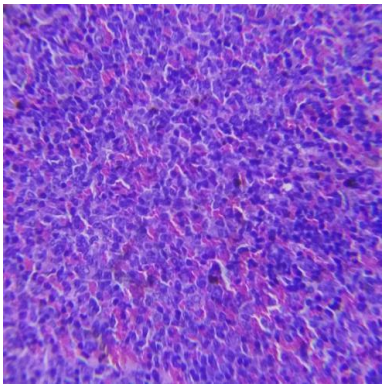

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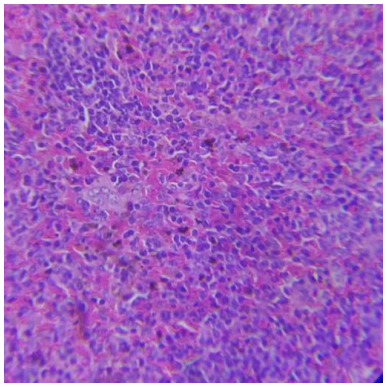

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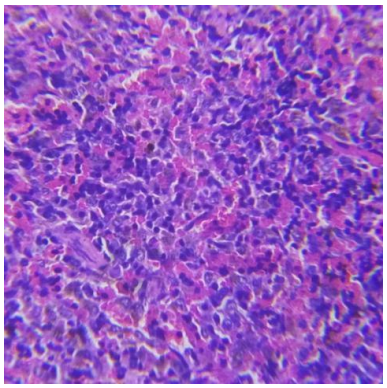

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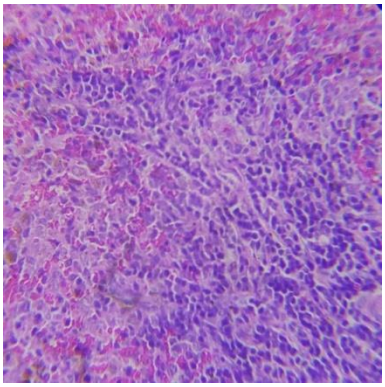

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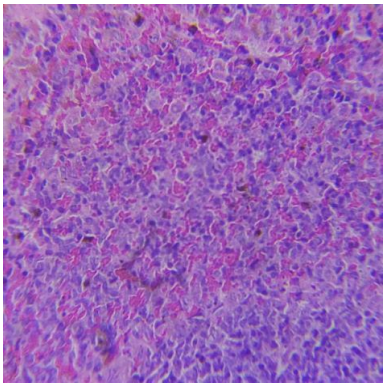
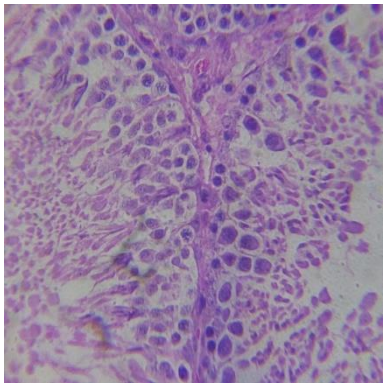


HISTOPATHOLOGICAL FINDINGS AND RESULTS OF 28 DAYS REPEATED ORAL TOXICITY STUDY OF *NILAVEMBU KUDINEER*

HISTOPATHOLOGY OF TESTIS

CONTROL



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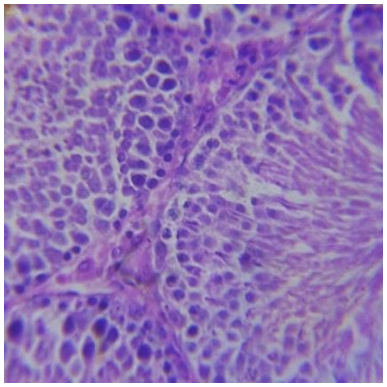

