A scientific approach on the validation of *Naaval Kottai  Mathirai* for its
Safety and Efficacy in the management of Non Insulin Dependent
Diabetes Mellitus (*Madhumegam*) - a Preclinical approach

**THESIS**

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<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>AYUSH</td>
<td>Ayurvedha, Yoga, Unani, Siddha, Hemopathy</td>
</tr>
<tr>
<td>OECD</td>
<td>Organization for Economic Co-operation and Development</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<td>NKM</td>
<td>Naaval Kottai Mathirai</td>
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<tr>
<td>AE</td>
<td>Adverse Event</td>
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<tr>
<td>ALP</td>
<td>Alkaline Phosphatise</td>
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<tr>
<td>CPSCEA</td>
<td>Committee for the purpose of control and supervision of Experimental on animal</td>
</tr>
<tr>
<td>ESR</td>
<td>Erythrocyte sedimentation rate</td>
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<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>HDL</td>
<td>High Density Lipoprotein</td>
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<tr>
<td>ICMR</td>
<td>Indian Council of Medical Research</td>
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<tr>
<td>ID</td>
<td>Identity Card</td>
</tr>
<tr>
<td>LDL</td>
<td>Low Density Lipoprotein</td>
</tr>
<tr>
<td>NIS</td>
<td>National Institute of Siddha</td>
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<td>LFT</td>
<td>Liver function test</td>
</tr>
<tr>
<td>NOEL</td>
<td>No observed effect level</td>
</tr>
<tr>
<td>NOAEL</td>
<td>No observed adverse effect level</td>
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<tr>
<td>PCV</td>
<td>Packed Cell Volume</td>
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RFT - Renal function test
RBC - Red Blood Cells
SGOT - Serum Glutamate Pyruvate Transaminase
SGPT - Serum glutamate Oxalo Acetic Transaminase
SD - Standard Deviation
SEM - Standard Error Mean
SAE - Serious Adverse Event
VLDL - Very Low Density Lipoprotein
WBC - White Blood Cells

g - Gram
L - Litre
mL - Milliliter
µl - Microlitre
dl - Deciliter
ng - Nanogram
mg - Milligram
µg - Microgram
kg - Kilogram
Cumm - Cubic milliliter
Cm - Centimeter
µm - micrometer
nm - Nanometer
hr - hour
ppm - parts per million

% - Percentage

Rf - Retardation factor / Retention factor

v/v - volume per volume

w/w - weight per weight

NIDDM - Non Insulin Dependent Diabetes Mellitus

STZ - Streptozotocin

DM - Diabetes Mellitus

mmol/L - millimoles per liter

FPG - Fasting Plasma Glucose

MD - Mid Diabetic

SD - Severe Diabetic

SEEJ - Seed Extract of Eugenia Jambolana

DPPH - Di-Phenyl 2 Picrylhydrazyl

TBARS - Thio Barbiteric Acid Reactive Substances

GTT - Glucose Tolerance Test

TC - Serum Total Cholesterol

NDF - Neutral Detergents Fibre

HESC - Hydroalcoholic Extract of Syzygium Cumini

IAEC - Institutional Animal Ethics Committee

MHB - Muller-Hinton Broth

MHA - Muller-Hinton Agar
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According to Thirumoolar,

- Medicine is the one which heals disease
- Medicine is the one which heals the mental disorders
- Medicine is the one by which we can prevent disease
- Medicine is the one which prevents death.

Siddha system of medicine is a holistic approach to human being. Since it deals with body, mind and soul, giving a fullness in life.

An absolute state of physical, social, mental and spiritual well being but not merely the absence of disease and infirmity is defined as health. It is the desire of every human being, to have good health and long life. In order to maintain the good health and to recover from the diseases, the advancement of science and technology has provided various types of treatments which include Traditional and Modern systems of medicine. Every human race has its own traditional system of medicines. Our country has the oldest, richest and most diverse traditional medicine cultures in the world. In India several thousand types of plant species are being used by thousands of ethnic communities.

The traditional medical systems of India are Siddha, Ayurveda and Unani. The Tamil (Siddha) system of medicine is the oldest of the three Indian medical systems. Siddha system of medicine is unique and ancient of its kind originating from Tamilnadu in India. Siddha system could be considered as the crown of all the traditional arts of the ancient Tamil. The medical works were bestowed by the great Siddhars, after attaining spiritual knowledge through perfection and spiritual salvation. Typically, Siddhars were saints, doctors, alchemists and mysticists all at once. They laid the foundation for the system of medicine called “SIDDHA”.
The Siddha system of medicine is a traditional Indian system which had been evolved with the development of mankind and is more of an evolution rather than invention. Siddha system describes the health of an individual as an ideal perfect state of the physical, social, psychological, and spiritual components of a human being. According to this system of medicine the three bio regulating principles such as Vatham, Pitham and Kapham are the governing constitution responsible for physical, emotional and mental well being of an individual. The five elemental (earth, water, fire, air and space) and three humoral theory of Siddha system of medicine forms the basis for human physiology, disease classification, drug formulation, drug selection and treatment. Any imbalance in these vital humors results in ill health.

As per the above quote, the equilibrium of three humors is considered as health and its disturbance and imbalance lead to the morbid state. The factors which affect the equilibrium are diet, stress, environment, physical activities and climatic condition. The ratio between Vatham, Pitham and Kapham are in the ratio of 4:2:1 respectively under normal body condition.\(^{(1)}\)

The below verses states clearly about the composition of our body and the derangement of 10 Naadis, 10 Vayus and 3 Uyirthathu leads to disease which are classified as 4448.

In Siddha system of medicines the drugs were broadly classified into 3 different groups i.e., Thathu (metals and mineral substances) Thavaram (Herbal product), and Sangamam (Animal
products). Siddha medicine could be categorized into 2 classes based on the route of administration and the pharmaceutical dosage forms.

**I.** Internal medicines administered through oral route are classified into 32 categories owing to their method of preparation, shelf life, etc.

**II.** There are various external therapies in Siddha system of medicines which include medicines used as nasal, ear and eye drops etc. Among these Leech therapy and Karanool therapy are the major therapies. These external therapies are then classified into 32 types (2).

The main aim of Siddha medicine is to balance the three humors of human body. So in disease conditions patients are advised to follow proper diet, medicine and disciplined regimen of life are advised for healthy living and to restore equilibrium of the three humors.

In Siddha system of medicine, *Madhumegam* is one of the disease mentioned by siddhars and it is classified under *Neerinai Perukkal Noi*; which is correlated with the symptoms of Diabetes Mellitus. The condition of *madhumegam* is characterized by increased frequency and output of urine (poly urea), presence of insects and ants which is attracted to the place of urination, while boiling the urine the urine imparts a sweaty odour and the body goes on emaciating day by day (3).

As per the above quote, Siddhars followed a treatment regimen. According to that the first line of treatment is to cure the disease by using the herbs (roots and leaves) and then if it is not cured by the plant kingdom, they used higher order medicines (*Parpam* and *Chenduram*). As per the siddhars first line of treatment, we use *Naaval Kottai* and *Aadutheendapalai* (both are plants) in the formulation of study drug *Naaval Kottai Mathirai* (4,5).

In our siddha system many more traditional formulations are available. Most of them are clinically used by Siddha physicians but have not been evaluated scientifically. So, researcher would like to evaluate the siddha formulation *Naaval kotai Mathirai*, which is indicated for diabetes mellitus in many siddha literature, in which the ingredients are easily
available, can be prepared easily and the cost of the medicine is very cheap. *Naaval kottai Mathirai* is a classical siddha herbal formulation. *Naaval kottai* (seed of *Syzygium cumini*) is processed (rubbed in the *kalvam*-stone mortar) with leaf juice of *Aadutheenda paalai* (*Aristolochia bracteolata*) and it is made into small pills of 500 mg (*Pattany size*), and preserved in an air tight container.

In view of the phytomedicine research, many compounds are identified and these are still under evaluation against diabetes mellitus. But most of the compounds will cause adverse reaction while consuming for long period. So we want to establish a new test drug which is under practice in the management of *madhumegam* as a safe and potential anti-diabetic agent which is widely used in our clinical practice, and we observe that this drug can control the accelerated glycemic level (upto300 mgm/dl) and minimize the complication of *madhumegam*. For universal acceptance of this siddha formulation, we are in need to explore the safety and efficacy of this medicine by conducting experimental study in animal model.
AIM AND OBJECTIVES

AIM

To validate the safety and efficacy of Siddha herbal formulation Naaval Kottai Mathirai in the management of NIDDM (Madhumegam).

OBJECTIVES:

- To perform the Botanical and Physico-chemical analysis of the Naaval Kottai Mathirai
- To standardize the Naaval kottai mathirai including heavy metal analysis as per AYUSH Guidelines.
- To evaluate the active compounds of the Naaval kottai mathirai using HPTLC/HPLC fingerprinting
- To evaluate the Acute oral toxicity, Repeated Dose 28-day Oral Toxicity and Repeated Dose 90-day Oral Toxicity study of Naaval kottai mathirai as per OECD guidelines.
- To Study the anti-diabetic activity of Naaval kottai mathirai by in-vitro glucose uptake in L6 myotubes.
- To Study the anti-diabetic potential of Naaval kottai mathirai in high fat diet and low dose Streptozotocin (STZ) induced diabetic model in rats.
3.1. Siddha aspect- Madhumegam

In Siddha system of medicine the disease Madhumegam \(^{(3)}\) is correlated with the symptoms of Diabetes Mellitus. The condition of madhumegam is characterized by increased frequency of micturition (poly urea), presence of insects and ants which is attracted to the place of urination, while boiling the urine the urine imparts a sweaty odour and the body goes on emaciating day by day.

In Siddha literature, the causative factors for diabetes is described as

- Increased sexual activities
- Increased consumption of fatty meat products
- Increased consumption of milk, ghee, sweetened and oily food products
- Changes in blood and seven physical constituents (udal thathukkal)

It may cause for development of Diabetes mellitus.
The pre-monitory symptoms of *Madhumegam* are increased frequency and volume of urine output, the urine is clear in appearance and on drying has a sticky nature. There will be a diminished strength which progress day by day, dryness of the tongue etc.

The common clinical features are,

- Urinary output and frequency are increased.
- The basic character, colour, odour, density, deposit also altered.
- The urine becomes watery (clear) in appearance, increased density with the presence of deposit and sweet odour resembling honey. On heating the urine will impart a honey odour.
- The body will be emaciate day by day, the skin will loosen its oily nature and becomes over dried, wrinkled and whitish yellow in colour
- Dryness of tongue, over thirst (Polydypsia)
- Increased appetite (Polyphagia) or hunger intolerance. Even if they consume energizing food or food having tonic properties like milk products, the body strength is not improved.
- The breath, sweat of the person will impart a honey odour.
- Diminished vision and occurrence of cataract.

The disease primarily occur during *Kapha* period (3rd stage of life span) as if it occurs in young people and middle age persons it can be completely cured or controlled with medications. If the disease is uncontrolled with medication on long standing it may complicate to other medical conditions. Due to persistent polyuria the body will loosen its valuable salts (electrolytes) or nutrients which are eliminated through urine. Feeling of dizziness, giddiness may dominate the individual and the person will be over exhausted and lose his self consciousness.

On advanced stages, the urination which was on higher output and frequency is slowly decreased day by day resulting in *Neerkattu* (Anuria) accompanied with nausea, vomiting, indigestion, loss of appetite, abdominal pain, depression and giddiness, with this condition the person become bed ridden shows respiratory distress, wheezing and finally dies or it may complicate to either longstanding cough, wheezing, *Kaphakshayam* (Tuberculosis), cardiac diseases, nerve weakness or secondary skin disease (*Sori, Sirangu, Madhumega katti* - Diabetic carbuncle).
In Siddha literature *Megha Vakada Thirattu*, the disease *madhumegam* (Diabetes) is described as follows.

- The affected victim of *Madhumegam* will have urine resembling tender coconut water or like very clear urine. Insects and flies will be attracted towards the urine, if the above features are shown; it is the Diabetes resulting due to vitiation of all the three *doshas*. So with proper judgement and prescription the condition should be controlled wisely.
- In the condition of *Madhumegam* there will be increased urinary output, and the body’s strength will be reduced progressing to further complications slowly.
- The urine will have fleshy odour which on boiling resembles honey like in consistency.
- The persons with *Madhumegam* will have voracious hunger, burning sensation of extremities, and urine odour as like unripped coconut. With the chronicity, vitiation of all the three humours may result in loss of strength.
- The feature of *Madhumegam* is as follows; on boiling the urine, it will impart a honey odour or ghee like odour.
- The common symptoms of *Madhumegam* patients will be altered sensorium like increased feeling of pain, and burning sensation of extremities.

**Aetiology - Madhumegam**

- Over consumption of food or excessive intake of carbohydrate diet.
- Taking uncooked meat.
- Due to drinking of contaminated water or salty water.
- Untimely food intake.
- Excessive Sexual indulgences.
- Increased body heat.
- Obesity
  - *Karma Vinai*: occurring as a result of previous ancestral deeds.

This may manifest as over dryness of the body and bodily fluids including blood and progress to *Madhumegam*.
Mukutra Verupadu (Three humoural pathology):

In the sequence of disease, due to dietary or habitual variations or other secondary causes, the first humour that is vitiated is phlegm (Kapha dosham), affecting abanan (vital air with downward force) and stepwise degeneration of seven physical constituents occur one by one. During the chronicity diminished digestive power and inability of the body to absorb or utilize nutrients from food even with normal dietary intake, the body won’t gain strength or vitality. The vitiated kapha humour affects the other two doshas (Vatham and Pitham) of its respective zones. So that so many secondary diseases will arrives along with the primary condition of diabetes. “It is the vital constituent (seven physical constituent) that is going to be degenerated at final stages” as per Pathinen Siddhar Naadi Nool.

Description of the above poem, the conjoined Vatha and Pitha Naadi if perceived, the person shows polyuria or increased urinary flow as like of heavy rains. The normal musculature of the body changes. The person feels voracious hunger even after heavy food intake. All the vital essences from the physical constituents will be eliminated through urine. Urine becomes sweet in taste.
Description of the above poem, if *Kapha* pulsation is perceived with in the left side of normal *Vatha Naadi*, polyuria is the feature and the urine may resemble toddy or fermented juices. The body become emaciated and pale. Secondary *Kapha* diseases will affect the victim

*Kapha* புளிக்கும் விலக்கம் பிள்ளைக் காணல்

மூட்டுப்பாதையின் வளர்ப்பபாதையான காரணத்தாக்காம்

When *Madhumegam* reaches uncontrolled or chronic stage all the three pulsations (*Naadi*) will appear extremely weak.

*Naadi* புளிக்கும் விலக்கம் பிள்ளை காணல்

மருத்துவ காரணத்தாக்கும் விலக்கம் பிள்ளை

*Madhumegam* may occur in persons with hyper dominant *Pitha Naadi*

*Naadi* புளிக்கும் விலக்கத்தாகும் பிள்ளை காணல்

மருத்துவ காரணத்தாக்கும் விலக்கம் பிள்ளை

அருகிலும் விலக்கும் பிள்ளை விலங்குவிடும் காணல்

In *Madhumegam* the body will lose its strength as like shade from dark clouds which blocks the sunlight, so many diseases will accompany and the condition won’t make the person to regain his strength. All the pulsation will be weak and is perceived like flowing of water or like crawling and struggling of a worm in fire.

*Naadi* புளிக்கும் விலக்க பிள்ளை விலங்குவிடும் காணல்

மருத்துவ காரணத்தாக்கும் விலங்குவிடும் பிள்ளை விளை

எடைகிய விலங்கு மட்டும் விலங்குவிடும் காணல்

*Naadi* காரணத்தாக்கும் விலங்குவிடும் பிள்ளை விளை

During the stage of extreme dryness of the body *Pitha Naadi* is perceived and the *Naadi* won’t alter its location to right or left and may resemble crawling of a worm (head to tail movement).
**Neerkuri and Neikuri: (Oil on urine Test)**

The urine variations of 4 types of *Vatha Mega Neer* is as follows

- colour, consistency, odour and taste like ghee
- colour, consistency and odour like cow’s urine
- colour, consistency and odour like goat’s meat washed water
- colour, consistency and odour like toddy

Such type of urine is collected in a round porcelain vessel and monitored for *Neikuri* changes through oil drop method. If the oil drop is scattered the disease is non-cureable according to one concept or can be cured with proper medications.

*Pitha* natured urine will be yellowish in colour and its presence may attract insects or flies within.

*Kapha* natured urine will be viscous, white frothy with sedimentation. The oil drop sinks down without floating.

**Stools**

For *Vatha* disease the stool appears hard, blackish or may resemble like goat’s excreta. The stools will be yellowish and loose in consistency with burning sensation at anal region after evacuation of stools in *pitha* diseases. For *Kapha* diseases the stools will be pale and starchy in nature with increased frequency.

**Management**

As in the disease which shows the vitiation of all the three *Doshas and Dasa vayus* (Ten Vital Airs) with simultaneous degeneration of *Saptha Thathus* (7 Physical Constituents). It is very optive as the first step to normalise the derangement and to strengthen each constituents one by one. Curative approach includes treatment for *Madhumegam* caused due to *Megha* diseases.
3.1.1. Sequential Stepwise Progression of *Madhumegha Noi*

“..."Madhumegha Noi" is a term used in ayurvedic literature to describe the progression of diabetes mellitus. The symptoms and complications of diabetes mellitus will occur in a sequential manner from initial to the final stage."