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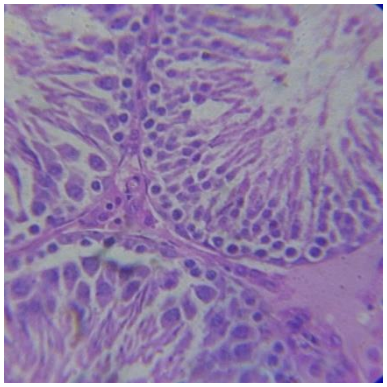
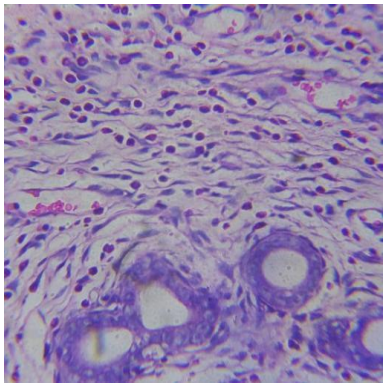


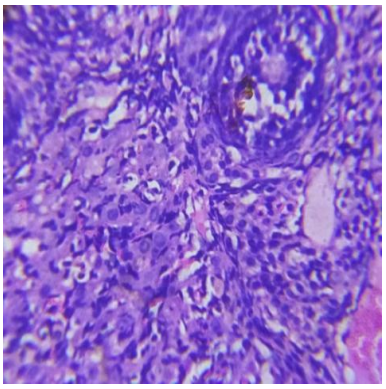
HISTOPATHOLOGICAL FINDINGS AND RESULTS OF 28 DAYS REPEATED ORAL TOXICITY STUDY OF *NILAVEMBU KUDINEER*

HISTOPATHOLOGY OF UTERUS AND OVARY

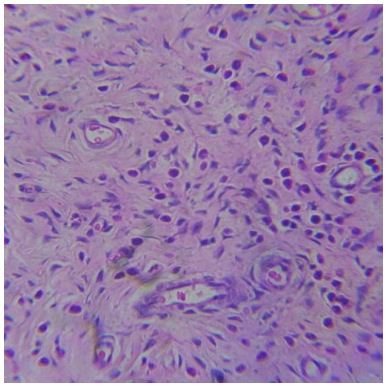
CONTROL UTERUS



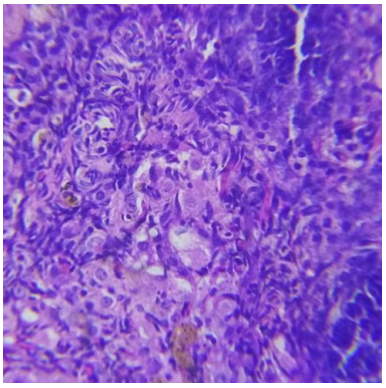
CONTROL OVARY



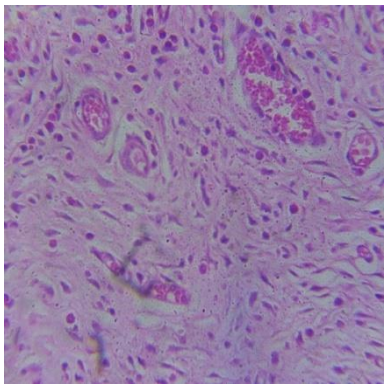
HIGH DOSE UTERUS



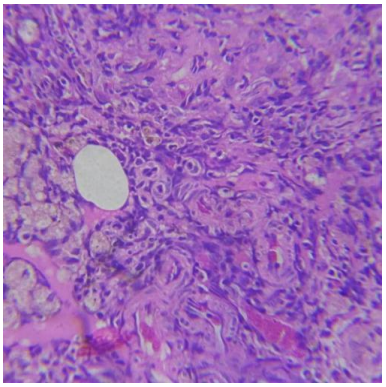
HIGH DOSE OVARY



SATELLITE UTERUS



SATELLITE OVARY



HISTOPATHOLOGICAL FINDINGS OF CONTROL GROUP

TISSUES	FINDINGS		RESULTS
	MALE	FEMALE	
BRAIN	Shows normal histology of striatum	Marginal alignment on the neurons with promising histology were observed	**Normal**
HEART	Showing normal histological structure of myocardium	Showed normal appearance of heart fibres	**Normal**
LUNGS	Normal alveoli with quidistant arrangement and prominent histology	Lung parenchyma appears normal	**Normal**
STOMACH	Normal gastric glands and gastric pits	Gastric epithelium and mucosa appears normal	**Normal**
LIVER	Normal hepatocytes with no signs of necrosis	Showing normal hexagonal hepatic lobules	**Normal**
KIDNEY	Section showed Glomerular Basement Membrane	Appearance of glomerular basement membrane was normal	**Normal**
SPLEEN	Morphology of capsule, nodes, red and white pulp appears normal	Three dimensional meshwork of splenic cords and venous sinuses were observed	**Normal**
TESTIS	Section of testis of showing normal interstitial connective tissue	--	**Normal**
UTERUS	--	Endometrial gland appears normal	**Normal**
OVARY	--	Section of ovary showing well follicular development	**Normal**

HISTOPATHOLOGICAL FINDINGS OF HIGH DOSE GROUP

TISSUES	FINDINGS		RESULTS
	MALE	FEMALE	
BRAIN	Appearance of Hippocampal neurons was normal with dense network	Dentate gyrus and CA3 pyramidal cells of the hippocampus appears normal	**Normal**
HEART	Normal appearance of myocytes	Normal histology of myocardial tissue with prominent inter fiber distance	**Normal**
LUNGS	Normal alveoli with equidistant arrangement and prominent histology	Regular arrangement of alveoli and alveolar sac	**Normal**
STOMACH	Mucosal wall appears normal	Appearance of Sub-mucosa and gastric glands appear normal	**Normal**
LIVER	Normal central vein, sinusoids and hepatocytes	Regular radiated hepatic cords from the central vein to the peripheral of lobule , central vein .	**Normal**
KIDNEY	Appearance of Proximal Convoluted Tubule was normal	Appearance of glomerular basement membrane was normal	**Normal**
SPLEEN	Appearance of splenic red pulp was normal	Appearance of splenic sinuses was normal	**Normal**
TESTIS	Appearance of leydig cells, interstitial tissue , seminiferous tubule, Sertoli cells and spermatogonia were normal	--	**Normal**
UTERUS	--	Uterine epithelium and blood vessels appears normal	**Normal**
OVARY	--	Follicular cells, cytoplasm and nucleus appears normal	**Normal**

HISTOPATHOLOGICAL FINDINGS OF SATELLITE GROUP

TISSUES	FINDINGS		RESULTS
	MALE	FEMALE	
BRAIN	The cerebral sections showed normal architecture	Cerebral region shows the neuronal population s	**Normal**
HEART	Showed normal appearance of heart fibres	Normal histology of myocardial tissue	**Normal**
LUNGS	Normal parenchyma with no signs of inflammation and lesions	Inter alveoli septum and bronchioles appears normal	**Normal**
STOMACH	Normal gastric glands and gastric pits	Normal surface epithelium , mucosa and sub-mucosa	**Normal**
LIVER	Normal hepatocytes with no signs of necrosis	Normal central vein, sinusoids and hepatocytes	**Normal**
KIDNEY	Arrangement of glomerular loop was normal	Showing normal, intact renal tubules as well as renal glomeruli	**Normal**
SPLEEN	Appearance of Splenic cord and endothelial orientation was normal	Regular appearance of red pulp	**Normal**
TESTIS	Sperm oriented towards the center of sertoli cells	.--	**Normal**
UTERUS	--	Appearance of endometrium, myometrium and uterine glands was normal	**Normal**
OVARY	--	Follicular cells, cytoplasm and nucleus appears normal	**Normal**

RESULTS:

The microscopic findings observed in various organs such as Brain, Heart, Lungs, Stomach, Liver, Kidney, Spleen, Testis and Uterus, Ovary of Control group showed no pathological changes. High dose treated group and Satellite group showed no pathological changes in Brain, Heart, Lungs, Stomach, Liver, Kidney, Spleen, Testis and Uterus, Ovary.

These results suggest *Nilavembu Kudineer* did not induce any lesions of toxicological significance in the organs examined under the experimental conditions employed as per the study plan.