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In *Madhumegam* (Diabetes mellitus), the following symptoms, conditions or complications will occur in a sequential manner from first stage to the tenth stage (*Meghathin dasa avathaigal*) indicating the progression and severity of the disease.
Megathin dasa avathaigal

1. Body becomes obese and heavy with the dilatation of urinary passage

2. Increased urinary output (polyuria) associated with seminal discharge (spermatorrhoea) skin complexion is reduced

3. Dryness of the tongue, abdominal distension due to flatus

4. Excessive thirst (polydypsia) with delirial features (sannipatham)

5. Continuation of Polyuria associated with seminal loss (Thathu nattam)

6. Patient unable to lie in bed becomes dyspnoeic or may lead to syncope (Moorchai)

7. Extreme tastelessness (Ageusia), dyspnoea and emaciation

8. Occurrence of secondary skin diseases or infections like abscesses or carbuncles (Madhumegha pilavai, kiranthi)

9. Infections and infestations with features of malnourishment and emaciation

10. Occurrence of pulmonary tuberculosis leads to death.

3.1.2. Madhumegam in Theran Maruthuva Bharatham

“புரத்தேவநிதா புராணமால் பிர்காரி வயிற்றிய

பல்லனை ஒலிக்கு பல்லுறுக்கா பிற்கன் விழுந்த”
The description about Diabetic conditions (Neerilivu, Madhumegam) and its curative medicines has been beautifully narrated in the classical text called “Theran Maruthuva Bharatham”. 

The verses with excellent literary work and grammatical implications (Tamil Ilakana Nayam) are uniform with comparative meanings specifies the story line of epic Mahabharatham. The symptoms or diabetic features is compared with that of kauravas (Disease or enemy part) and its curative approach with that of Pandavas (Medicine part).

As per the versatile meaning of this poetry story, Megha Noigal (20 types) is depicted as Keechagan and one among the Megha Noigal that is Madhumegham is compared as Sainthavan. A descriptive quote of Maruthuva Bharatham says “like the strong desire of Keechagan towards Panjali, the Megha disease primarily occur due to immoral and excessive indulgence of sexual practices.

It is like how Bheeman, killed his sole enemy Keechagan its wise and optive to use mercurial medications (as compared with Bheema) in Megha Noigal (as compared with Keechagan). Simultaneously the conditions of Neerilivu (as compared with Sainthava) will be cured with medicines prepared with Iron (Ayam), Steel (Urukku), Silver (Velli), Gold (Thangam), Lead (Karuvangam), Copper (Thamiram) as compared with Krishna and Pandavas.

3.1.3. Selection of medicines for curing Megha Noigal and to balance and nourish the deranged physical constituents: (7 Udal Thathukal)

With the quotes in Theran Maruthuva Bharatham which explains the chronological usage of medications for correcting each physical constituent which overall results in good prognosis for Neerilivu. As a first step Sulphur medications (Gandhaga Marundhugal) and
Mercurial preparations (*Rasa Marundhugal*) are insisted for correcting the primary *thathu* - *Saram* (Chyme). The following medicines are administered with each order corresponding to that of deranged physical constituents.

Table No: 3.1.1: Selection of medicines for curing *Megha Noigal*

<table>
<thead>
<tr>
<th>S. No</th>
<th>Physical constituent (Devitalised / Deranged)</th>
<th>Medications chosen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Saram</em> (Primary nourishing juice)</td>
<td>To give nourishment to the body fluids sulphur preparations are used.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eg: <em>Gandhaga Parpam, Gandhaga Chenduram</em> followed by Mercurial preparations with suitable adjuvants.</td>
</tr>
<tr>
<td>2</td>
<td><em>Senneer</em> (Blood)</td>
<td>To enrich the blood the Iron preparations are used.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eg: <em>Aya Chenduram, Aya parpam</em> Steel (Urukku) preparations Eg. <em>Urukku parpam</em> with adjuvants like <em>Naval pazha charu</em> (Fruit juice of Syzygium cumini) or <em>Avaaarai kudineer</em>.</td>
</tr>
<tr>
<td>3</td>
<td><em>Oon</em> (Muscle)</td>
<td>To develop the muscle constituent and to correct the excessive heat due to <em>Megham</em>, Silver (Velli) preparations eg. <em>Velli Chenduram, Velli Parpam</em> are given with coolant or diuretic herbal preparations made with <em>Neermulli</em> (Hygrophiла auriculata), <em>Manathakkali vithai</em> (Solanum nigrum).</td>
</tr>
<tr>
<td>4</td>
<td><em>Kozhuppu</em> (Fatty Tissue)</td>
<td>To strengthen the fatty tissues Gold preparations are advised with suitable adjuvants.</td>
</tr>
<tr>
<td>5</td>
<td><em>Enbu</em> (Bone)</td>
<td>Note: The medications should continue without stopping even with good prognosis like improved vitality, strength and complexion otherwise complete result won’t be obtained</td>
</tr>
</tbody>
</table>

15
Medications Prepared with Lead (Karuvangam) is advised twice a day with suitable adjuvant, after the course Copper (Chembu) preparations are started which cures the disease completely as per Theran.

3.2. Modern aspect- Diabetes mellitus

3.2.1. Definition

Diabetes mellitus comprises a group of common metabolic disorders that share the phenotype of hyperglycemia.

3.2.2. Pathogenesis

Type 1 DM

It develops as a result of the synergistic effects of environmental, genetic, and immunological factors that destroy the beta cells of the pancreas.

Genetically susceptible individual have normal beta cell mass at birth but it begins to lose beta cells secondary to autoimmune destruction that occurs months to years which is triggered by environmental stimulus or infections and is sustained by a beta cell specific molecule.

Beta cell mass begins to fall results in impaired secretion of insulin, although glucose tolerance is maintained in normal level. Features of diabetes do not exist until a vast number of beta cells are destroyed. At this time, residual functional beta cells still exist but it is not sufficient to maintain the glucose tolerance.

After the initial clinical phase, a honeymoon phase may develop in which glycemic control is achieved by insulin and in some cases insulin is not needed. As the auto immune process destroy the remaining beta cells this fleeting phase of insulin production from pancreatic beta cells disappears. Finally, the individual becomes completely insulin deficient.
Prevalence

The prevalence of diabetes in people has risen from 108 million in 1980 to 422 million in 2014. The global prevalence of people with diabetes above 18 years has risen from 4.7% in 1980 to 8.5% in 2014. (7) In 2012 approximately 1.5 million deaths were caused by diabetes and another 2.2 million deaths were attributable to high blood glucose. According to International Diabetes Federation (IDF) report, there are 100 million people with diabetes worldwide that is about 6% of all adults. (8) The prevalence of diabetes is high in Asia that is about 20% of the global diabetic population resides in South-East Asia. People with diabetes in India is likely to double in two decades that is from 39.9 million (in 2007) to 69.9 million by 2025. (9,10) In 1970, Indian Council of Medical Research (ICMR) reported a prevalence of 2.3% in urban area, which had increased to 12-19% in 2000. Simultaneously, in rural area, prevalence rate of 1% had increased to 4-10%, and it was reported to be 13.2% in other study. (11-13) From the above report it was clear that from the last 2-3 decades the prevalence of diabetes are increasing rapidly in both urban and rural population at a ratio of 2:1 to 3:1. (14-17) WHO reported that diabetes will be the 7th major cause of death in 2030. (18) Almost half of all deaths attributable to high blood glucose occur before the age of 70 years.

Autoimmune factors

After the destruction of beta cells, the immunological markers disappear and islets become atrophic.

The autoimmune process are studied in human and animal models of type 1A DM and the abnormalities in both cellular and humoral arms are given below

- Islet cell auto antibodies
- Activated lymphocytes in the peripancreatic lymphnodes and systemic circulation
- T lymphocytes proliferate when stimulated with islet proteins
- Release of cytokines within the insulitis

Beta cells are more susceptible to the toxic effect of some cytokines like interferon γ, α TNF-α and interleukin-1 (IL-1). Insulin, decarboxylase (GAD), ICA-512 / IA-2, glutamic acid and phogrin are the pancreatic islet molecules targeted by the auto immune processs.
Genetic considerations

The major susceptible gene is located in the HLA region of chromosome 6. Most of the individuals have HLA DR3 and/or DR3 haplotype in type 1 A DM. Polymorphism in HLA complex is the major genetic risk for developing type 1 A DM. The haplotypes DQB1*0302, DQA1*0301, DQA1*0501 and DQB1*0201 are strongly associated with type 1 A DM.

Environmental factors

Various environmental factors have been proposed to trigger the autoimmune process but none have been conclusively linked to diabetes. Putative environmental trigger include viruses (rubella, and coxsackie most prominently), bovine milk proteins and niosourea compounds.

Type 2- DM

It is characterized by 3 patho-physiological abnormalities

- Insulin resistance
- Impaired insulin secretion
- Excessive hepatic glucose production

Obesity particularly central or visceral is very common in type 2 DM. Leptin, free fatty acids, TNF-α, adiponectin, resistin are the biological products secreted by adipocytes that modulate insulin secretion, insulin action and body weight and may contribute to insulin resistance.

As hyperinsulinemia and insulin resistance progress, the pancreatic islets are unable to sustain the hyperinsulinemic state. Elevation in postprandial glucose then develops. Further fall in the insulin secretion and increase in hepatic production leads to diabetes with fasting hyperglycemia. This ultimately results in beta cell failure.

Insulin resistance

The decreased ability of insulin to act effectively on peripheral target tissues is a prominent feature of type 2 DM resulting from a combination of obesity and genetic susceptibility. Both impaired glucose utilization by insulin sensitive tissues and increased hepatic glucose output contributes to hyperglycemia. Increased hepatic glucose output results
in increased fasting blood glucose level while decrease in peripheral glucose usage results in postprandial hyperglycemia. Elevated Free fatty acid is a common feature of obesity which decreases the glucose utilization in the skeletal muscle and promotes the production of glucose in the liver resulting in impaired beta cell function.

**Impaired insulin secretion**

Insulin secretion and insulin resistance are interrelated. The reason for impaired insulin secretion in type2 DM is unclear. But it is assumed that a second genetic defect superimposed upon insulin resistance leads to beta cell failure.

**Increased Hepatic Glucose Production**

In type 2 DM, insulin resistance in liver results in fasting hyperglycemia and decreased glycogen storage by the liver in postprandial state, which reflects the failure of hyperinsulinemia to suppress gluconeogenesis.

**Genetic considerations**

- Type 2 DM has a strong genetic component
- In identical twins the concordance of type 2 DM is between 70 and 90%
- One parent with type 2 DM have an increased risk of diabetes
- If both parents have type 2 DM , the risk approaches 40%
- Mutations in insulin receptors and enzymes involved in glucose homeostasis
- Genome wide scanning for polymorphisms or mutations associated with type 2 DM is used to identify the genes associated with type 2 DM
- The gene for calpain 10, protease are associated with type 2 DM

**3.2.3. Diagnosis**

Criteria for the diagnosis of DM issued by The National Diabetes Data group & World Health Organisation is given below
- Symptoms of diabetes plus random blood glucose concentration ≥11.1 mmol/dL (200mg/dL) or

- Fasting plasma glucose ≥7.0 mmol/L (126 mg/dL) or

- Two hour plasma glucose ≥ 11 mmol/L (200 mg/dL) during an oral glucose tolerance test.

Based on Fasting Plasma Glucose level (FPG), Glucose tolerance is classified into 3 categories

- FPG< 5.6 mmol/L (100 mg/dL)
- FPG≥5.6 mmol/L (100 mg/dL) but <7.0 mmol/L (126 mg/dL) - IFG
- FPG≥7.0 mmol/L (126 mg/dL) - warrants the diagnosis of DM

IGT is defined as plasma glucose levels between 7.8 and 11.1 mmol/L (140 and 200 mg/dL) 2 hour after 75 g oral glucose test.

A random plasma glucose concentration ≥ 11.1 mmol/L (200 mg/dL) with classical symptoms of DM (polyuria, polydypsia, weight loss) is sufficient for the diagnosis of DM

The revised criteria for the diagnosis of DM assumes that FPG as a convenient and reliable test for diagnosing DM in asymptomatic individuals.
3.3. **Naaval Kottai Mathirai**\(^4, 5\)

**Mathirai (Urundai) - Definition**\(^19\)

The raw drugs are triturated with the juices of leaves or *kudineer* (decoction). They are rolled into different sizes of pills, dried and stored.

*Naaval Kottai Mathirai* is a herbal based Siddha formulation, prepared from *Naaval kottai* (*Syzygium cumini* seed) and *Aadutheenda paalai* leaf juice (*Aristolochia bracteata*) which has been used for DM by traditional healer and Siddha physicians. NKM is very effective for the management of DM and preventing the complication of DM.

### 3.3.1. **Syzygium cumini** (*Naaval*)

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subkingdom</td>
<td>Tracheobionta</td>
</tr>
<tr>
<td>Subdivision</td>
<td>Spermatophyta</td>
</tr>
<tr>
<td>Division</td>
<td>Magnoliphyta</td>
</tr>
<tr>
<td>Class</td>
<td>Dicotyledons</td>
</tr>
<tr>
<td>Subclass</td>
<td>Rosidae</td>
</tr>
<tr>
<td>Order</td>
<td>Myrtales</td>
</tr>
<tr>
<td>Family</td>
<td>Myrtaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Syzygium</td>
</tr>
<tr>
<td>Species</td>
<td><em>Syzygium cumini</em> (L.) Skeels</td>
</tr>
</tbody>
</table>

**Other names**

**Tamil** : *Naval, Navval, Nambu, Sambu, Saathavam, Aarukatham, Neredu, Neredam, Sattuvalam, Saambal, Surabipaththirai.*\(^20\)

**Telugu** : Neradu

**Hindi** : Jamuna
Malayalam : Gnaval
Sanskrit : Jambu
Kannada : Neralu
English : Eugenia jambul
Part used : All parts
Taste : Astringent
Nature : Neutral
Division : Hot

**Action:**

- Astringent
- Stomachic
- Diuretic
- Tonic

Plate 3.3.1: Syzygium cumini
Occurrence and Distribution

Found almost throughout India. This plant ascending up to an altitude of 1800 m.

Description

A moderate sized, glabrous tree.

Leaves coriaceous, shining, entire, oval, lanceolate- oblong or oval-oblong, long acuminate, numerous lateral nerves, parallel, confluent near the margin.

Flowers greenish, tetramerous, in 3 flowered cyme, arranged in broad trichotomous panicles.

Calyx- tube funnel shaped. Berry oblong or subglobose purple or black succulent, smooth when ripe. (21)

The Syzygium cumini tree is found almost in many places in Tamilnadu and is quite popular among Tamil people. It finds a place even Tamil literature from literary and medical implications thereof- point of view. The tree grows up to 10 to 15 meter in height. The leaves may resemble that of mango tree with 10 to 15 cm long and oval in shape. It has small white flowers that blossom normally in March in Tamilnadu. The fruits are small and round and purple in colour. The fruit, kernel and bark are all astringent in taste. The fruits are smooth and shining and when crushed have a camphor odour and on distillation yield a bright green oil.

Normally, the fruits will be mixed with some common salt and kept for an hour. By doing this not only the taste of the fruit is improved but will also prevent the increase of phlegm humour and cold which the fruit is liable to cause. It should be cautioned that the fruits should not be given to pregnant women. The whole tree has astringent property. The fruit with the kernel used to be eaten during famine. The parasite of the tree is good in arresting premature ejaculation. Though there are more than twenty varieties of jambul, only two varieties are quite common namely the white one (where the fruit is white in colour) and the purple one. However the purple one is commonly available and used as such. (22)

A large evergreen tree with white flowers and purple fruit, the latter in cultivation oblong and edible. Bark smooth, light grey; wood hard, reddish- grey; Leaves thin, obovate; Flowers small; in axillary or lateral cymes. Fruit globose 2 inch in diameter. (23)
Chemical constituents

Fruits

Delphinidin -3- gentiobioside, malvidin-3- laminaribioside, petunidin-3- gentiobioside; glucose , fructose; gallic acid, malic acid; Eugenia triterpenoids A & B; oleanolic acid.

Seeds

Caffeic and ferulic acids, corilagin, guaiacol, resorcinol dimethyl ether, veratrole; jamboline (a glycoside), myricyl alcohol, quercetin; 1 & 3 - galloyl glucose, 3,6-hexahydroxy diphenoyl, glucose and its isomers, 4,6-hexahydroxy diphenoyl glucose.\textsuperscript{(21)}

Phytochemicals

Fruit

- Malvidine-3 - laminaribioside
- Anthocyanins
- Delphinidin -3- gentiobioside

Leaves

- Oil containing $\infty$ - pinene
- Cis- ocimene
- Trans - ocimene

Flowers

- Acetyl oleanolic acid
- Ellagic acid
- Myricetin

Root

- Myrecetin -3- O- glucoside
- Myrecetin -3- robinoside

Stem bark

- Betulinic acid \textsuperscript{(22)}
General Characters

“In excess dyspepsia (maantham) and painful eczema (karappan) will adhere
But diabetes mellitus will never come near
Wind diseases, hot flush and polydipsia will be cured
By the fresh and pure Eugenia jambolana fruit”

Method of Use

- After eating the flesh of the fruit of Syzygium cumini please do not throw away the hard nut inside. Collect as much nuts as possible. Dry and pulverize them. Taking 2 grams of the powder with warm water will reduce sugar in the blood and over urination especially in the night - a situation most annoying to people suffering from Diabetes mellitus.

- Taking 100 ml at a time and two times a day of the decoction of the bark of Syzygium cumini will reduce blood sugar.

- Grind equal amount of the tender leaves of Syzygium cumini and mango tree. Take 20 grams of this with butter milk. This will cure dysentery, loose motion attended with blood and irritation around anus.

- Extract the juice from the tender leaves of Syzygium cumini and take 1 tablespoon of this juice. To this add a pinch of cardamom and clove. Drinking this twice a day will cure irritable diarrhoea.