DISCUSSION

6. DISCUSSION

Nilavembu Kudineer is one of the famous Siddha herbal formulation which is used for Suram (Fever). First to evaluate the safety profile of the test drug - Nilavembu Kudineer. The present study with an objective of finding whether this drug has got any adverse effect in long administration or not. Nilavembu Kudineer consist of Nilavembu *(Andrographis paniculata),* Vettiver *(Vertiveria zieanioides),* Vilamichuver *(Plectranthus vettiveroides),* Santhanasirai *(Santalum album),* Peippudal *(Tricosanthes cucuerino),* Parppadagam *(Hedyotis corymbosa),* Koraikkizhangu *(Cyperus rotundus),* Sukku *(Zingiber officinale),* Milaku *(Piper nigrum)*

Literature review of all the ingredients of Nilavembu Kudineer was collected in Siddha aspects and evaluated pharmacological actions and toxicological aspects. It revealed the ingredients of Nilavembu Kudineer have properties to treat Suram as per Siddha literature. Then literature review revealed that there was no repeated oral toxicity study has been done on Nilavembu Kudineer.

Qualitative assessment and safety profiles has been confirmed through Organoleptic Characters, Physico-chemical analysis, Microbial contamination, Aflotoxin, Instrumental analysis (HPTLC analysis), Pesticide residue, Phyto-chemical analysis, Bio-chemical analysis, Acute and 28 days repeated oral toxicity studies.

Organoleptic characters of Nilavembu Kudineer showed it has decoction - water extraction state, dark brown in colour, mild characteristic odour and slightly viscose in nature **(Table 13)**

Physicochemical analysis of the Nilavembu Kudineer Chooranam was done as per standard procedure **(Table 14)**

The **loss on drying test** is used to determine the amount of volatile matter (i.e water drying off from the drug). Moisture is one of the major factors responsible for deterioration of the drugs.[179] The percentage of loss on drying of Nilavembu Kudineer Chooranam was **3.53 %** (Normal range 1 - 20 %). Low moisture content is always desirable for higher stability of drugs.[179] As per the results the loss on drying of Nilavembu Kudineer Chooranam is low, so the stability of the Nilavembu Kudineer Chooranam is higher.

The **total ash** value is useful in determining the purity of the sample. A high ash value is indicative of contamination, substitution, adulteration, in preparing the drug. And also this value indicates the amount of minerals and earthy materials present in the plant material.[179] The total inorganic contents (ammonium, potassium, calcium, chloride, iron, etc.) present in the drug are measured through this value. The total ash value of Nilavembu Kudineer Chooranam is **5.37 %** (normal range 1 -24 %). The total ash value of Nilavembu Kudineer Chooranam is low, it implies that the inorganic constituents are low and indicates the purity of the drug.

The **acid insoluble ash** limit test is designed to measure the amount of ash insoluble to dilute hydrochloric acid. The acid insoluble ash value of Nilavembu Kudineer Chooranam was **1.49 %** and it shows that a very small of inorganic constituents is insoluble in acid.

The percentage of soluble matters present in the drug is determined by the values of **water extractive and ethanol extractive**. Based on the extractive value suitable solvent can be selected. It also gives the percentage of drug which correlates with the metabolism reactions. Water-soluble extractive value plays an important role in evaluation of crude drugs. The alcohol-soluble extractive value serves the same purpose as the water-soluble extractive value. The extract value of alcohol in Nilavembu Kudineer is **20.15 %** (normal range 4 - 85 %) and water is **13.61 %** (Normal range 4 – 85 %).

P^H of the Nilavembu Kudineer is **4.05 %**. It denotes that it is acidic. Hence in the oral administration, the drug is expected to be absorbed quickly in the stomach. It reveals that Nilavembu Kudineer is expected to have better bio availability.

Nilavembu Kudineer Chooranam was quantitatively analysis for **Heavy metal** content by AAS method. This analysis showed the below detection limit and **lead (Pb)** shows **0.11** (10 ppm) but it is also within limit.

Microbial contamination analysis is determining the free from the presence of viable microorganism. **Microbial contamination analysis** of Nilavembu Kudineer Chooranam revealed the absence of total bacteria content, specific pathogens and presence of minimal amount of fungi **(Table 16)** It denotes quality and purity of Nilavembu Kudineer Chooranam.

Aflotoxin are poisons produced by certain fungi that are found on agricultural crops at harvest and during storage. It particularly causes liver carcinoma[182]. The presence of Aflotoxins assessed by TLC method. The results indicate **(Table 21)** that the drug is free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2.

Pesticide residue analysis of Nilavembu Kudineer Chooranam revealed Organophosphoras, Organochlorine and Phyrethroid contents are below detection limit **(Table 18)**. It indicates the purity of Nilavembu Kudineer Chooranam.

The **Phyto chemicals** are chemical compound produced by plant through primary and secondary metabolites. Phytochemical is required for normal physiological functions so must be obtained from the diet in humans. Phytochemical analysis to detect the presence of various phyto constituents in formulation.[183] Phytochemical analysis of Nilavembu Kudineer reveals the presence of Alkaloids, Carbohydrate, Glycoside, Saponin, Phytosterol, Tannins, Diterpenes, Gum and Mucilage.

Alkaloids are important secondary metabolite that is known to possess curative properties. These are important therapeutic molecules that inhibit the growth and development of microorganisms including bacteria, fungi and protozoan.[184]

Carbohydrates are ubiquitous and perform a wide array of biological roles. Carbohydrate-based or - modified therapeutics are used extensively in cardiovascular and hematological treatments ranging from inflammatory diseases and anti-thrombotic treatments to wound healing. Carbohydrate-based therapeutics include polysaccharide and oligosaccharide anti-inflammatory, anti-coagulant and anti-thrombotic agents from natural and synthetic sources, some as an alternative to heparin and others which were designed based on known structure-functional relationships. Some of these compounds have multiple biological effects, showing anti-adhesive, anti-HIV and anti-arthrithic activities.[185]

Glycosidic compounds currently used in medicine but also with biological activity of some glycosidic metabolites of the known drugs. It involves glycosides of vitamins, polyphenolic glycosides (flavonoids), alkaloid glycosides, glycosides in the group of antibiotics, glycopeptides, cardiac glycosides, steroid and terpenoid glycosides etc.[186]

Due to the great variability of their structures, **saponins** always display anti-tumorigenic effects through varieties of antitumor pathways. Some special saponins with strong antitumor effects have also been exhibited.[187]

Phytosterols are steroid compounds present in plants which are similar to cholesterol in structure and functions. Several animal and human studies show that phytosterols lower plasma total and LDL-cholesterol levels. It is generally accepted that cholesterol-lowering effect of phytosterols is due to direct inhibition of cholesterol absorption,

through displacement of cholesterol from mixed micelles. Saturated phytosterols (stanols) are found to be more efficient in lowering cholesterol levels than sterols (unsaturated). It may produce health benefits in animals/humans such as reduction of cholesterol levels with decreased risk of coronary heart diseases, anti-inflammatory activities, and induction of apoptosis in cancer cells, disease prevention and treatment.[188]

Tannins antimicrobial activities of tannins are well documented. The growth of many fungi, yeasts, bacteria, and viruses was inhibited by tannins. Tannins have also been reported to exert other physiological effects, such as to accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver necrosis, and modulate immunoresponses.[189]

Most natural foods contain Terpenoids, Diterpenes, and Tetraterpenes. **Diterpenes** have been used in traditional medicine for anti-cancer, anti-diabetic and various other ailments.[190]

The **Bio chemical analysis** of Nilavembu Kudineer indicates **(Table 20)** the presence of Alkaloids, Calcium, Carbonate, Ferrous Iron, Phosphate, Sulphate, and Tannic Acid.

Alkaloids are important secondary metabolite that is known to possess curative properties. These are important therapeutic molecules that inhibit the growth and development of microorganisms including bacteria, fungi and protozoan.[184]

Calcium is the major constituent of bones, teeth, muscle contraction and nerve transmission. Calcium also plays a role in platelet aggregation. So this calcium has been implicated in the immunopathogenesis of dengue. Derangements of calcium homeostasis are likely to be associated with myocardial dysfunction and cardiac arrhythmias observed in dengue as suggested by in vitro studies.[191]

The total content of iron in an adult body is 3-5 g. About 70 % of this occurs in the erythrocytes of the blood as the constituents of haemoglobin. Peroxidase is the iron contained enzymes which is present in lysosomes. These enzymes are requied for phagocytosis and killing of bacteria by neutrophil. Iron is associated with effective immune competence of the body so this iron is used to cure bacterial infection and boost the immunity.[184]