- Dry the bark of Syzygium cumini under shade and make powder of it. Mix sufficient quantity of the powder with “kaar arisi” in Tamil (red rice) and bake it. Eating this with brown sugar (palm sugar) will be advantageous in case of menorrhagia, haemorrhoids, cough and asthma.
- Collect sufficient amount of the seeds of Syzygium cumini and grind them with the juice of the leaves of Aristolochia bracteolate (“Aadutheendaa paalai” in Tamil) as nicely as possible and make tablets of 150 mgm each. Dry them under shade and then store in an air tight jar. Dose: 2 or 3 tablets at a time and twice a day. Within 15 days diabetes will come to normal and the sugar in the urine will be reduced. Over urination will be stopped. 

3.3.2. Aristolochia bracteolata - Aadutheenda paalai

Kingdom : Plantae
Subkingdom : Tracheophyta
Division : Magnoliopsida
Class : Dicotyledons
Order : Piperales
Family : Aristolochiaceae
Genus : Aristolochia
Species : Aristolochia bracteolata Lam.

Synonyms
- Worm killer
- Ulcer plant
- Black blood plant

Other Names

Tamil : Aadutheenda paalai
Telugu : Kadapara
Hindi : Gandan
Malayalam : Atuthendapala
Sanskrit : Ajasparsha
Kannada : Sanjali hallu
English : Birthwort
Latin : Aristolochia bracteolata
Part Used : Leaf, Root, Seed
Taste : Bitter
Nature : Hot
Division : Hot

**Action**

- Stimulant
- Tonic
- Purgative
- Emmenagogue
- Anthelmintic
- Antiperiodic
- Alterative

**Habit and Habitat**

A slender, decumbent, glabrous, perennial twiner. (26)

**Description**

A slender perennial stems 30 - 45 cm long, weak, prostrate, branched, striate, glabrous. Leaves 3.8 - 7.5 cm long and as broad as long, reniform or broadly ovate, usually obtuse, cordate at base with a wide shallow sinus, glaucous; petioles 1.3 - 3.2 cm long. Flowers solitary, pedicles with a large sessile orbiculate or subreniform bract at the base. Perianth 2.5 - 4.5 cm long, base subglobose, tube cylindrical with a trumpet shaped mouth, dark purple
lip- linear, having revolute margins, finely reticulately veined, as long as the tube. Capsules 1.3 - 2 cm long, oblong, ellipsoid 12 ribbed, glabrous. Seeds 6 mm long, deltoid with a slightly cordate base. \(^{(27)}\)

A perennial herb with greenish tube and dark purple lip to the perianth, which is linear with revolute margins, the capsule about 75 inch long, oblong- ellipsoid, 12 ribbed. A nauseously bitter plant, used as an anthelmentic.\(^{(28)}\)

A medicinal plant commonly grows in hedges, roots perennial, and leaves alternate and kidney shaped, curled at the margin, flowers solitary; dark and purple. This is also a bitter plant capable of curing poisons. Flowering nearly all the year. \(^{(25)}\)

**Phytochemicals**

**Plant**
- N- acetyl nor-nuciferine
- Aristololactum \(^{(29)}\)

**Root**
- Aristolochic acid

**Leaves & Fruits**
- β- sitosterol

**Seed**
- Alkaloids
- Non-dryingiol \(^{(22)}\)
Plate 3.3.2: Aristolochia bracteolata

Properties and Uses

- The plant is bitter, purgative, anthelmintic, and useful in Vatha; kapha fevers, painful joints, applied to soles to kill maggots.

- It is well-known by its Hindustani name Kira-mar, from its supposed anthelmintic properties, and also probably from the fact of the expressed juice of the leaves being applied to foul and neglected ulcers, for the purpose of destroying the larvae of insects. A belief in the anthelmintic virtues of the leaves is common amongst the natives, but facts based on European experience are wanting to substantiate its claims to this character. (30)

- The plant is spoken of as having a merited reputation as an antiperiodic in intermittent fevers. Emmenagogue properties are also assigned to it. Dr. J. Newton reports that in scinde the dried root, in doses of about a drachm and a half, in the form of powder or in infusion, is administered during labours to increase uterine contractions. (31)

- The leaves are applied to the navel to more the bowel of children, and are also given internally in combination with castor oil as a remedy for colic.
• The root and leaf are remarkably bitter, and yield a thick yellowish juice which is given in the treatment of syphilis with boiled milk and when combined with opium is used with great success in gonorrhoea.

• This plant is used by Hindu physicians on account of its bitter purgative and anthelmintic properties. Bruised leaf mixed with castor oil is applied externally in obstinate cases of eczema of the legs of children.

• A decoction of the root 1 in 10 in doses of 1 to 2 ounces was given three times a day to suspected cases of round worms followed by castor oil and was found to be generally efficacious in expelling round worms.\(^{(27)}\)

• The juice of the plant leaves mixed with water is given in snake - bite as an antidote and also for colic mixed with castor oil. It is also applied to sores. The powdered root is useful in ague. A paste of the leaves is applied as a poultice for children’s costiveness.

• The plant is an insecticide, anthelmintic, emmenagogue, febrifuge and parturificient. The leaves are applied to foul and neglected ulcers to kill the worms in them.\(^{(25)}\)

• This plant is used in the treatment of snake bites, amenorrhoea, dysmenorrhoea and tedious labour.

• The juice of the leaves is applied to foul and neglected ulcers

• The leaves are stomachic, tonic and anti periodic

• The root decoction is reported to expel round worms.\(^{(26)}\)

• The plant is so named since even goats will not eat its leaves because of their bitterness. \((Aadu\) in Tamil means goat + \(theendaa\) means not to touch + \(paalai\) means the plant). The Aristolochia bracteata has the property of kill all worms in the intestine, and to cure diseases concern with nerves, poison in the body, black spots over the body, eczema and 80 types of diseases arising due to deranged wind humor and diseases that make the skin thick like a pig. The decoction of the root of Aristolochia brateata expels ascaris from the stomach. It also gives strength to the body and consolidateds semen.
General Characters

The old Siddha text says

White leprosy and black vitiligo one may contract
Eighty types of diseases due to deranged wind humor
Chronic eczema and shaking nervous problem cured by Birthwort
Which is also anthelmintic and aphrodisiac so tell the elder.

Method of Use

- Take 10 grams of the leaves of Birthwort and put it in quarter liter of hot (nearly boiling) water and keep it for about two hours. Then filter. Drink the filtered water at a time about 15 to 10 ml, three times a day. This will cure diseases arising due to deranged wind humor.

- Grind the leaves of Aristolochia bracteata to a paste form and apply over the stomach of children. This will remove constipation.

- Dry the leaves of Aristolochia bracteata and powder them. Take 1 gm of this powder with hot water to cure snake bite, black coloration of the skin and psoriasis.

- Take 5 grams of the seeds of Aristolochia bracteata and give along with a spoon of castor oil. This will result in loose motion and with that problem associated with menstruation; worms in the intestines will be cured. This is good for the womb.
• Extract juice by pounding the entire plant of Aristolochia bracteata and take equal amount of gingelly oil. Boil the mixture to a consistency. The oil is used for external application for all skin diseases.

• The root of Aristolochia bracteata can be powdered and 5 grams of this can be taken twice a day for three days. During these days diet should be free of salt and the person should not be allowed to sleep for 24 hours. This will remove snake poision from the body.

• Mix equal amount of the powder of the roots of Aristolochia bracteata and Azima tetracantha. From this mixture 2 grams twice a day. This will stop black spots appearing on the face and spreading skin diseases.

• Take 20 grams of the root bark of Aristolochia bracteata and equal amount of Cinnamom bark. Grind them together with lemon juice to a paste form. Form tablets of 2 grams each and dry under shade. Now, take the entire plant of Evolvulus alsinoides, the outer skin of one Terminalia chebula and 5 peppers. Put them all in 100 ml of water and boil till the volume is reduced to quarter. Filter and add 1 tablet made above into this decoction and drink. This will cure many types of fevers almost 64 varieties. Eat only gruel made of rice.

• Soak 4 grams of the root bark of Aristolochia bracteata into 150 ml of cold water for an hour. Then, filter and drink. This will induce labor pain again. That is, it will cure uterine inertia. (22)

**Ethno botanical studies**

• Used for flatulence, wounds and as antidote to scorpion stings

• As an anthelmintic, purgative for fever and joint pain

• Seeds are used as purgative

• The leaves are used as appetizer, wormicidal, in piles, ulcers, eczema and other skin diseases, for dysentery in cattles and in menstrual disorders

• Root is used for expelling round worms

• Root is used in liver enlargements (26)

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3.3.3. Scientific Review

a. Syzygium cumini

This study was performed to assess the anti-diabetic effect of ethanol extract of Eugenia jambolana seeds using alloxan induced mid diabetic (MD) and severely diabetic (SD) rabbits. The extract when subjected to purification gives hypoglycemis principle (SIII). This yielded an active compound LH II on further purification by sephadex LH 20. The phytochemical analysis of LH showed the presence of $\Delta^5$ Lipid, saturated fatty acid and sterol. The anti diabetic activity was evaluated by the oral administration of LH II to MD and SD at a dose of 10 mg/kg b.w showed significant fall in fasting blood glucose at 90min (21.2% MD; 28.6 %SD), 7th day and 15th day 35.6% MD and 59.6 % SD respectively. Plasma insulin values were increased significantly (p $\leq$ .001). After 15 days treatment HBA\(_1\)C level showed significant fall of 50.5% in SD. In vitro studies on pancreatic $\beta$ - cells showed 3 times rise in insulin level than the untreated group. In addition to this, LH II resulted in significant rise in the key enzymes involved in glycolysis and fall in the key enzymes involved in gluconeogenesis. There was an elevation of liver and muscle glycogen by 52 % and 47 % for SD 36.6% and 30% for MD respectively. These findings concluded that the LH II has significant anti diabetic activity in both MD and SD rabbits. (32)

Godwin Selvaraj Esther et al studied the effect of ethanol extract of seeds of Eugenia jambolana (SEEJ) by using in vitro and in vivo models to assess the anti-diabetic activity with its protective effect on diabetic nephropathy. To determine the anti diabetic effect, in vitro study was carried out in lymphocyte culture by glucose uptake assay which showed marked increase in glucose uptake when compared with control rats. In vivo study was carried out in male albino wistar rats to determine the anti diabetic effect and protective effect on diabetic nephropathy by induction of type II DM using streptozotocin (STZ) - nicotinamine, when compared to diabetic control group resulted in significant fall in the blood glucose level. In addition to that, there was a marked reduction in the renal enlargement, HbA\(_1\)C, urea, Blood Urea Nitrogen, uric acid, serum creatinine, volume of urine and micro albumin values than the diabetic control group. There was a profound increase in body weight in diabetic rats treated with SEEJ compared to diabetic control rats. The histopathological analysis of kidney tissues firmly suggested the protective effect of SEEJ in diabetic nephropathy. The results of this study strongly concluded that SEEJ has both anti-diabetic activity and significant protective effect in diabetic nephropathy. (33)
Pragati Baghel et al. studied the antioxidant effect and in-vitro Sun Protection Factor value of Eugenia jambolana. In this study, the phytochemical analysis showed the presence of glycosides, alkaloids, tannin, saponin, flavonoids, herbal dyes in Eugenia jambolana. The antioxidant property was determined by spectrophotometric method by using a reagent 1,1 Diphenyl 2 picrylhydrazyl (DPPH). The result of absorption inhibition range was found 91.59 ±0.25 %. The Sun Protection factor value was determined by using UV-Visible spectrophotometer, the results of SPF value are 2.278±0.127. Finally, this study concluded that the extract of Eugenia jambolana have good anti-oxidant as well as significant SPF value.\(^{(34)}\)

Stanley Mainzen Prince et al. did a study to prove the reduction of tissue damage in the brain of diabetic rats by the seed extract of Syzygium cumini. They stated that jamun seed has been used in different parts of India for the treatment of diabetes and there is a marked reduction in lipids thiobarbiteric acid reactive substances (TBARS) and the catalase and superoxide dismutase has been increased in the brain tissue of diabetic induced rat, after an oral administration of aqueous jamun seed extract for six weeks. They also stated that both alcoholic and aqueous extract had better action than glibenclamide and they reduce tissue damage in the brain of diabetic rats.\(^{(35)}\)

A study was conducted by R. Bhaskaran Nair et al. to screen the antidiabetic activity of seed kernel of Syzygium cumini at the dosage of 1g, 2g, 4g and 6 g / kg body weight. The result showed that there was a maximum hypo glycemic effect (42.64 %) in rabbits at the dose of 4g/kg, 3 hours after medication. This drug might promote endogenous release of insulin like Tolbutamide. And it produced a significant reduction in blood sugar level is 17.04% in the diabetic rats induced by Alloxan.\(^{(36)}\)

Stanley.P et al. Studied that the effect of extract of Syzygium cumini seeds on Glucose-6-phosphatase and hepatic hexokinase in alloxan induced experimental diabetic rats. The aqueous extract of seeds of Syzygium cumini at a dose of 2.5 g/kg of b.w was given to alloxan diabetic rats for one month. The results showed a significant elevation in hexokinase activity and depletion in glucose -6- phosphatase activity. It also decrease the leakage of serum alkaline phosphatise, Lactate dehydrogenase and acid phosphatise.\(^{(37)}\)

SB Sharma, A.Nasir et all had a study in diabetic rats induced by alloxan to prove the hypoglycemic and hypolipidemic effects of ethanolic seed extract of Eugenia jambolana. The hypoglycemic effect was monitored by decrease in fasting blood glucose level (FBG)in 90 min and fall in the peak blood glucose level in (GTT) glucose tolerance test in the mild(MD) and
sub-diabetic rats (AR) and by decrease in FBG at 90 min in severe diabetic (SD) rabbits. There was a significant reduction in FBG at 90 min (12% AR, 18.9% MD and 29% SD) and 16.9% reduction in peak blood glucose in AR and 21% in MD rabbits during GTT when ethanol extract (100mg/kg body weight) was given orally to sub-diabetic (AR) for 1 day, MD for 7 days and SD for 15 days. It showed a significant reduction in FBG (41.3% MD, 31.6% SD) and GHb (glycosylated haemoglobin) values (23.3% MD, 26.6% SD), while showed significant upgradation (32.8% MD, 26.9% SD) in serum insulin level. When the extract was administered daily for 15 days to MD and SD rabbits. There was an increase in liver and muscle glycogen content. There was a reduction in total serum cholesterol (TC)/high density lipoprotein cholesterol (HDL-c) ratio, serum LDL cholesterol levels and the activity of HMG-CoA reductase was reduced. This proves its hypolipidimic effect and there was a normal appearance of liver, pancreas and aorta in histopathological studies. (38)

Singh N, Gupta M. reported that, the ethanol extract of the powder of Syzygium cumini seeds in alloxan induced diabetic rats has the effect of lowering the blood glucose level on the islets of pancreas. It also caused increase in body weight. There was a significant result in lowering the blood glucose level in the rats after feeding with that extract. The sugar level which had been dropped to normal, has not been elevated even after the withdrawal of the extract feeding and this is the peculiar finding of this study. (39)

P. Stanley Mainzen prince et al. studied the Hypoglycaemic effect of Jamun seeds, effect of antioxidant on lipid peroxidation in alloxan induced diabetic rats. In this study, the aqueous seed extract of Eugenia jambolana was orally administered for 6 weeks at a dose of 2.5 and 5.0 b.w showed marked increase in blood haemoglobin level and decrease in blood glucose level but there was no significant effect at a dose of 7.5 g/kg b.w. The result of this study showed that the aqueous extract of Jamun seed possess hypoglycaemic action. In addition to this, decrease in body weight was also prevented.