An adult body contains about 1 kg phosphate and it is found in every cell of the body. Phosphate is present in bones, teeth, muscles and blood. It is important for the maintenance of p^H in the blood as well as in the cell. It plays a central role for the

formation and utilization of high energy phosphate compounds. It is also an essential component of several nucleotide coenzymes. And several proteins and enzymes are activated by phosphorylation.[184]

Sulphate is used to detoxify the toxic substance of the body by the conjugation process. So it eliminates the bacterial toxins in the body.[184]

Many tannin molecules have also been shown to reduce the mutagenic activity of a number of mutagens. Many carcinogens and / or mutagens produce oxygen-free radicals for interaction with cellular macromolecules. The anticarcinogenic and antimutagenic potentials of tannins may be related to their antioxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation. The generation of superoxide radicals was reported to be inhibited by tannins and related compounds.[189]

Alkaloids, Calcium, Carbonate, Ferrous Iron, Phosphate, Sulphate, and Tannic Acid are present in Nilavembu Kudineer which suggests that this drug can be used to cure fever.

WHO has need for quality assurance of herbo mineral products, includes microbial contamination, pesticide residue, Heavy metal analysis and aflotoxin. These tests should be within the permitted and specified limits for micro-organism, pesticide and heavy metals. These analyzes are useful in determining the purity of the drug.

The **HPTLC analysis** of the Nilavembu Kudineer showed that presence of eight prominent peaks corresponds to presence of eight versatile phyto - components present within it. Rf value of the peak ranges from 0.06 to 0.88. Further the peak four and two occupies the major percentage of are of 35.95 and 15.71 % which denotes the abundant existence of phytochemical compounds.

Acute and 28 days repeated oral toxicity study of Nilavembu Kudineer was carried out as per OECD guideline 423 and 407.

In Acute Toxicity study there was no abnormal behavioural changes noted at the dose level of 5000 mg / Kg b.wt of Nilavembu Kudineer within 24 hours observation in female Wistar albino rats **(Table 22)** No mortality and morbidity was observed throughout the study period. And also there was no pathological changes seen in all the internal organs of sacrificed test animals at the end of the study period. These results shows LD_{50} of Nilavembu Kudineer was greater than 5000 mg / Kg b.wt. and Nilavembu Kudineer classified under category 5 as per Globally Harmonized Classification system.

In Repeated 28 days oral toxicity study was conducted as per OECD guideline 407. Totally 5 groups of Wistar albino rats in both sexes were used, each group contain 5 males and 5 females. Group I received RO water and Group II, III, IV and V received 6, 12, 48 and 48 ml / Kg b.wt of Nilavembu Kudineer respectively by oral administration. All the test animals were observed throughout the study period. Nilavembu Kudineer did not produce any behavioural changes in all groups of animals. The body weight of the all the male and female treatment animals were gradually increased during the study period and significantly increased when compared with control group **(Table 23 - 24).** But body weight within physiological limit. Feed intake of the treatment animals low dose and satellite group was significantly changes when compared with control group. **(Table 25 - 26)** water consumption of all the treatment animals not significantly changes when compared with control group. **(Table 27 - 28)**

Hematological parameters results suggest that there were no significant changes in complete blood count. **(Table 29)** when compared with control group. It results revealed that no alteration in their components and all parameters within physiological level. Bio - chemical parameters results also no significant changes when compared with control group. And it suggests there is no alteration in the serum components. **(Table 30 - 32)**

At the end of the study period all the animals sacrificed and all the vital organs and cavities were observed. There were no gross pathological changes noted. The histopathology of Brain, Heart, Lungs, Liver, Kidney, Stomach, Testis, Uterus and Ovary were done in control, High dose treatment group, and Satellite group. The histopathology results of revealed all the studied organs are normal. The post treatment observation group (satellite group) also showed no abnormalities. Finally the 28 days repeated oral toxicity study of Nilavembu Kudineer reveals No Observed Adverse Effect Level (NOAEL) of Nilavembu Kudineer is Greater than 48 ml / Kg b.wt.