The elevation of reduced glutathione (GST), catalase and superoxide dismutase and depletion of free radical formation in tissues, thiobarbituric acid reactive substances (TBARS) strongly shows the antioxidant activity of seed extract of Eugenia jambolana. It showed the significant anti oxidant activity in animals given with Jamun seed extract at a dose of 5.0 g/kg b.w when compared to glibenclamide. From this study, it was concluded that the seed extract of Eugenia jambolana posses significant hypoglycaemic effect and good antioxidant property. (40)
Pandey M and Khan A gave the study report of hypoglycaemic effects of defatted seeds and water soluble fibre from the Syzygium cumini seeds in diabetic rats induced by alloxan. In this study, the effect of oral administration of different fractions of S. cumini seeds was tried in glucose tolerance and fasting blood glucose in normal and diabetic rats. It was determined that 40% of water soluble gummy fibre and 15% of water insoluble neutral detergents fibre (NDF), were present in the Syzygium cumini seeds. This study was proved that 21 days feeding with the diets having 15% unextracted seed powder containing water soluble gummy fibre, 15% defatted seed powder from which lipid and saponins were removed and 6% gummy fibre which is soluble in water, significantly reduced the blood glucose level and improved oral glucose tolerance but feeding with 15% powdered degummed seeds from which the gummy fibre soluble in water was removed but has the neutral detergent fibre and 2.25% of water insoluble neutral detergent fibre isolated from the seeds, had no effect in the lowering blood glucose or increase the oral glucose tolerance in both normal and diabetic rats. This showed that the seeds of S. cumini had the hypoglycaemic effect and it was due to water soluble gummy fibre and water insoluble neutral detergent fibre.\(^{(41)}\)

Prince PS and others had a study report Syzygium cumini seeds which act in alloxan induced albino rats to check the Properties of hypolipidimic and hypoglycaemic effect. In this study, first the rats were induced by single intra peritoneal injection of alloxan (150mg, kg (-1) b.w 0 to cause diabetes. There was a significant reduction in blood and urine sugar level, lipids in serum and tissues showed a marked change when alcoholic Jambolona seed extract (JSEt) was administered orally at the dose of 100mg kg (-1) body weight. This extract had increased the total Haemoglobin level. The effect of alcoholic JSEt was equivalent to insulin and all the altered parameters became normal.\(^{(42)}\)

Antidiabetic activity and Pharmacognostic standardization of the barks of Syzygium cumini on Streptozotocin induced diabetic rats, Tripathi et al, did the phytochemical and pharmacological screening of the Syzygium cumini barks. As per the OECD guidelines, the acute oral toxicity was done and the dose of the bark was made as 300-500mg/kg.b.w. A dose of Streptozotocin 50 mg/kg was administered through intra peritoneoealy and the rats were made diabetic. In the fasted diabetic and normal rats, the effective dose of postprandial was determined as 500 mg/kg. In the OGTT study after glucose administration, the glucose levels in blood was measured at 0, 30, 90 min. The chronic study was carried by administering the bark extract for 21 days at dose 500 mg/kg to compare its activity; Glibenclamide (2.5 mg/kg) was used. The result of this study shows that, the significant reduction of postprandial blood
glucose (p ≤ 0.001) when Syzygium cumini extracts were taken orally, ½ an hour before meals when compared to control/ and less significant than the standard drug glibenclamide.\textsuperscript{43}

Rachel Melo Ribeiro et al. studied the antihypertensive effect of hydroalcoholic extract of Syzygium cumini leaves (HESC) in normotensive and spontaneously hypertensive wistar rats (SHR) as well as its in vitro effect vascular reactivity of resistant arteries. HESC was administered orally at a dose of 0.5 g/kg/day for 8 days to SHR and the heart rate, mean arterial pressure and vascular activity were evaluated. Continuous administration Of HESC showed a time dependent reduction in the blood pressure of maximum 62% in SHR. HESC treated endothelium deprived arterial ring showed a maximum reduction of 40% induced by NE. The results showed that the antihypertensive effect of Syzygium cumini leaves extract was probably due to the inhibition of arterial tone and extracellular calcium influx.\textsuperscript{44}

Faiyaz Ahmed et al. reported that the radical scavenging and anti lipidperoxidative effects of Eugenia jambolana aqueous extracts using 3 in vitro methods – DPPH free radical scavenging assay, lipid peroxidation inhibition and reducing power in which rat liver homogenate was used as substrate. In addition to that heat treatment on antioxidant activity was also studied. When compared to synthetic anti oxidant (Butylated hydroxyl toluene) both heat treated and untreated extracts showed significant radical scavenging activity. Both the extracts exhibited similar reducing power which was significantly lower than that of ascorbic acid. Both the extracts significantly inhibited the lipid peroxides formation and restored the glutathione content in the liver in anti lipid peroxidation assay. These findings concluded that E. jambolana possesses a strong antioxidant activity.\textsuperscript{45}

Jamun seed fortified cookies were prepared by fortifying jamun seed powder in different ratios 20%, 30%, 40% to the cookies flour whereas cookies without adding jamun was kept as control. The cookies were analyzed for chemical, sensory and self life evaluation after baking. Among these 3 ratios 30% jamun powder showed highest score for organoleptic characters such as colour, appearance, crispiness, flavor and overall acceptability even up to 30 days of storage. Protein and fat content were increased in the jamun seed powder cookies. As well as in shelf life study both control and jamun seed powder cookies showed best shelf life of 30 days but after 30, 45,60 days they showed sudden increase in the moisture content. These findings suggested that jamun seed powder cookies can be recommended to diabetic patients because of its high jamboline content.\textsuperscript{46}
Sahana D.A et.al studied an open labeled randomized clinical trial was conducted in 30 freshly diagnosed type 2 diabetic patients to validate the Effect of E. jambolana seed based drug *Madhuhara churna*. In this study patient were enrolled in three groups they have no significant difference in their baseline characteristic - Group 1 received *Madhuhara churna*, Group 2 received metformin; Group 3 were on diet restriction and exercise only for 6 months. The results showed a significant reduction in the fasting blood glucose level at 3rd and 6th and a highly significant rise in the high density lipoprotein level at 3rd month in group 1. The results concluded that the *madhuhara churna* has a beneficial effect in improving glycemic profile within 6 months of treatment in freshly diagnosed type 2 diabetes patients. (47)

*Bhavana Srivastava et.al* carried out a research study to determine the hypoglycemic effect of Eugenia jambolana pulp and seed extract in streptozotocin induced diabetic albino rats. In albino rats diabetes was induced by intraperitonital injection of streptozotocin 50mg/kg. The animals were divided into 5 groups – Group 1 – control; Group 2 received vehicle; Group 3- received ehanolic pulp extract of 200mg/kg; Group 4 received ethanolic seed extract of 200mg/kg and Group 5 received standard anti diabetic drug glibenclamide. The biochemical analysis such as blood sugar, lipid profile blood urea and glycosylated hemoglobin levels were estimated on 0,7,14 and 21st day of the study and also effect on the body weight also observed. The results showed a significant increase in the body weight of diabetic rats and significant reduction in the blood glucose, blood urea and lipid profile in E. jambolana pulp and seed extract treated diabetic rats (200mg/kg.bw). Therefore this investigation concluded that E.jambolana pulp and seed extract possesses hypoglycemic and hypolipidemic effect. (48)

*Shivani Sidana et.al* had an attempt to validate the dyslipidemic effect of Syzygium cumini seed powder in patients with type 2 diabetes in which both patient and investigators were blinded about the treatment. Patients were randomly divided into 2 groups- Group A was provided with 10 gms/day jamun seed powder and Group B was given with placebo powder. The lipid profile was estimated at baseline and 30th, 60th and 90th day of the treatment. The results showed a significant reduction in the cholesterol levels by 10.55% and 15.79%, LDL levels by 10.29% and 14.50% and mean triglyceride levels by 8.28% and 13.66% at 30th and 60th day respectively. Statistically significant reduction in VLDL levels was noted at 30th,60th,90th day by 9.38%,12.90% and 20.69% respectively.HDL level increased significantly after 30 and 60 days of supplementation with S.cumini seed powder by 11.1% and 13.89% in males and 10.81% and 16.21% in females respectively. By the above reports it was concluded that supplementation of S. cumini to type 2 diabetic patients improved their lipid profile. (49)
Ravi.K. et.al carried out a research study to determine the antioxidant defense system of Eugenia jambolana seed kernel in streptozotocin induced diabetic rats. A significant increase in the plasma glucose, vitamin E, lipid peroxides, ceruloplasmin and decrease in vitamin C level and reduced glutathione were observed in pancreatic tissue in diabetic rats compared to control group. Histopathological studies showed protective effect of E. jambolana seed extract on pancreatic β cells. This study shows decreased oxidative stress in diabetic rats which may be due to its hypoglycemic activity.\(^{(50)}\)

Anwesa Bag et.al had an attempt to determine the in vitro antibacterial potential of Eugenia jambolana seed extracts against multidrug- resistant human bacterial pathogens. Antibacterial susceptibility was determined by agar well diffusion method and microbroth dilution assay. Rate and extent of bacterial killing was determined by kill-kinetics study. Phytochemical analysis and TLC were performed in ethyl acetate fraction to determine the putative compounds responsible for antibacterial activity which shows the presence of phenols as major active components. Acute toxicity study was done in mice and cytotoxic potential was determined by hemolytic assay method. The ethyl acetate fraction does not possess hemolytic activity as well as no toxic effect at recommended dosage. The ethyl acetate fraction obtained from ethanol extract possesses maximum antibacterial effect against all the test isolates.\(^{(51)}\)

Ravi et.al had an attempt to analyze the inorganic trace elements in Eugenia jambolana seed on streptozotocin– induced diabetes in rats. E.jambolana seeds were reduced to ash and the inorganic elements were analyzed. The hypoglycemic activity was assessed by glucose tolerance test on streptozotocin– induced diabetic rats. Elements possessing hypoglycemic activity such as zinc, potassium, chromium, vanadium were present in the E. jambolana seed. Eugenia jambolana seed ash treated diabetic rats exhibited normoglycemia and better glucose tolerance. From the results of this study it was concluded that the inorganic elements plays a major role in the hypoglycemic nature of the seed kernel E. jambolana.\(^{(52)}\)

Tehzeeb-ul-Nisa et.al carried out a research study to evaluate the hypoglycemic activity of flower extracts as well as ash obtained from the flowers of Eugenia jambolana. Flower extract of about 100 mg/kg b.w was orally administered to streptozotocin induced diabetic rabbits and ash content of about 4.8 mg/kg b.w to nicotinamide- streptozotocin induced diabetic rabbits to detect whether the organic or inorganic constituent in the extract was responsible for its hypoglycemic activity. The results showed that the alcoholic and the aqueous extract of E. jambolana have no effect on fasting blood glucose level in streptozotocin
induced diabetic rabbits. However the alcoholic extract of the flower showed significant reduction in fasting blood glucose level in nicotinamide- streptozotocin induced diabetic rabbits and also a highly significant reduction in fasting blood glucose level of ash treated rabbits. The results concluded that the alcoholic extract of E. jambolana flowers possesses hypoglycemic effect on nicotinamide- streptozotocin induced diabetic rabbits because of the presence of inorganic metals in it. 

Muhammad Shahnawaz et.al was carried out a study, to determine the anti oxidant effect and total phenolic compounds in various products of 2 jamun fruit cultivators endogenous to the tropical region of sindh. Cultivars are V1- improved and V2- indigenous. Jam, squash, ready to drink, juice, pulp powder and seed powder were the 5 products made from cultivars and Folin-Ciocalteu colorimetric method was used to analyze total phenolic compounds wheras the anti oxidant effect was analyzed by DPPH assay. The results showed a highest phenolic content of about 42 and 40mg gallic acid equivalent (GAE)g-1 (DW) while lowest content was found in squash in both cultivars. The highest antioxidant capacity was found in seed and lowest in squash.

Prakash R. Patel and T.V.Ramana Rao, Studied the antibacterial activity of Syzygium cumini fruits against gram positive and gram negative bacterial strains. Highest zone of inhibition against Bacillus cereus was obtained using extract of diethyl ether. Diethyl ether extract of preripened fruit was effective against Bacillus cereus at a lowest minimum inhibitory concentration of about 0.25 mg/ml.Zone of inhibition were obtained for all bacterial strains except salmonella paratyphi using ethyl acetate and diethyl ether and Micrococcus luteus against ethyl acetate fractions. The study revealed that jamun fruit, possess rich bioactive compounds which was responsible for its anti microbial activity.

Adelia F.et.al determined the phenolic compounds and carotenoids from the jambolana fruits by HPLC-DAD-MS/MS. All-trans- lutein 43.75% and all- trans- b carotene (25.4%) were the 2 carotenoids found in the fruits of jambolana. The anthocyanin composition was characterized by the presence of 3, 5 diglucosides. This pattern was also observed for other flavanoids identified such as diglucosides of dihydromyricetin, methyl dihydromyricetin and dimethyldihydromyricetin, a galloyl glucose ester and myricetin glucoside. In addition to that the antioxidant capacity of the extract was evaluated by ABTS scavenging assay, peroxyl radical and protective effect against singlet oxygen. The TEAC values indicated that the quinonoidal and hemiacetals species possess higher radical scavenging capacity compared to
flavylium cation. The functional extract exhibited 60% of dimethylantracene protection against 102 and 16.4 l mol Trolox/g of ORAC. (56)

**Jamaludin, M.et.al** studied the effect of homeopathy remedy *Syzygium jambolanum* on glucose level, lipid profile and histology of pancreas of streptozotocin induced SD diabetes rat (32 male rats) divided into 4 groups with body weight 250-300g. STZ injection was given to 2 groups at a dose of 45mg/kg intravenously and on the 3rd day diabetes was confirmed by measuring the blood glucose level. Homeopathy remedy *Syzygium jambolanum* was administered for 28 days through force feeding. The results showed significant reduction in the mean plasma glucose level, total cholesterol, triglyceride and LDL cholesterol level in treated diabetic rats compared to non treated diabetic group rats and also significant increase in the plasma HDL level in treated diabetic rats compared to non treated diabetic group rats. The histological study revealed the presence of larger islet of Langerhans and denser beta cell distribution in treated diabetic rats compared to non treated diabetic group rats. (57)

**Rabiea Bila et.al** conducted a randomized control study on 75 male albino rats and compared the effect of fruit extract from *E. jambolana* with simvastatin on liver enzymes such as aspartate aminotransferase, alanine transferase, and creatinine phosphokinase in diet induced hyperlipidaemic rats. The animals were divided into 5 groups of 15 each, Group A – kept as control; Group B,C,D and E were provided with hyperlipidaemic diet for 6 weeks and then Group B kept as toxic control; Group C- received ethanolic extract of *E. jambolana*; Group D- received simvastatin; Group E- received the combination of both for 8 weeks. Total cholesterol, LDL, HDL, TGL, AST, ALT and CPK were measured at 0,6,14 weeks of treatment. At 14th week there was a significant reduction in the serum AST, ALT and CPK levels in group C compared to group B, D and E. serum ALT level returned to normal level after 8 weeks in Group C, which is considered as most important marker of hepatotoxicity. From the investigation reports it was concluded that ethanolic extract of *E. jambolana* caused a reduction in ALT, AST, CPK levels when compared to simvastatin. (58)

**Das S, and Sarma G.** evaluated the hepato protective activity of ethanolic pulp extract of *E. jambolana* on paracetamol induced hepatotoxicity in albino rats. Albino rats of either sex weighing 100-150 gm were divided into 5 groups of 6 animals in each group. Group A- normal control ; Group B – paracetamol treated control received 3% gum acacia 5ml/kg; Group C and D – received *E. jambolana* pulp extract 100 mg/kg and 200mg/kg respectively, and Group E- received Silmymarin 100 mg/kg for 10 days. On the 8th day of the study a single dose of
paracetamol 2gm/kg was given to Group B, C, D and E to induce hepatotoxicity. At the end of the study liver function test and histo pathological examinations were done and the reports revealed a significant reduction in total protein and significant increase in all serum biomarkers in Group B compared to Group A. when compared to Group B, Group C & D showed significant increase in total protein and reduction in liver enzymes and total bilirubin. In histopathological studies fatty changes, fibrosis and necrosis were observed in Group B whereas it was normal in Group C, D, E. Thus the results concluded that E. jambolana possesses significant hepatoprotective activity at a dose of 100 and 200 mg/kg/day.\(^{(59)}\)

**Ahmad Raza et.al.** carried out this study and investigated the antihyperlipidaemic effect of ethanolic seed and fruit extract of Syzygium cumini in hypercholesterolemic rats. High cholesterol diet (1.5% cholesterol) was given to normal rats to raise their lipid profile. Then diet containing 3% of Syzygium cumini extract was given. The results showed a maximum reduction in triglycerides, cholesterol, LDL up to 7.09%, 9.32%, 11.46% and a significant increase in HDL level up to 2.62%. From this research it was concluded that Jamun SE possesses anti hypercholesterolemic effect.\(^{(60)}\)

**Akansha Mishra, A.K. Jaitly and Arvind Srivastava** studied the anti hyperglycaemic efficacy of 6 edible plants –dried fruit powder of Momordica charantia and Coccinia indica, dried seed of Syzygium cumini, seed oil of Aegle marmelos , dried powder of root and rhizome of Curcuma longa and seeds of Trigonella foenum graecum in normoglycaemic and STZ induced diabetic rats. The results showed that the aqueous extract of Momordica charantia, Syzygium cumini, Aegle marmelos, Curcuma longa produced significant hypoglycaemic activity at a dose of 250-500 mg/kg in both normal and diabetic animals.\(^{(61)}\)

**Kasiappan Ravi, et.al.** Evaluated the anti hyperlipidemic efficacy of E. jambolana seed kernel in STZ induced diabetic rats. Ethanolic seed kernel extract (EJs – kernel) was administered at a dose of 100mg/kg b.w and were monitored for biochemical analysis. The plasma lipoprotein and free fatty acid level came back to normal in animals treated with EJs extract. These results suggest that the hypolipidemic effect of E. jambolana seed kernel may be due to the presence of triterpenoids, flavonoids and saponins in the extract.\(^{(62)}\)
3.3.3. b. Aristolochia bracteolata

S. Ramachandran et al studied the effect of anti diabetic activity of poly herbal extract which consists of ethanolic extract of Madhuca lotifolia (iluppai), Aristolochia bracteata (Aaduthinda paalai), and Aristolochia indica (Echuramooli) in rats by alloxan induced diabetic method. In this study, the anti diabetic effect was compared with standard oral anti diabetic drug Glibenclamide. The result of this study, the decreased level of blood glucose was significantly found in twelfth and twenty fourth hours than that of initial. Finally, this study concluded that, the poly herbal extract of above said plant combination possessed good potential anti diabetic activity. (63)

P. Bharathajothi et al studied the anti inflammatory and anti pyretic activity and phytochemical screening for the plant extract of Aristolochia bracteolata. The above studies were done as per the standard procedure. The result of this study, the plant Aristolochia bracteolata shows the significant effect of anti inflammatory and anti pyretic action when compared with standard drug Paracetamol and the phytochemical screening also done to identify the presence of phyto constituents. The result indicates the presence of tannin and flavonoid in rich status. (64)

Alagesaboopathi. C studied the effect of anti microbial properties of acetone, ethanol and petroleum ether extract of Aristolochia bracteolata against selected human pathogens, Escheria coli, Bacillus aureus, Pseudomonas aeruginosa and Klebsiella pneumonia by the method of agar well diffusion. The results of this study, the petroleum ether extract possess highest anti microbial effect when compared with other samples. Conclusion of this study, the three extracts of Aristolochia bracteolata possess significant inhibitory effect against tested pathogenic microorganism. This study, recommended that the plant Aristolochia bracteolata can be used as a novel broad spectrum anti bacterial traditional medicine. (65)

Trayee Sarkar Das et al, studied the Glucosidase inhibition effect of methanol, Ethyl acetate and hexane extract of Aristolochia bracteolata. The inhibitory effect was studied by the method of Dahlqvist with slight modification. The inhibition effect was studied for the three extracts with the concentration of 200 - 1000 µg/ml and compared with the standard acarbose. The results of this study reveal that the methanol extract possessed 78.27% of alpha-Glucosidase inhibition. Finally, concluded that the plant Aristolochia bracteolata showed significant effect of Glucose inhibition activity. (66)
D.Kavitha and R. Nirmaladevi, studied the bio therapeutic potential of Aristolochia bracteolata through the anti bacterial assay by agar well diffusion method and the anti fungal activity by radial growth inhibition assay method. The anti bacterial activity was performed for the aqueous, methanol and chloroform extract of Aristolochia bracteolata against the selected pathogens of Escherichia coli, Bacillus subtilis, salmonella typhimurium, klebsiella pneumonia, shigella flexneri, staphylococcus aureus, salmonella typhi, Proteus vulgaris, and pseudomonas aeurginosa. The results of this study, the methanol extract shows the significant effect while chloroform extract possess moderate activity. Aqueous extract didn’t show any anti bacterial. Anti fungal activity also done against the Aspergillus niger, Aspergillus terreus, Penicillium notatum and Rhizopus. In this study the water extract are more susceptible followed by methanol and chloroform extracts. Rhizopus pathogen was inhibited only by the methanol extract of Aristolochia bracteolata. The chloroform extract had the significant effect against only the Aspergillus terreus fungus. Finally this study concluded that the plant Aristolochia bracteolate possessed good potential of anti bacterial and anti fungal effect. (67)
METHODOLOGY OF PRECLINICAL STUDIES

IAEC APPROVAL

Collection of plant materials
Authentication of plant materials
Purification of plant materials
Preparation of Naaval Kottai Mathirai
Standardization of Naaval Kottai Mathirai

Quantitative Analysis

Microbial Load
Total Bacterial count
Total Fungal Count
Enterobacteriaceae
Escherichia coli
Salmonella.Spp
Staphylococcus Aureus

Heavy Metal Analysis
Lead
Cadmium
Arsenic
Mercury

Qualitative Analysis

Siddha clasical Method
Organoletic Characters
Colour
Odour
Taste
Physico - chemical Analysis
Loss on Drying
Total Ash
Water Soluble Ash
Acid insoluble Ash
Alcohol Soluble Extractive Value
Water Soluble Extractive Value
Bulk Density
## MATERIALS AND METHODS

### Table 5.1: Study names and study places

<table>
<thead>
<tr>
<th>S. No</th>
<th>Study name</th>
<th>Done at</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Procurement of raw drug</td>
<td>Dr. Murugesan MD (siddha), Orathanadu, Tamilnadu, India</td>
</tr>
<tr>
<td>2</td>
<td>Collection of raw materials</td>
<td>The field of Seeganendal, Pudukkottai district, Tamil nadu, India</td>
</tr>
<tr>
<td>3</td>
<td>Purification of plant materials</td>
<td>National Institute of Siddha, Chennai-47</td>
</tr>
<tr>
<td>4</td>
<td>Preparation of <em>Naaval Kottai Mathirai</em></td>
<td>National Institute of Siddha, Chennai-47</td>
</tr>
<tr>
<td>5</td>
<td>Physico chemical analysis</td>
<td>Regional Research Institute of Unani Medicine, Chennai, India.</td>
</tr>
<tr>
<td>6</td>
<td>Heavy metal analysis</td>
<td>Regional Research Institute of Unani Medicine, Chennai, India.</td>
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<td>7</td>
<td>Phytochemical analysis</td>
<td>Regional Research Institute of Unani Medicine, Chennai, India.</td>
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<td>8</td>
<td>HPTLC analysis</td>
<td>Regional Research Institute of Unani Medicine, Chennai, India.</td>
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<td>9</td>
<td>Microbial load</td>
<td>Regional Research Institute of Unani Medicine, Chennai, India.</td>
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<td>Aflatoxin and Pesticide value</td>
<td>Bureau Veritas Consumer Products Services (I) Pvt. Ltd, Chennai-32</td>
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<td>11</td>
<td>Toxicity studies</td>
<td>National Institute of Siddha, Tambaran sanatorium, Chennai, India</td>
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<td>12</td>
<td>Pharmacology study</td>
<td>K.M.College of Pharmacy, Madurai, India</td>
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<td>13</td>
<td>In-vitro Glucose uptake activity</td>
<td>KMCH college of Pharmacy, Coimbatore, India</td>
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<tr>
<td>14</td>
<td>In-vitro Alpha Glucosidase and Alpha amylose inhibitor assay study</td>
<td>Satyabama University, Chennai, India.</td>
</tr>
<tr>
<td>15</td>
<td>In- vitro anti oxidant studies</td>
<td>Satyabama University, Chennai, India.</td>
</tr>
</tbody>
</table>
5.1. Naaval Kottai Mathirai(4,5)

5.1.1. Collection of Plant materials

The seeds of Syzygium cumini and leaves of Aristolochia bracteolata were collected from the field of Seeganendal village, Pudukkottai District, Tamilnadu, India.

5.1.2. Authentication of Plant materials

The seeds of Syzygium cumini and leaves of Aristolochia bracteolata were appropriately authenticated by Prof. P. Jayaraman, Director, Plant Anatomy Research Centre, Chennai-45, after studying its anatomical structure. The authentication number is PARC/2016/3255 and 3256. The cross sectional anatomical studies also done for this plant materials.

5.1.3 Preparation of Naaval kottai mathirai

Ingredients

- Naaval Kottai (seeds of Syzygium cumini) - 500mgm
- Leaf juice of Aadutheenda paalai (Aristolochia bracteolata) - 4000ml

Purification of Plant materials

Naaval Kottai

The fruits of Jambolana were taken and the flesh were removed and dried in sun shadow. The outer coat or skin of the seeds were peeled off and powdered.

Aadutheenda paalai

The leaves of Aristolochia bracteolata were taken and washed in fresh water. Then the leaves were wiped with clean cloth and juice was taken.

Procedure

500 gms of seed powder of Syzygium cumini was grounded in the stone mortar. To this, 4000ml leaf juice of Aristolochia bracteolata was added little by little over the seed powder simultaneously during grinding and triturated well for 24 hours continuously to attain waxy consistency without sticking in the finger. Then, it was made into small pills of 500 mg (Pattany size), and preserved in an air tight container.

Dose

One tablet two times a day with warm water.

Indication: Madhumegam (Diabetes Mellitus)
Plate 5.1.1. *Syzygium cumini* seed

Plate 5.1.2: *Aristolochia bracteolata*
Plate 5.1.3. *Syzygium cumini* inner seed  
Plate 5.1.4. *Aristolochia bracteolata* leaf juice

Plate 5.1.5. & 5.1.6: Grinding process of *Naaval Kottai Mathirai*
Plate 5.1.7 & 5.1.8: Different stages of Naaval Kottai Mathirai karkkam

Plate 5.1.9: Naaval Kottai Mathirai
5.2. Plant Anatomical Studies (68-79)

This study was carried out in Plant Anatomy Research Centre, Chennai-45.

Collection of plant specimen

The plant specimens used for this study were collected from Seeganendal village, Pudukkottai District. The healthy plants and normal organs were selected with care. The required samples were cut and removed from different organs of the plant and fixed in FAA (5 ml Formalin + 5 ml Acetic acid + 90 ml - 70% ethylalcohol ). The specimens were dehydrated after 24 hours of fixing with tertiary butyl alcohol as per the time schedule given by sass 1940. The specimens were infiltrated by the addition of paraffin wax at a melting point of 58- 60°C. When they attained super saturation, they were cast into paraffin blocks.

Sectioning

The sectioning of the paraffin casted specimens were done by using rotary microtome at a thickness of 10 - 12 µm. The sections were dewaxed by customary procedure (Johansen, 1940). As per the procedure published by O’Brien et al., the sectioned specimens were stained by polychromatic stain, Toluidine blue which gives remarkably good results as well as cytochemical reactions also. This dye rendered blue colour to lignified cells, pink colour to cellulose walls, violet to the mucilage, dark green to subrein, blue to the proteinbodies. If necessary staining were also done with Fast- green, safranin and Ikl.

The stomatal morphology, trichome distribution and venation pattern were studied by paradermal section and also by clearing of leaf with sodium hydroxide 5 % (or) epidermal peeling by partial maceration using Jaffrey’s maceration fluid were prepared. (sass, 1940). Temporary preparations were done for macerated or cleared materials using Glycerine mounting. The powdered samples of different parts of the plant were cleared with sodium hydroxide and mounted in glycerine after staining. Then, the different components of cell were studied and measured.

Photomicrographs:

Wherever necessary, microscopic description of tissues were supplemented with micrographs. Photographs were taken at different magnifications with Nikon labphoto 2 microscopic unit. Bright light was employed for normal observation and polarized light was used for the study of starch grains, crystals and lignified cells. As these structure have
birefringent property they appear bright against dark background under polarized light. Magnifications were indicated by scale-bars. The descriptive terms of anatomical features were given in standard anatomy books. (Esau, 1964).

5.2.1. Syzigium cumini

The seeds of Syzigium cumini are spherical smooth white when fresh and turn black on drying. The fruit is a berry.

Structure of the Seed

In sectional view, the seed consists of a thin seed coat or testa and a thick cotyledon. The testa is free from the cotyledon (Plate 5.2.1. & Plate 5.2.2.).

Plate 5.2.1. T.S of the seed showing two halves with Cotyledons and seed coat

[Cot- Cotyledons; Te- Testa]
The seed coat (testa) is uniformly thick and encloses the cotyledon all around; the seed coat is 230 µm thick. It consists of outer epidermis which includes tangentially elongated and thick walled. The outer surface is coated and thick dark cuticle. Inner to the epidermis is the parenchymatous sarcotesta. This zone includes polygonal, thin walled compact parenchyma cells. Scattered in this parenchymatous zone are quite large and prominent scleroids which are irregular in distribution (Plate 5.2.3. & Plate 5.2.4.).
Plate 5.2.3. T.S of Seed showing portion seed coat and Cotyledons

Plate 5.2.4. T.S of seed - Enlarged

[ Cot- Cotyledons ; Epc- Epidermis of the Cotyledons ; EpT- Epidermis of the Testa ; Cu- Cuticle ; IE- Inner Epidermis ; Pa-Parenchyma ; OEp-Outer Epidermis ; Scl- Scleroids ; Te- Testa (seed coat); VT-Vascular Tissue]
Plate 5.2.5. Seed coat with Elongated Sclereids

Plate 5.2.6. Seed coat with Brachy Sclereids

(Cot- Cotyledons; Bscl- brachy sclereids; CL- Cell lumen; Ep-Epidermis; CW-Cell wall; Pa- Parenchyma; EScl- Elongated Sclereids)
The scleroids are either horizontally elongated (Plate 5.2.5.) or cuboidal (Plate 5.2.6.). The cuboidal scleroids are known as brachy scleroids or stone cells. The scleroids have thick, lignified secondary walls and wide lumen (Plate 5.2.6.).

**Cotyledons:**

The cotyledons have thick squarish epidermal cells and thick cuticle (Plate 5.2.6.). The inner portion of the cotyledon includes large, fairly thick walled, elliptical or ovate parenchyma cells. Large starch grains are abundant in the cells of the cotyledon (Plate 5.2.7.) The starch grains vary in size and shape. Some are circular, some are ovate and others are elliptical. When the starch grain are viewed under polarized light, they appear bright with +, Y and X shaped dark polarimarks.

Along the periphery of the cotyledons are located wide, elliptical or circular secretory cavities (Plate 5.2.8. & Plate 5.2.9.). The outer part of the cavity is surrounded by secretory epithelial cells. The cavity is 130µm in diameter.

Plate 5.2.7. Cells of the Cotyledons with Starch grains
Plate 5.2.8. Cotyledons with secretory cavity

Plate 5.2.9. Starch grains as seen under Polarised light

(Cot- Cotyledons; Ep-EEpidermis; PM- Polarimark; SG- Starch grain; SC- Secretory Cavity).
5.2.2. *Aristolochia bracteolata*

The leaf in cross section exhibits thin lamina and prominent midrib. (Plate 5.2.10.) The midrib is 500 µm thick and 430 µm wide. It enclose a prominent circular vascular bundle, parenchymatous ground tissue and thick epidermal layer of small highly thick walled cells. The cell above the vascular bundle and beneath the ad axial epidermis are circular and thick walled. The ground cells in the abaxial part of the midrib are angular thin walled and compact.

Plate 5.2.10. T.S of leaf through midrib.

[ AdS- Adaxial side; Ep- Epidermis; GT- Ground Tissue; La- Lamina; MR- Midrib; Ph- Phloem; X- Xylem ]

**Vascular bundle**

The vascular bundle is circular and 270 µm in diameter. It consists of several circular highly thick walled xylem elements. Beneath the xylem strand occurs phloem elements. The phloem elements are angular thick walled and are arranged in compact layer. (Plate 5.2.11.)
Lamina:

The lamina as a lateral vein which is slightly concave on the adaxial side and prominently hemispherical on the abaxial side (Plate 5.2.12.).
The vascular bundle is collateral with adaxial duster of thick walled xylem elements and small group of abaxial phloem elements (Plate 5.2.13.). The ground cell the abaxial part of the lateral vein are thick walled and sclerenchymatous.

Plate 5.2.13. Vascular bundle of the lateral vein enlarged

[ AD- Adaxial side; GT- Ground Tissue; La- Lamina; LV- Lateral Vein; Ph- Phloem;
VB- Vascular bundle; X- Xylem ]

Leaf margin

The marginal part of the lamina becomes gradually tapering into a short cylindrical part with blunt end. The marginal part is 70 µm thick. Within this narrow end occur a compact mass of thick walled angular cells. (Plate 5.2.14).

Leaf

The middle part of the leaf is 130µm thick. It consists of wide cylindrical or elliptical thin walled cells with thick cuticle. The abaxial epidermis consist of small cylindrical cells. The mesophyll tissue is differentiated into adaxial single layer of palisade cells and abaxial zone of small lobed spongy mesophyll cells. (Plate 5.2.15).
Plate 5.2.14. T.S of leaf through marginal part

Plate 5.2.15. T.S of lamina

[ AbE- Abaxial Epidermis; AdE- Adaxial Epidermis; Cu- cuticle; LM- Leaf Margin; PM- Palisade mesophyll; SM- Spongy mesophyll ]
5.3. Standardization of Naaval Kottai Mathirai

The huge increase in population, modern lifestyle, increasing cost of the drugs, unavailability, scarcity of chemicals, and their side effects lead the people to get remedy from plant sources. Most of the Siddha formulations are in crude extracts and show activities similar or different from their individual activity when analysed.

This shows that the activity of a formulated medicine is the synergistic action of its various components. The medicinal value of the plants lies in the bioactive phytochemical constituents that produce definite physiological effects on human body. The study of both molecular and phytochemical analysis would help to understand the plants and help to conserve the plants in future.

To export a drugs strict quality parameters, quality control and safety and efficacy are required. Hence the following standardization parameters have been done as per AYUSH guidelines.

For standardization of the drug, three samples of the Naaval Kottai Mathirai were taken (sample I, II and III).

5.3.1. Qualititative analysis of Naaval Kottai Mathirai

a. Siddha classical method

The quality of Naaval Kottai Mathirai was accessed by the parameters cited in the Siddha pharmacopoeia

Vanthidu sarakkaik kallil

Kallura varaiththal Karkkam

The quality assessment of mathirai (tablet) formation according to Siddha is that the material for tablet with adjuvant liquid medium when rubbed in mortar should not be sticky. It should be soft to touch and should be taken fully from the mortar without leaving any matter in the mortar.

b. Organoleptic evaluation

It is the evaluation of drug by the sense organs like skin, eye, tongue, nose and ear or macroscopic evaluation and it includes evaluation of drugs by colour, taste, odour, size, shape
and special features like touch, texture etc. It is the technique of qualitative evaluation based on the study of morphological and sensory profile of whole drugs. In this study organoleptic evaluation was carried out for identification of sensory characteristics like colour, taste, odour, texture, etc

c. Physico chemical analysis (82)

The physico-chemical test was carried out at Regional Research Institute of Unani Medicine, Chennai, India. The procedures recommended in WHO guidelines (Anonymous, 1998) were followed.

Loss on Drying

3 g of NKM was kept in a previously weighed 100 mL beaker and heat in an oven at 105°C for 5 h. Cool in a desiccator and weighed. Repeat the procedure till constant weight is obtained. The percentage of loss in weight of the sample was calculated.

\[
\text{Percentage of Loss on Drying at 105°C} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the NKM}} \times 100
\]

Total Ash

3g of NKM was accurately in a previously ignited and tarred Silica dish. Spread the material evenly and ignite in a muffle furnace at 600°C until free from carbon. Then it was cooled and weighed. Percentage of ash with reference to the air-dried powder was calculated.

\[
\text{Calculation of total Ash} = \frac{\text{Weight of ash}}{\text{Weight of the NKM taken}} \times 100
\]

Water Soluble Ash

The above obtained ash was boiled with 25mL water for 5 minutes. The insoluble ash was collected using filter paper and washed with hot water and transferred to the silica crucible then ignites for 15 minutes at temperature not exceeding 450°C. The silica crucible and residue were weighed until constant weight was attained for determination insoluble ash weight. The
weight of the water insoluble ash was subtracted from the weight of total ash to determine the water soluble ash.