SUMMARY

7. SUMMARY

The experimental formulation Nilavembu Kudineer has been choosen for my dissertation work based on Siddha Vaithiya Thirattu. Nilavembu Kudineer prepared with the ingredients of Purified Nilavembu *(Andrographis paniculata)*, Vettiver *(Vertiveria zieanioides)*, Vilamichuver *(Plectranthus vettiveroides)*, Santhanasiraai *(Santalum album)*, Peippudal *(Tricosanthes cucuerino)*, Parppadagam *(Hedyotis corymbosa)*, Koraikkizhangu *(Cyperus rotundus)*, Sukku *(Zingiber officinale)*, Milaku *(Piper nigrum)*, it has been mentioned for Suram.

The aim of the research work was to study the safety of the experimental formulation by acute and 28 days repeated oral toxicity study in the wister albino rat as per OECD Guidelines. The ingredients such as Nilavembu were collected from National Institute of Siddha, Herbal Garden, Chennai - 47 and other drugs were purchased from standard raw drug market. The drugs were identified and authenticated by Assistant Professor of Medicinal Botany, National Institute of Siddha, Chennai - 47. All the ingredients have been purified as per Siddha literature and formulation was prepared in Gunapadam Lab of National Institute of Siddha, Chennai - 47.

Nilavembu Kudineer Choornam was analyzed quantitatively and qualitatively with Organoleptic Characters, Physico-chemical analysis, Aflotoxin, Pesticide residue, Microbial contamination, Nilavembu Kudineer was analyzed quantitatively and qualitatively with Phyto-chemical analysis, Bio-chemical analysis, Instrumental analysis (HPTLC analysis), Acute and 28 days repeated oral toxicity studies.

Initially the trial drug - Nilavembu Kudineer Chooranam was subjected to physico - chemical analysis. It reveals the increase in bio availability and purity of the drug. And Nilavembu Kudineer was subjected to Phyto-chemical investigation results showed the presence of **Alkaloids, Carbohydrates, Diterpenes, Glycoside, Phytosetrol, Saponins, Tannins** and **Mucilage.**

Then the Nilavembu Kudineer was analyzed for chemical constituents. It showed the presence of **Alkaloids, Calcium, Carbonate, Ferrous Iron, Phosphate, Sulphate, and Tannic Acid.** Presence of the above components in phyto – chemical and bio-chemical analysis increase the therapeutic value of Nilavembu Kudineer.

Microbial load, pesticide residue, aflotoxin level was quantitatively measured in Nilavembu Kudineer Chooranam the results indicates the below detectable limit of them. Nilavembu Kudineer Chooranum. From the results we concluded that the below detection limit of microbial contamination, aflotoxin, pesticide residues indicated the quality of Nilavembu Kudineer Chooranam.

The HPTLC analysis of the sample reveals that presence of eight prominent peaks corresponds to presence of eight versatile phytocomponents in which peak 4 and 2 occupies the major percentage of are of 35.95 and 15.71 % which denotes the abundant existence of phytochemical compounds.

Acute toxicity study there was no abnormal signs reported at the dose level of (5000 mg / Kg b.wt.) within 24 hours in wistar albino rats. No mortality, morbidity and no pathological changes have been seen in internal organs of treated groups in the 14 days study period.in acute toxicity study revealed LD_{50} of NilavembuKudineer is greater than 5000 mg/kg.b.wt.

28 days Repeated oral toxicity study of Nilavembu Kudineer showed did not produced any behavioral changes, mortality and morbidity with in the study period. The body weight of the all the animals were gradually increased during the study period and no significant to compare with control group. The water consumption of all treated animals was gradually increased during the study period and no significant to compare with control groups. The feed intake of female rats of low dose treated group, and satellite group was significant to compare with control group and male rats of satellite group significant to compared with control group.

Hematological parameters of all the test animals were normal when compared with control group. Bio-chemical analysis also within normal level when compare with control group. Histopathological examination of Nilavembu Kudineer treated animals, and post toxicity test group (satellite group) results revealed, Nilavembu Kudineer did not induce any lesions of toxicological significance in all the vital organs examined under the experimental conditions.

This 28 days repeated oral toxicity study results suggest the No Observed Adverse Effect Level (NOAEL) of Nilavembu Kudineer is greater than 48 ml / Kg b.wt.