**Acid insoluble Ash**

The above obtained ash, the dish containing the total ash, add 45 mL of 1:5 Hydrochloric acid in three portions of 15 mL each time, boil gently for 5 minutes and filter. The insoluble ash was collected using filter paper and washed with distilled water until the residue is free from acid. Transfer the filter paper containing the insoluble matter to the original dish, dry and ignite to constant weight. Cool the dish in a desiccator and weighed. The percentage of Acid insoluble-ash of the air dried material was calculated.

\[
\text{Percentage of Acid insoluble-ash} = \frac{\text{Weight of the acid insoluble residue}}{\text{Weight of the NKM}} \times 100
\]

**Alcohol Soluble extractive Value**

3g of NKM in a glass stoppered flask and add 100 mL of distilled Alcohol, shake occasionally for 6 h and then allow standing for 18 h. filter rapidly taking care not to lose any solvent and pipette out 25 mL of the filtrate in a pre weighed 100 mL beaker and evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 h, cool in a desiccator and weighed. Calculated the percentage of alcohol extractable matter of the NKM. Repeat the experiment twice and take the average value.

\[
\text{Percentage of Alcohol- soluble extractive} = \frac{\text{Weight of the extract}}{25 \times \text{weight of the NKM taken}} \times 100
\]

**Water soluble extractive value**

3g of NKM in a glass stoppered flask and add 100 mL of distilled water, shake occasionally for 6 h and then allow standing for 18 h. filter rapidly taking care not to lose any solvent and 25 mL of the filtrate is pipette out in a 100 mL pre weighed beaker and evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 h, cool in a desiccator and weighed. Repeat the experiment twice and take the average value.
Weight of the extract

Percentage of Water-soluble extractive = \underline{__________________________} \times 100

25 \times \text{weight of the sample taken}

d. Preliminary Phytochemical Analysis of Naaval Kottai Mathirai:

Test for Terpenoids

Two hundred mg of NKM extract was taken separately in a tube; 3 ml of petroleum ether was added and filtered. The filtrate was evaporated and the residue was dissolved in small amount of chloroform and 1 ml Tin and Thionyl chloride solution was added.

Test for Flavonoids and their glycosides

Two hundred mg of NKM extract was dissolved in 10 ml of alcohol separately and it was hydrolyzed with ten percent v/v sulphuric acid and cooled. Then, it was extracted with diethyl ether and divided into 3 portions in 3 separate tubes. One ml of dil. Na$_2$CO$_3$ sodium carbonate, 1 ml of 0.1M NaOH and 1 ml of dil. NH$_3$ were added to all the 3 tubes.

Shinoda’s Test

Two hundred mg of NKM extract was dissolved in 2ml of alcohol and a little part of magnesium ribbon was added and 2 drops conc. HCL was added.

Zinc-HCl Reduction Test

Two hundred mg of NKM extract was separately taken in a tube, and add a drop of conc. HCl and a pinch of zinc dust.

Lead Acetate Solution Test

Two hundred mg of NKM extract was separately taken in a tube, and 10% w/v lead acetate was added to it.

Test for Alkaloids:

Two hundred milligram of NKM extract was treated separately in test tube with five milliliter of dilute Hcl and filtered. The filtrates were treated separately with different alkaloid reagents Mayer’s reagent, Dragendorff’s reagent, Wagner’s reagent and Hager’s reagent.

Test for Saponins

Two hundred mg of NKM extract was taken separately in a tube and dissolved in 5 ml of distilled water, shaken in a graduated cylinder for 15 min.

Test for Tannins

Two hundred mg of NKM extract was separately dissolved in a test tube with 3 ml of distilled water and filtered. The filtrate was treated separately in a test tube with 10% w/v
aqueous potassium dichromate solution, 10% w/v aqueous lead acetate solution and 1 ml of 5% ferric chloride solution.

**Test for Anthraquinone glycosides**

**Borntrager’s test**

Two hundred mg of NKM extract was taken in a tube alone and boiled with three milliliter of dil. H₂SO₄ filter and to the filtrate; benzene was added and shaken well. The inorganic layer was separated and NH₃ was added slowly.

**Modified Borntrager’s test**

Two hundred mg of NKM extract was taken in a tube alone with boiled for two minutes with dil. Hcl and three drops of ferric chloride was added, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volumes of dilute ammonia solution were added to the benzene extract and shaken well.

**Test for Amino acids**

Two hundred mg of NKM extract was dissolved in 3 ml of distilled water separately in a test tube and treated with Ninhydrin reagent (Triketohydrindene hydrate) at the pH range of 4 to 8.

### 5.3.2. Quantitative analysis of Naaval Kottai Mathirai

**a. Heavy Metals Analysis**

Heavy metals concentration such as Lead and Cadmium were observed by Atomic Absorption Spectroscopic study. The procedures recommended for analysis of Heavy metals like Lead and Cadmium in WHO, 1998 and AOAC, 2005 and study was done at Regional Research Institute of Unani Medicine, Chennai – 600013.

The procedures recommended for analysis of Heavy metals like Lead and Cadmium in WHO, 1998 and AOAC, 2005 and study was done at Regional Research Institute of Unani Medicine, Chennai – 600013.
**Instrument details**

Thermo Fischer M Series, 650902 VI.27 model Atomic Absorption Spectrometer (AAS) was used for the analysis. The operating parameters were as follows:

- **Instrument technique**: Flame technique
- **Wavelength (Lead)**: 217 nm
- **Wavelength (Cadmium)**: 228.8 nm
- **Slit width**: 0.5 nm
- **Lamp current (Lead)**: 4.0 mA
- **Lamp current (Cadmium)**: 3.0 mA
- **Carrier gas and flow rate**: Air and Acetylene, 1.1 L/min
- **Flow rate**: 2 ml/min

The Hallow cathode lamps for Lead and Cadmium analysis were used as light source to provide specific wave length for the elements to be determined.

**b. Microbiological contaminent study**

TVC of the sample was observed using series dilution method. NKM was ground and dissolved in water and diluted with phosphate buffer at pH 7.2. Test for micro organisms like E. coli, Pseudomonas aeruginosa, Staphylococcus aureus and Salmonella Sps. in the test sample were studied.

**Total viable aerobic Count (TVC)**

TVC of NKM was observed using series dilution method. In these method 12 tubes containing 9-10 ml of soybean-caseing digest medium was taken. 1 ml of NKM solution was added to all the tubes and incubated at 30-35° C for 72 hrs. Microorganisms produced were determined.

**Total bacterial count**

To a Petri dish one millilitre of drug solution and 15 millilitre of liquefied casein-soybean digest agar was added and incubated at 30-35° C for 72 hours. Bacteria produced were counted.
Total fungal count

One millilitre of NKM and 15 millilitres of antibiotics was added in a petri dish and incubated at 20-25°C for 120 hrs. Fungi count produced were counted.

E. coli

A small amount of the homogenized lactose broth was prepared and 1 ml of NKM solution in 100 ml of Mac Conkey broth was added and incubated at 43-45°C for 1 ½ to 2 days. (18-24 hours). The presence of E.Coli was observed.

Pseudomonas aeruginosa

The NKM solution was mixed with buffered sodium chloride peptone solution and inoculated with 100 ml of medium incubated at 35-37°C for 1-2 days. The appearance greenish fluorescence colour indicates the occurrence of Pseudomonas aeruginosa.

Staphylococcus aureus

A subculture with Baired-Parker agar was prepared and 1 ml of NKM solution was added and incubated at 35-37°C for 24 - 48 hours. The presence of S. Aureus was observed.

c. Pesticide residues

The pesticide residues in *Naaval Kottai Mathirai* has been analysed in Bureau Veritas Consumer Products Services (I) Pvt. Ltd, Chennai-32 by the testing method of AOAC 2007.01 by GC MS MS /LC MS MS.

d. Aflatoxin

The Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Aflatoxin G2 in *Naaval Kottai Mathirai* has been analysed in Bureau Veritas Consumer Products Services (I) Pvt. Ltd, Chennai-32 by the testing method of AOAC 2008.02.

5.4. Toxicity studies

Safety studies of *Naaval Kottai Mathirai* in animal model

The toxicity studies of *Naaval Kottai Mathirai* (NKM) in animal model were conducted after obtaining prior approval (1248/AC/09/CPCSEA-9/Dec2013/17) for animal studies from CPCSEA, Government of India through the Institutional Animal Ethics Committee (IAEC) of National Institute of *Siddha*, Chennai and conducted at animal house, National Institute of *Siddha*, Chennai, Tamil Nadu, India.
**Experiment animals**

Species/Strain : Albino rat / Wistar

Sex : Male and Female, Female rats were nulliparous and non-pregnant

Age : 8-12 weeks

Weight : 140±20 g

Source of procurement : Tamilnadu Veterinary and Animal Sciences University, Madhavaram, Chennai.

The animals were maintained in the animal house of National Institute of Siddha, Chennai following the guidelines for care and use of animals in scientific research drafted by Indian National Science Academy, New Delhi, India.

**Laboratory condition maintained**

Room temperature : 22±2°C

Relative humidity : 40 – 65%

Ventilation : Air cycles: 15/min; 70:30 Exchange ratio

Illumination : By Fluorescent Lamp 60 Lumens/Watt (325 Lux)

Photoperiod : 12-h light/dark cycle by time controlled lighting system

Noise control : Constructed with Concrete walls

**Husbandry**

Housing : Same sex of three animals were housed in polypropylene cages

Bedding : With husk

Feed : Amruth Rodent pellet, Pranav Agro Industries Ltd, Sangli, Maharastra, India

Water : Purified water by Reverse Osmosis procedure was supplied *ad libitum* by Rodent water feeder.
Identification

Cage : Cage card was tagged in each cage and indicated with animal numbers, markings and sex.

Animal : Each animal has marked with picric acid on the fur for identification (Head, Neck, Body and Base of tail) and it was indicated in cage card along with number.

Test sample

*Naaval Kottai Mathirai*

5.4. 1. Acute oral toxicity study *(83, 84)*

a. Methodology

The acute oral toxicity test was performed following 423 guidelines of Organization for Economic Co-operation and Development (OECD) for testing of chemicals.

Procedure

Selection of Animals: Six female Wistar rats were randomly selected and acclimatized for one week prior to the study. The rats were fastened overnight before the administration of test drug.

Dose calculation

The body weight of each fasted rat was weighed and an individual dose of test drug NKM was calculated according to the initial body weight.

Dosing

The acute toxicity study was done at the starting dose of 2000 mg/kg body weight of a rat as per Annexure 2d of OECD guidelines. For the first step, 2000 mg/kg NKM was suspended in 2 mL vehicle and administered to three animals using oral gavage as single time on 0 day. After single time administration of NKM, rats were deprived from feed and water for 4 h. The three animals were observed for mortality and abnormal clinical signs periodically for 14 days. Since there was no mortality and abnormal signs in the first step, further three animals were administered with NKM at the same dosage of 2000 mg/kg as single time for second step
and observed for mortality and clinical signs of toxicity. The Median Lethal dose (LD<sub>50</sub> cut-off value) for NKM was determined in agreement with Globally Harmonized System of Classification and Labelling of Chemicals cited in Annex 2d of OECD Guidelines 423.

**b. Observations**

**Body weight**

Each animal was weighed and recorded prior to the administration of test drug (On 0 day) and again on 7<sup>th</sup> and 14<sup>th</sup> day.

**Cage Side observations**

After administration of NKM, all animals were observed for mortality and clinical signs of toxicity and behavioural changes at 30 min, 1, 2 and 4 hours and thereafter once a day for the next 14 days. The observations include general behaviour, cardiovascular signs, respiratory pattern, reflexes, motor activities and changes in skin and fur texture, changes in eyes and mucous membrane of oral cavity, salivation, lethargy, sleep, coma, convulsion, tremors, diarrhoea, morbidity and mortality.

**Necropsy**

The animals which were died were planned for gross necropsies. The organs present in the thoracic and abdominal cavities were panned for examination.

**5.4.2. 28 days repeated oral toxicity**<sup>(85)</sup>

**a. Methodology**

A 28-day repeated oral toxicity study was performed according to the OECD guideline - 407 with minor modifications in dosage levels of test drug.

**Selection of animals**

Twenty male and twenty female Wistar rats were randomly selected and acclimatized for 7 days prior to the conduction of study. At the end of acclimatization period, animals were examined for good health condition.

**Dose calculation of test drug**

In the literature of Siddha, 500 mg of NKM as two times a day was recommended as therapeutic dosage for adult human. For the study, the dose of NKM in rat was estimated by
conversion of dose of NKM in 70 kg human on the basis of relative body surface area. The therapeutic dose in rat was calculated as follows:

200 g rat dose = Daily therapeutic dose in Human x Surface area conversion factor

\[ = 1000 \text{ mg} \times 0.018 = 18 \text{ mg} \]

1000 g rat dose = 18 mg x 5 = 90 mg

In the present study, NKM was administered at three dose levels as below

Low dose: The therapeutic dose of NKM was fixed – 90 mg/kg (x)

Intermittent dose: 5 times the therapeutic dose of NKM was fixed – 450 mg/kg (5x)

High dose: 10 times the therapeutic dose of NKM was fixed – 900 mg/kg (10x)

**Experimental design**

Number of groups: 4

Number of animals in each group: 5 Male and 5 Female

Treatment period: 28 days

Frequency: Single time per day. Daily at morning time

Route: Oral

**Table: 5.4.1 - Grouping and treatment details for 28 days repeated oral toxicity study**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I – Control</td>
<td>Vehicle – distilled water 1mL</td>
</tr>
<tr>
<td>II – Test group at low dose level</td>
<td>NKM at 90 mg/kg b. wt suspended in 2 mL vehicle</td>
</tr>
<tr>
<td>III – Test group at intermittent dose level</td>
<td>NKM at 450 mg/kg b. wt suspended in 2 mL vehicle</td>
</tr>
<tr>
<td>IV – Test group at high dose level</td>
<td>NKM at 900 mg/kg b. wt suspended in 2 mL vehicle</td>
</tr>
</tbody>
</table>
b. Observations

The experimental animals in all groups were observed throughout the course of period of 28 days treatment of drug.

Body weight

Each animal was weighed and recorded on 0 day, at weekly intervals through-out the course of study period and at the sacrifice day. Mean body weight and percentage of body weight gain were calculated for each group of both sexes.

Food consumption

The quantity of food consumed by the animals in each cage was recorded at weekly intervals. The mean value of food consumption was calculated for each group of both sexes.

Clinical signs

After one hour of treatment on each day throughout the study course, all animals were observed for signs of toxicity. The clinical signs were examined at the same time in each day. If any clinical signs were observed, the time of onset, intensity and duration were recorded. The observations include general behaviour, respiratory pattern, cardiovascular signs, motor activities, reflexes and changes in skin and fur texture, changes in eyes and mucous membrane of oral cavity, salivation, lethargy, sleep, coma, convulsion, tremors and diarrhoea.

Mortality

All the experimental animals were observed for mortality and morbidity twice daily during the entire course of study.

Ophthalmologic Examination

The eyes of each animal of group I (Control) and IV (High dose) were examined on 29th day. After induction of mydriasis with 0.5% Tropicamide solution, examination was carried out using a hand slit lamp.

Urine Analysis

Animals of control, high dose groups were kept in metabolic cages in last 7 days of observation. 1 mL of urine sample was collected without faecal contamination from each
animal and used for urine analysis. The following parameters were analyzed using routine appropriate methodology.

- Colour
- Transparency
- Specific gravity
- pH
- Presence of Protein, Glucose, Bilirubin, Ketones, Blood and Urobilinogen

c. Terminal studies

Euthanasia

Animals of control, low, intermittent and high dose groups were fasted over-night at the end of 28th day of treatment and observation period. On 29th day animals were sacrificed under CO₂ inhalation.

Blood collection

After scarification, immediately a volume of 5 mL blood was collected from each animal by a sterile disposable syringe through cardiac puncture. 2 mL of blood was transferred into a tube containing anticoagulant Potassium EDTA (1.5 mg/mL) and used for haematological investigations. 3 mL of blood was transferred into a tube without anticoagulant and used for biochemical investigations.

Haematological Investigations

The following haematological parameters were analysed using Erba Mannhein® haematology analyser.

- Haemoglobin (Hb)
- Red Blood Cell count (RBC)
- Red cell Distribution Width (RDW)
- White Blood Cell count (WBC)
Differential count - Lymphocyte, Granulocyte and Monocyte

Haematocrit (HCT)

Mean Corpuscular Volume (MCV)

Mean Corpuscular Haemoglobin (MCH)

Mean Corpuscular Hemoglobin Concentration (MCHC)

Platelet count

Platelet Crit (PCT)

Platelet Distribution Width (PDW)

Mean Platelet Volume (MPV)

The reticulocyte count was estimated microscopically.

**Biochemical Investigations**

The following biochemical parameters were analysed using Erba system Pack kits in Fully Automated Biochemistry analyzer.

Glucose

Cholesterol

Triglyceride (TGL)

Protein

Urea

Creatinine

Bilirubin

Serum glutamic-oxaloacetic transaminase (SGOT)

Serum glutamic pyruvic transaminase (SGPT)

Alkaline Phosphatase (ALP)
**Necropsy study**

After blood collection, body weights of all animals were recorded. All rats were dissected for gross necropsy study. Cranial, thoracic and abdominal cavities were opened and viscera’s were dissected out. Organs such as brain, trachea, lungs, heart, liver, kidney, stomach, spleen, intestine, testis, uterus and ovaries were studied for gross study by viewed under magnification glass to find the presence of macroscopic pathological lesion. Each organ was weighed and expressed in terms of absolute organ weight. With respect to body weight, relative organ weight was calculated by the below formula.

\[
\text{% Relative organ weight} = \left[ \frac{\text{Absolute organ weight}}{\text{Body weight}} \right] \times 100
\]

**Histo-pathological study**<sup>(86)</sup>

**Collection of organs**

Since no abnormalities found during necropsy study, the organs of one animal showing high value of blood renal and hepatic parameters among the animals from control, high dose were subjected to histo-pathological studies. Organs such as brain, liver, kidney, lungs, stomach, heart, spleen, and femorotibial joints were collected and placed in 10 % Formalin. 10 % Formalin was prepared by mixing 100 mL of 40 % Formaldehyde in 900 mL of 0.9 % Normal saline. Further below procedures were carried out in Liveon Biolabs Pvt. Ltd. Tumakuru, Karnataka.

**Collection of tissues**

Thin pieces of 3 – 5 mm thickness of tissues were cut from the organs collected.

**Fixation**

The collected tissues were kept in 10 % Formalin at room temperature for 48 h to harden the tissues by coagulating the cell protein, to prevent the structure and to prevent the shrinkages. The volume of formalin added was 10 times the volume of the tissues.

**Hydration**

After fixation, the tissues were washed completely in running water.
Dehydration

The tissues were dehydrated by passing it in ascending grades of alcohol as below to prevent undue shrinkage of tissues.

- Ethyl alcohol 50 % for 8 h
- Ethyl alcohol 70 % for 2 h
- Ethyl alcohol 90 % for 2 h
- Absolute alcohol – I for 1 h
- Absolute alcohol – II for 1 h

Clearing

After dehydration, the tissues were cleared from alcohol by keeping in Xylol – I and II each with 30 min.

Infiltration

The tissues were completely impregnated with Paraffin wax (Melting point 50 - 56°C) kept in the cups and melted in a paraffin oven. The tissues were kept for 30 min in each cup.

Embedding

Two L shaped moulds were arranged in the form of a rectangle over a porcelain slab. The melted paraffin was poured into the mould and the tissue was so oriented that the cutting surface of the tissue faces the porcelain slab. The moulds were removed as soon as paraffin sets and the blocks were sectioned.

Sectioning

The blocks were trimmed by removing the excess paraffin all around. Sections of 4 – 5 µm thickness were cut in a Rotary microtome. The sections were transferred from the cutting edge of the microtome knife with the help of spatula to a tissue floatation bath having warm water (40 - 45°C). Then the sections were spread out uniformly and were taken on the clean glass slides coated with Meyer’s albumin – glycerine mixture.
Staining of sections

Haematoxylin and eosin method of staining (H & E) was employed.

H & E staining procedure

1. Deparaffinised the sections by passing in Xylol for 5 to 10 min.
2. Removed the Xylol by passing in Absolute alcohol.
3. Washed in tap water.
4. Stained with haematoxylin for 3 to 4 min.
5. Washed in tap water.
6. Allowed the sections in acid alcohol for 15 to 30 sec.
7. Washed in tap water for 5 to 10 min.
8. Counterstained with 0.5 % Eosin until the sections appeared light pink (15-30 sec).
9. Washed in tap water.
10. Dehydrated in alcohol.
11. Cleared with Xylol for 15 to 30 sec.
12. Mounted in DPX mountant
13. Slides were dried and cover slipped without the presence of air bubble.
14. Examined under a light microscope and microscopic features were observed.

Statistical analysis

All data were expressed as mean ± standard error of mean (SEM). The test groups were compared with control for testing significance by one way ANOVA followed by Dunnet test using GRAPH PAD INSTAT version 3 software programmes. P values less than 0.05 were considered significant.

5.4.3. 90 days repeated oral toxicity (87)

a. Methodology

90-days repeated oral toxicity study was performed according to the OECD guideline - 408 with minor modifications in dosage levels of test drug.
Selection of animals

Forty male and forty female Wistar rats were randomly selected and acclimatized for 7 days prior to the conduction of study. At the end of acclimatization period, animals were examined for good health condition.

Experimental design

Number of groups: 4

Number of animals in each group: 10 Male and 10 Female

Treatment period: 90 days

Frequency: Single time per day. Daily at morning time

Route: Oral

Table:5.4.2 - Grouping and treatment details for 90 days repeated oral toxicity study

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I – Control</td>
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<tr>
<td>IV – Test group at high dose level</td>
<td>NKM at 900 mg/kg b. wt suspended in 2 mL vehicle</td>
</tr>
</tbody>
</table>

b. Observations

The experimental animals in all groups were observed throughout the course of period of 90 days treatment of drug. The observations have been done as per 28 days toxicity study. Food intake of the animals, body weight, clinical signs were observed as per the guidelines. At the end of 90 days toxicity study, Euthanasia, Blood collection, Haemotological Investigations, Biochemical Investigations, Necropsy study and Histo-pathological study were done by the methodologies followed in 28 days toxicity study.
5.5. In-vitro studies:

5.5.1. Anti-bacterial Activity (88)

The anti-bacterial activity of the sample NKM was carried out by disc diffusion method. The concentrations of the test compounds were used at the concentration of 100, 200, 300µg. The target microorganisms were cultured in Muller-Hinton broth (MHB). After 24 h the suspensions were adjusted to standard sub culture dilution. Then the diluted bacterial stains were cultured in petri dishes containing Muller-Hinton Agar (MHA) medium. Disc made of Whatman No.1, diameter 6mm was pre- sterilized and was maintained in aseptic chamber. Each concentration was injected to the sterile disc papers. Then the prepared discs were placed on the culture medium. Standard drug streptomycin (20 µg) was used as a positive reference standard to determine the sensitivity of each microbial species tested. Then the inoculated plates were incubated for 24h at 37°C. The diameter of the clear zone around the disc was measured and expressed millimetres as its anti-microbial property.

5.5.2. Determination of Cell viability by MTT Assay: (89) (Francis and Rita 1986).

Procedure:

The monolayer cell culture was trypsinized and DMEM containing 10 % FBS was used for cell count at 1.0 × 10^5 cells/ml. In a 96 well microtitre plate, diluted cell suspension of 0.1 ml was added, a partial monolayer was formed after 24 hours then the supernatant was removed. The monolayer was washed once with the medium and different concentrations of test drug were added at 100µl to the partial monolayer in microtitre plates. Then the plates were incubated for 3 days at 37°C in 5 % CO₂ atmosphere. The microscopic examinations were done and observations were readed at 24 hours. The test drug was discarded from the wells then MTT in FBS (Fetal Bovine Serum) of 50 µl was added to each well. The plates were shaken gently and incubated at 37°C for 3 hours in 5 % CO₂ atmosphere. The supernatant was replaced and propanal was added at 100µl. Then the plates were shaken gently to solubilise the formazan. A microplate reader was used to measure the absorbance wavelength of 540 nm. The following formula was used to calculate the percentage growth inhibition and the dose response curves were generated to determine the concentration of test drug required to inhibit the cell growth by 50 % (CTC50).
Mean OD of individual test group

Percentage growth inhibition = 100 - \frac{\text{Mean OD of control group}}{\text{Mean OD of test group}} \times 100

Mean OD of control group

**In-vitro Glucose uptake assay:** (90, 91) (Hisako et al.; 2003; Angeline et al.; 2007)

Differentiated L6 cells were used to determine the Glucose uptake activity in test substance. The 24 hr cell cultures were done in 40mm petri plates with 70 - 80 % confluency. Then they were maintained in DMEM with FBS 2 % to differentiate the cells for 4 - 6 days. The extent of differentiation was analysed by observing multinucleation of cell. The over night serum starved cells were once washed with HEPES buffered KRP buffer (Krebs Ringer Phosphate solution) at the time of the experiment. Then incubated at 37°C for 30 min with different concentration of non - toxic test and standard drugs at 37°C along with negative controls.

D- glucose solution was simultaneously added at 20 µl to each well and incubated for 30 min at 37°C. After incubation, glucose uptake was stopped by aspiration of solution and washing the well thrice with ice cold KRP buffer solution. 0.1µl NaOH solution was used for lysis of cell and the cell associated glucose was measured by aliquot of cell lysates.

ERBA - Glucose assay kit was used to measure the glucose level in cell lysates. The percentage of glucose uptake was determined by taking 2 independent experiment values in duplicates.

**5.5.3. In-Vitro α- Glucosidase Enzyme Inhibition Study**

The test and standard sample of about 5µl each was added to 20mM p-nitro phenyl α-D glucopyranoside and 100 mM phosphate buffer (PH-7.0) of about 250 µl and 495µl respectively. It was pre incubated for 5 mins at 37°C. Then the reaction was started by addition of about 250 µl of α- glucosidase enzyme solution by α-glucosidase 0.5mg in 10 ml phosphate buffer (PH-7.0) containing bovine serum albumin 20 mg, after that it was incubated for exactly 15 min at 37°C. Instead of enzyme phosphate buffer of about 250µl was added to blank. Then the reaction was stopped by the addition of 200 mM Na2 CO3 solution 1000µl. By using UV visible spectrophotometer, the amount of p- nitrophenol released was ascertained by reading the absorbance of sample drug against a blank containing PBS with no sample.
5.5.4. *In- vitro* Alpha Amylase Inhibition Study \(^{(93)}\)

The test and standard sample (acarbose) of about 600µl were added to \(\alpha\)- amylase enzyme solution of 30 µl and incubated for 15 min at 37°C. To this reaction mixture, a substrate of about 370µl of CNPG\(_3\) (2-chloro-4-Nitrophenyl- \(\alpha\)-Maltotrioside) was added and incubated for 10 min at 37°C. Finally, by using the spectrophotometer absorbance was measured against blank at 405 nm. A control reaction was performed without the test sample and the percentage inhibition was calculated.

5.5.5. *In-Vitro* Anti-oxidant assay

a. DPPH (2,2- Diphenyl 1-2 picrylhydrazyl) Assay \(^{(94)}\)

DPPH free radical scavenging assay was used to determine the antioxidant activity of the test drug NKM. The stock solution was prepared in required concentration (100µg/ml or 10mg/100ml) by mixing the extract of sample with 95% methanol. 1ml, 2ml, 4ml, 6ml, 8ml, 10ml of solution was taken from the stock solution in 5 test tubes. Then the serial dilution were made with the same solvent and the final volume of each test tube was 10ml whose concentration was then 10 µg/ml, 20 µg/ml, 40 µg/ml,60 µg/ml,80 µg/ml,100 µg/ml respectively. Ascorbic acid was used as standard and it was prepared in same concentration by using methanol as solvent as that of the sample extract. To the different concentration of test sample solution of about 2.5 ml the final reaction mixture containing 1ml of 0.3mM DPPH methanol solution was added and allowed to react at room temperature. After 15 min of incubation at 37°C absorbance at different concentration of the sample (10 µg/ml, 20 µg/ml, 40 µg/ml,60 µg/ml,80 µg/ml,100 µg/ml) was noted. Absorbance was measured by double beam UV Spectrophotometer using methanol as blank.

\[
\frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100
\]

% scavenging =

b. Hydrogen Peroxide Scavenging Assay (H\(_2\) O\(_2\))

H\(_2\) O\(_2\) is decomposed into water and oxygen rapidly and this produce OH\(^-\) radicals that initiate lipid per oxidation which results in DNA damage in the body. The ability of test drug to scavenge H\(_2\) O\(_2\) can be estimated by H\(_2\) O\(_2\) scavenging assay. A solution of about
40mM hydrogen peroxide is prepared in 50 mm phosphate buffer at PH 7.4. The Spectrophotometer is used to determine the concentration of hydrogen peroxide at 230nm by absorption. The extract of sample drug NKM prepared in different concentrations is added to H₂O₂ and absorbance is determined after 10 min against a blank solution (phosphate buffer without hydrogen peroxide) at 230nm.

c. Nitric oxide Radical Scavenging Assay (95)

Serial dilutions were done at different concentration of the test sample from 10-100µg/ml and the standard gallic acid. Griess reagent was prepared immediately before use by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid. A volume about 0.5 ml of sodium nitroprusside in phosphate buffered saline was added and mixed with 1mL of the sample extract at different concentrations (10-100µg/ml) and incubated for 180 mins at 25°C. Freshly prepared Griess reagent with equal volume was added to the plant extract. Control samples were prepared with an equal volume of buffer without the extracts in similar way as done for the test drug samples. The absorbance was measured by using a Spectra Max Plus UV- Vis microplate reader (Molecular Devices, GA,USA) at 546nm. Gallic acid was used as the positive control. The percentage inhibition of the test drug NKM and standard was calculated and recorded.

d. ABTS Assay (96)

ABTS assay was carried out for the purpose of evaluating the anti–oxidant potential of test drug NKM against ABTS radicals or 2, 2’-azino-bis (3- ethylbenzothiazoline-6- sulphonic acid). To evaluate the free radical scavenging effect of 100 pure chemical compounds the ABTS radical method was modified. The ABTS reagent was prepared by mixing 88µL of 140mM Potassium persulfate with 5mL 7mM ABTS. Then the mixture was kept in the dark at room temperature. At that time the free radicals were generated which was then diluted with 1:44, v/v of water. The scavenging activity was determined by mixing 100µL of ABTS reagent with 100µL of test drug sample at different concentrations (10-100µg/ml) and incubated at room temperature. After 6 minutes of incubation, the absorbance was measured at 734 nm. 100 percentage methanol was used as control. Gallic acid was used as positive control and it was measured by similar manner as done for the test drug.
5.5.6. Pharmacological Study

Anti-diabetic activity

Animals

Male wistar albino rats of weight between 180-220gm were procured from the central animal facility of the institution. The animals were housed in large spacious standard polypropyle cage having 3 rats /cage and acclimatized at controlled room temperature of about 22±2°C and humidity 55±5% with 12 hour dark and 12 hour light cycle. The animals were fed with commercially available normal pellet diet and water ad libitum, prior to the dietary manipulation. CPCSEA guidelines were followed and prior permission was sought from the institutional animal ethical committee for conducting the study.

Development of HFD fed and STZ induced diabetic rats

The rats were divided into two groups (36 and 54 rats), one group provided with normal pellet diet and the other with High fat diet (fat-58%, protein-25%,Carbohydrate 17%, as a percentage of total k.cal) ad libitum for first 2 weeks.

After 2 weeks of dietary manipulation, a sub group of rats (12 and 30) were taken. Among these 30 rats were fasted for 24 hours and injected with low dose streptozotocin 25mg/kg intraperitonelly dissolved in physiological saline. The animals were allowed to drink glucose solution overnight to overcome the hypoglycemia induced by the drug. The control rats (12rats) received the vehicle alone. All the animals in the STZ induced sub group (30 rats) were screened for diabetes after 1 week and the animals with blood glucose level above 250mg/dl and glycosuria were considered diabetic and selected for the further pharmacological study.
Experimental Design

The wistar albino rats were divided into 6 groups of 6 rats in each group.

Group I : Normal control administered with 10ml/kg of normal saline orally;

Group II : STZ (Streptozotocin) induced diabetic rats as toxic control;

Group III : Diabetic rats administered with glipizide at a dose of 10 mg/kg orally;

Group IV : Diabetic rats control administered with NKM (200mg/kg) orally for 28 days.

Group V : Diabetic control rats administered with NKM (400mg/kg) orally for 28 days.

Biochemical Analysis

Plasma insulin was determined by ELISA method using a Boehringer–Mannheim kit with an ES300 Boehringer analyzer (Mannheim, Germany). After 28 days of treatment, the animals were fasted for 16 hours and sacrificed by cervical decapitation and blood was collected from the eyes (venous pool) by sino-ocular puncture in EDTA coated plasma tubes for the estimation of blood parameters such as blood glucose, haemoglobin, glycosylated haemoglobin (HbA1C), total cholesterol, triglycerides, HDL-cholesterol and phospholipids and glycogen content and plasma insulin were determined. For the estimation of enzymes, a portion of liver tissue was dissected and washed immediately with ice cold saline, kept at 4°C. The liver tissues were homogenized twice with (0.01 cysteine and 1mM EDTA in 0.1 ml Tris-HCL, pH 7.4) and the supernant was analysed for Hepatic glucokinase and hexokinase. Glucose-6-phosphatase activity was measured by phosphate release by the method Marjorie. Glycogen content was estimated calorimetrically. (Morales et al., 1973).

Statistical analysis

The data for various biochemical parameters were analyzed using ANOVA (analysis of variance), and the group means were compared by Newman-Keul’s multiple range test (NKMRT). Values were considered statistically significant at p<0.01.
6.1 Qualitative analysis of *Naaval Kottai Mathirai*

**a. Siddha classical Method**

The *Naaval Kottai Mathirai* has the basic qualities of a *mathirai* (tablet).
- The karkkam (grounded material) is in waxy consistency and is not sticky in nature
- The surface of whole tablet is soft and uniform
- The colour of the tablet at any part is uniform
- It has no cracks on its surface after drying

**b. Organoleptic characters of Naaval Kottai Mathirai**

The following Organoleptic characters were performed in all the three samples of NKM (I, II and III) and the results were tabulated in table number 6.1.1.