CONCLUSION

8. CONCLUSION

Nilavembu Kudineer was taken as test drug and analyzed its quality parameters and safety profile as per standard procedure.

Quality parameters assessment of Nilavembu Kudineer results showed purity and bio availability of the drug. The results obtained by quality assessment of Nilavembu Kudineer will be used as standard for future research.

Safety profile of Nilavembu Kudineer was analyzed by acute and 28 days repeated oral toxicity study as per OECD guidelines 423 and 407.

In acute toxicity study results suggested the LD_{50} of Nilavembu Kudineer is greater than 5000 mg / Kg b.wt. So Nilavembu Kudineer falls under Category - 5 in Globally Harmonised System (GHS) Classification.

28 days repeated oral toxicity study of Nilavembu Kudineer was studied in different dose levels such as 6 ml/kg.b.wt, 12 ml/kg.b.wt, 48 ml/kg.b.wt and satellite group – 48 ml/kg.b.wt. 28 days repeated oral toxicity study result showed no significant changes in haematological, biochemical and histopathology of vital organs in all treated groups compared with control group. These result suggests NOAEL of Nilavembu Kudineer is great than 48ml/kg.b.wt. This dosage is fourfold of human human effective dosage of Nilavembu kudineer.

In conclusion the above toxicity study results suggest the human effective dosage of Nilavembu Kudineer 60 ml/ twice a day is safe for consumption.

Efficacy of Nilavembu kudineer will be analyzed by invivo methods in future is necessary to strengthen the therapeutic usage of Nilavembu Kudineer.

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9. BIBLIOGRAPHY

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ANNEXURE

10. ANNEXURE

The Scanned copy of following certificates to be enclosed in annexure

1. Research Methodology and Bio - Statistics Workshop participant certificate.

2. Laboratory Animal Care and Basic Research Techniques workshop participant certificate.

3. IAEC Approval Certificate.

4. Raw drugs Authentication Certificate.

1. Research Methodology and Bio - Statistics Workshop participant certificate.

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. M.R. SRINIVASAN

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

2. Laboratory Animal Care and Basic Research Techniques workshop participant certificate.

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.M.R.Srinivasan

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

3. IAEC Approval Certificate.

CERTIFICATE

This is certify that the project title: "Preclinical Safety Evaluation of **Nilavembu Kudineer** – A Siddha Polyherbal Formulation" **has been approved by the IAEC.** ~~Total~~ No of animal approved: 56 Rats (20M + 36F)
Approval No: NIS/IAEC-VII/28082018/18

V. Banumathi

Prof. Dr. V. Banumathi,
Chairman IAEC

Prof .Dr. K. Nachimuthu,
CPCSEA Nominee

28/8/2018

Signature with Date

Chairman IAEC **CPCSEA Nominee**

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

Name of the Research Scholar	:	Dr. M. R. Srinivasan,
Name of the Guide	:	Dr. V. Manjari MD(S)
Name of the Department	:	Nanju Maruthuvam

Received the original certificate

M.R. Srinivasan
20.11.18.
(Dr. M. R. SRINIVASAN)

4. Raw drugs Authentication Certificate.

NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

BOTANICAL CERTIFICATE

Certified that the following plant drugs used in the Siddha formulation **"Nilavembu kudineer"** taken up for Dissertation studies by **Dr.M.R.Srinivasan** 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

Andrographis paniculata (Burm.f.) Wall.ex Nees (Acanthaceae), Whole plant
Vettiveria zizanoides (Linn.) Nash (Poaceae), Root
Plectranthus vettiveroides (Jacob) Singh & Sharma (Poaceae), Root
Santalum album Linn. (Santalaceae), Heart wood
Trichosanthes cucumerina Linn. (Cucurbitaceae), Whole plant
Hedyotis corymbosa (L.) Lam. (Rubiaceae), Whole plant
Cyperus rotundus Linn. (Cyperaceae), Tuber
Zingiber officinale Rosc. (Zingiberaceae), Dried Rhizome
Piper nigrum Linn. (Piperaceae), Fruit

MEDICINAL BOTANY CHENNAI 600 047 National Institute of Siddha

Certificate No: NISMB3892019

Date: 15-05-2019

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