*Table: 6.1.1 - Organoleptic characters of Naaval Kottai Mathirai*

<table>
<thead>
<tr>
<th>Parameter</th>
<th><em>Naaval Kottai Mathirai</em> (Sample -I)</th>
<th><em>Naaval Kottai Mathirai</em> (Sample -II)</th>
<th><em>Naaval Kottai Mathirai</em> (Sample -III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Dark grey</td>
<td>Dark grey</td>
<td>Dark grey</td>
</tr>
<tr>
<td>Taste</td>
<td>Slightly astringent</td>
<td>Slightly astringent</td>
<td>Slightly astringent</td>
</tr>
<tr>
<td>Odour</td>
<td>Slightly pungent</td>
<td>Slightly pungent</td>
<td>Slightly pungent</td>
</tr>
</tbody>
</table>

**c. Physico- chemical Parameters of Naaval Kottai Mathirai**

The following Physico-chemical analysis were performed in all the three samples of NKM (I, II and III) and the results were tabulated in table number 6.1.2.
Table: 6.1.2 - Physico-chemical Parameters of Naaval Kottai Mathirai

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NKM -I</th>
<th>NKM -II</th>
<th>NKM -III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss on drying at 105°C</td>
<td>8.28 %</td>
<td>8.33 %</td>
<td>8.49 %</td>
</tr>
<tr>
<td>Total Ash Value</td>
<td>8.32 %</td>
<td>8.17 %</td>
<td>8.20 %</td>
</tr>
<tr>
<td>Acid Insoluble ash</td>
<td>1.26 %</td>
<td>1.23 %</td>
<td>1.24 %</td>
</tr>
<tr>
<td>Alcohol soluble extractive value</td>
<td>3.63 %</td>
<td>3.89 %</td>
<td>3.87 %</td>
</tr>
<tr>
<td>Water soluble extract</td>
<td>16.04 %</td>
<td>17.24 %</td>
<td>16.87 %</td>
</tr>
<tr>
<td>Bulk density (gm/ml)</td>
<td>1.61</td>
<td>1.79</td>
<td>1.78</td>
</tr>
</tbody>
</table>

d. Preliminary Phytochemical Analysis of Naaval Kottai Mathirai

The Phytochemical analysis was done for all the three samples of NKM (I, II and III) and the results were tabulated in table number 6.1.3.

Table: 6.1.3 - Preliminary Phytochemical Analysis of Naaval Kottai Mathirai

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NKM – I</th>
<th>NKM -II</th>
<th>NKM -III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Saponins</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Tannins</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Steroids</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Quinones</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Amino acids</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>
6.2 Quantitative analysis of *Naaval Kottai Mathirai*

a. Analysis of Microbial Load:

The microbial load has been analysed in the three samples of NKM (I, II and III) and the results were tabulated in table number 6.2.1.

**Table: 6.2.1 - Microbial Load analysis of *Naaval Kottai Mathirai***

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NKM- I</th>
<th>NKM -II</th>
<th>NKM – III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Bacterial Count</td>
<td>$3 \times 10^2$ cfu/ml</td>
<td>$1 \times 10^2$ cfu/ml</td>
<td>$3 \times 10^2$ cfu/ml</td>
</tr>
<tr>
<td>Total Fungal Count</td>
<td>Less than 10 cfu/g</td>
<td>$2 \times 10^2$ cfu/ml</td>
<td>Less than 10 cfu/g</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>$2 \times 10^2$ cfu/ml</td>
<td>$2 \times 10^2$ cfu/ml</td>
<td>$2 \times 10^2$ cfu/ml</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>$2 \times 10^2$ cfu/ml</td>
<td>$2 \times 10^2$ cfu/ml</td>
<td>$2 \times 10^2$ cfu/ml</td>
</tr>
<tr>
<td>Salmonella Spp</td>
<td>$2 \times 10^2$ cfu/ml</td>
<td>$2 \times 10^2$ cfu/ml</td>
<td>$2 \times 10^2$ cfu/ml</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>$2 \times 10^2$ cfu/ml</td>
<td>$2 \times 10^2$ cfu/ml</td>
<td>$2 \times 10^2$ cfu/ml</td>
</tr>
</tbody>
</table>

b. Heavy Metals Analysis

The heavy metals analysis has been done in the three samples of NKM (I, II and III) and the results were tabulated in table number 6.2.2.

**Table: 6.2.2 - Heavy metal analysis of *Naaval Kottai Mathirai***

<table>
<thead>
<tr>
<th>Name of the Element</th>
<th>NKM – I</th>
<th>NKM – II</th>
<th>NKM – II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead</td>
<td>0.0025 ppm</td>
<td>0.0012 ppm</td>
<td>0.0018 ppm</td>
</tr>
<tr>
<td>Cadmium</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
<tr>
<td>Arsenic</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
<tr>
<td>Mercury</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
</tbody>
</table>

c. Pesticide residues

The pesticide residues were analysed for the three samples of NKM (I, II and III) and the results were tabulated in table number 6.2.3.
Table: 6.2.3- Pesticide residual analysis of *Naaval Kottai Mathirai*

<table>
<thead>
<tr>
<th>S.no</th>
<th>Test Parameters</th>
<th>Results</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NKM I</td>
<td>NKM II</td>
</tr>
<tr>
<td>1</td>
<td>Aldrin</td>
<td>BLQ (LOQ-0.01)</td>
<td>BLQ (LOQ-0.01)</td>
</tr>
<tr>
<td>2</td>
<td>Dieldrin</td>
<td>BLQ (LOQ-0.01)</td>
<td>BLQ (LOQ-0.01)</td>
</tr>
<tr>
<td>3</td>
<td>Chlordane (cis &amp; trans)</td>
<td>BLQ (LOQ-0.01)</td>
<td>BLQ (LOQ-0.01)</td>
</tr>
<tr>
<td>4</td>
<td>Chlorothalonil</td>
<td>BLQ (LOQ-0.01)</td>
<td>BLQ (LOQ-0.01)</td>
</tr>
<tr>
<td>5</td>
<td>DDT (all isomers, sum of p,p'-DDT, o,p'-DDE and p,p'-TDE (DDD) expressed as DDT)</td>
<td>BLQ (LOQ-0.01)</td>
<td>BLQ (LOQ-0.01)</td>
</tr>
<tr>
<td>6</td>
<td>Dicofol</td>
<td>BLQ (LOQ-0.01)</td>
<td>BLQ (LOQ-0.01)</td>
</tr>
<tr>
<td>7</td>
<td>Endosulphan (All isomers)</td>
<td>BLQ (LOQ-0.01)</td>
<td>BLQ (LOQ-0.01)</td>
</tr>
<tr>
<td>8</td>
<td>Endrin</td>
<td>BLQ (LOQ-0.01)</td>
<td>BLQ (LOQ-0.01)</td>
</tr>
<tr>
<td>9</td>
<td>HCH (alpha &amp; beta)</td>
<td>BLQ (LOQ-0.01)</td>
<td>BLQ (LOQ-0.01)</td>
</tr>
<tr>
<td>10</td>
<td>Heptachlor</td>
<td>BLQ (LOQ-0.01)</td>
<td>BLQ (LOQ-0.01)</td>
</tr>
<tr>
<td>11</td>
<td>Lindane</td>
<td>BLQ (LOQ-0.01)</td>
<td>BLQ (LOQ-0.01)</td>
</tr>
<tr>
<td>12</td>
<td>4-bromo-2-chlorophenol</td>
<td>BLQ (LOQ-0.01)</td>
<td>BLQ (LOQ-0.01)</td>
</tr>
<tr>
<td>13</td>
<td>Acephate</td>
<td>BLQ (LOQ-0.01)</td>
<td>BLQ (LOQ-0.01)</td>
</tr>
<tr>
<td>14</td>
<td>Chlorfenvinphos</td>
<td>BLQ (LOQ-0.01)</td>
<td>BLQ (LOQ-0.01)</td>
</tr>
<tr>
<td></td>
<td>Chemical Name</td>
<td>Detection Level</td>
<td>Detection Level</td>
</tr>
<tr>
<td>---</td>
<td>-----------------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>15</td>
<td>Chlorpyrifos</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>16</td>
<td>Chlorpyriphos-ethyl</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>17</td>
<td>Chlorpyriphos-methyl</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>18</td>
<td>Diazinon</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>19</td>
<td>Dichlorvos</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>20</td>
<td>Dimethoate</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>21</td>
<td>Omethoate</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>22</td>
<td>Ethion</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>23</td>
<td>Etrimphos</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>24</td>
<td>Fenitrothion</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>25</td>
<td>Edifenphos</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>26</td>
<td>Fenthion</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>27</td>
<td>Iprobenphos</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>28</td>
<td>Malathion</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>29</td>
<td>Methamidophos</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>30</td>
<td>Monocrotophos</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>31</td>
<td>Oxydemeton-methyl</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BLQ ( LOQ- 0.01)</td>
<td>BLQ ( LOQ- 0.01)</td>
</tr>
<tr>
<td>---</td>
<td>----------------</td>
<td>------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>32</td>
<td>Parathion ethyl</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>33</td>
<td>Parathion methyl</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>34</td>
<td>Phorate</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>35</td>
<td>Phosalone</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>36</td>
<td>Phosphamidon</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>37</td>
<td>Profenophos</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>38</td>
<td>Primiphos-methyl</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>39</td>
<td>Propetamphos</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>40</td>
<td>Quinalphos</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>41</td>
<td>Temephos</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>42</td>
<td>Thiometon</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>43</td>
<td>Triazophos</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>44</td>
<td>Allethrin&amp;Bioallethrin</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>45</td>
<td>Bifenthrin</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>46</td>
<td>Cyfluthrin</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>47</td>
<td>Cypermethrin</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>48</td>
<td>Deltamethrin</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td></td>
<td>Chemical Name</td>
<td>BLQ (LoQ-0.01)</td>
<td>BLQ (LoQ-0.01)</td>
</tr>
<tr>
<td>---</td>
<td>-------------------------------------</td>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>49</td>
<td>Ethofenprox (Etofenprox)</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>50</td>
<td>Fenpropathrin</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>51</td>
<td>Fenvalerate &amp; Esfenvalerate (sum of RR&amp; SS isomers)</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>52</td>
<td>Fenvalerate &amp; Esfenvalerate (sum of RS&amp; SR isomers)</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>53</td>
<td>Lambda-cyhalothrin</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>54</td>
<td>Permethrin</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>55</td>
<td>Tau-Fluvalinate</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>56</td>
<td>Transfluthrin</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>57</td>
<td>Bendiocarb</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>58</td>
<td>Benfuracard</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>59</td>
<td>Benomyl</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>60</td>
<td>Carbaryl</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>61</td>
<td>Carbon di sulphide (Mancozeb, Maneb, Pro pineb, Metiram, Thiram and Ziram collectively estimated as CS2)</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>62</td>
<td>Carbosulfan</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>63</td>
<td>Diazometh</td>
<td>BLQ</td>
<td>BLQ</td>
</tr>
<tr>
<td>Sl.No</td>
<td>Test Parameters</td>
<td>Results</td>
<td>Units</td>
</tr>
<tr>
<td>-------</td>
<td>----------------------</td>
<td>--------------------------</td>
<td>-------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NKM I</td>
<td>NKMII</td>
</tr>
<tr>
<td>1</td>
<td>Aflatoxin B1</td>
<td>BLQ (LOQ:0.5)</td>
<td>BLQ (LOQ:0.5)</td>
</tr>
<tr>
<td>2</td>
<td>Aflatoxin B2</td>
<td>BLQ (LOQ:0.5)</td>
<td>BLQ (LOQ:0.5)</td>
</tr>
<tr>
<td>3</td>
<td>Aflatoxin G1</td>
<td>BLQ (LOQ:0.5)</td>
<td>BLQ (LOQ:0.5)</td>
</tr>
<tr>
<td>4</td>
<td>Aflatoxin G2</td>
<td>BLQ (LOQ:0.5)</td>
<td>BLQ (LOQ:0.5)</td>
</tr>
</tbody>
</table>

**d. Aflatoxin**

Aflatoxins B₁, B₂ and G₁ G₂ were analysed for the three samples of NKM (I, II and III) and the results were tabulated in table number 6.2.4.

Table: 6.2.4 - Aflatoxin analysis of *Naaval Kottai Mathirai*
e. TLC / HPTLC analysis of Naaval Kottai Mathirai

The HPTLC chromatogram peaks, Rf values and areas obtained for solvent extracts after scanning at UV 254 nm, 366 nm and after derivatization are depicted in respective figures and Table. Tracks 1 and 2 depict the sample Naaval Kottai Maathirai in increasing concentration i.e., 20µl and 25µl. The chromatograms of NKM at UV 254 and 366 nm revealed that, all samples constituents were clearly separated without any tailing and diffuseness. The fingerprints of two concentrations of NKM samples reveal obvious similarities. At UV 254 nm dark green weak zones were observed with different Rf values. At UV 366 nm presents a strong red zone followed by light red, red, blue and pink zone at different Rf values. In visible light after derivatization using Vanillin and Sulphuric acid, grey, light violet and violet zones were observed. The violet zones were in accordance with the Ayurvedic Pharmacopoeia of India (Part – I, Volume 2).

In the alcoholic extract of Naaval Kottai Maathirai, there are 7 peaks indicating the occurrence of at least 7 different components evident from the Table. Out of 7 components, the components with Rf values 0.28, 0.41, 0.45, 0.54, 0.60 and 0.70 were found to have area ranging between 244.1 and 1093.4. Moreover, the components with Rf values 0.45, 0.54, and 0.60 were found to be more predominant as the intensity of area was 733.4, 946.6, and 1093.4 AU respectively. The remaining components were found to be very less in quantity as the values of AU for all other peaks were less than 304.4. Comparatively, the densitometric results of the alcoholic extract of Naaval Kottai Maathirai made at UV 366 nm exhibited maximum of 6 peaks with Rf value ranging from 0.18 to 0.59 and the area ranging between 241.6 and 5978.8. 6 different peaks were observed at UV 366 nm with Rf values 0.18, 0.25, 0.39, 0.44, 0.54 and 0.59 with areas 858.0, 241.6, 849.5, 1499.0, 1935.8 and 5978.8 respectively. In the present study, HPTLC analysis revealed that, there were more number of peaks and corresponding area of components of the solvent extracts which were scanned at 366 nm than 254 nm. The appearance of peaks, Rf values and their areas provide corresponding chemical profiles for Naaval Kottai Maathirai.

The HPTLC analysis of Syzygium cumini using gallic acid as standard was studied by Itankar et al., in 2015, which indicated the presence of gallic acid at Rf value of 0.49. In our study, the alcoholic extract of Naaval Kottai Maathirai may indicate that the presence of gallic acid, Rf value of 0.47. The difference in Rf values in most of the appeared peaks reflect qualitative variation in the phytocompounds. It may be assumed that, the test formulation might have gallic acid alongside other secondary metabolites as well as due to the addition of
another ingredient *Aristolochia bracteolata*. Therefore, a comparative evaluation of peaks and Rf values with respective standard should be made to assess the identities of those compounds in the *Naaval Kottai Maathirai*.

Plate: 6.1.1 & 6.1.2 : TLC Photos of *Naaval Kottai Mathirai*
Plate: 6.1.3 HPTLC finger print *Naaval Kottai Mathirai* at UV-254 nm

Plate: 6.1.4 HPTLC finger print *Naaval Kottai Mathirai* at UV-366 nm
Plate: 6.1.5 HPTLC finger print *Naaval Kottai Mathirai* at UV-254 nm

Plate: 6.1.6 HPTLC finger print *Naaval Kottai Mathirai* at UV-366 nm
6.3. Toxicity Studies

a. Acute oral toxicity study

All animals were survived throughout the observation period of 14 days at the single dose of NKM at 2000 mg/kg and they were in good health and active. No behavioural changes or abnormal clinical signs of toxicity were observed. No gross pathological abnormality in the organs was found even at this high dose. LD50 value was found to be more than 2000 mg/kg body weight and therefore this test drug *Naaval Kottai Mathirai* falls under (Unclassified) category V with reference to Globally Harmonized classification System (GHS) (OECD, 2000).

Table 6.3.1: Allocation of Animal Identification code for acute toxicity study

<table>
<thead>
<tr>
<th>Step</th>
<th>Animal code</th>
</tr>
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<tbody>
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<td>NK/M/F1</td>
</tr>
<tr>
<td></td>
<td>NK/M/F2</td>
</tr>
<tr>
<td></td>
<td>NK/M/F3</td>
</tr>
<tr>
<td>II</td>
<td>NK/M/F4</td>
</tr>
<tr>
<td></td>
<td>NK/M/F5</td>
</tr>
<tr>
<td></td>
<td>NK/M/F6</td>
</tr>
</tbody>
</table>
Figure 6.3.1: Effect of *Naaval Kottai Mathirai* on body weight of Albino Wistar rats at single dose of 2000 mg/kg

Figure 6.3.2: Effect of *Naaval Kottai Mathirai* on body weight of Albino Wistar rats at single dose of 2000 mg/kg
Plate: 6.3.1. Necropsy findings of *NKM* on female control rat in acute toxicity study.

Plate: 6.3.2. Necropsy findings of *NKM* on female rat in acute toxicity study
Table 6.3.2: Cage side observation for the effect of *Naaval Kottai Mathirai* at 2000 mg/kg

<table>
<thead>
<tr>
<th>Observation</th>
<th>NKM/F1</th>
<th>NKM/F2</th>
<th>NKM/F3</th>
<th>NKM/F4</th>
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<tbody>
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<td>Respiration</td>
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<td>Motor activities</td>
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<td>Mortality</td>
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<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

N – Normal

b. 28 day - repeated oral toxicity study

**Randomization, Numbering and Grouping of Wistar Rats**

The rats allotted to different groups are noted in the table 6.3.1.

Table 6.3.3: Allocation of Animal Identification code for 28 day Repeated oral toxicity study

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I – Control</td>
<td>Vehicle – distilled water 1mL</td>
</tr>
<tr>
<td>II – Test group at low dose level</td>
<td>NKM at 90 mg/kg b. wt suspended in 2 mL vehicle</td>
</tr>
<tr>
<td>III – Test group at intermittent dose level</td>
<td>NKM at 450 mg/kg b. wt suspended in 2 mL vehicle</td>
</tr>
<tr>
<td>IV – Test group at high dose level</td>
<td>NKM at 900 mg/kg b. wt suspended in 2 mL vehicle</td>
</tr>
</tbody>
</table>
Body weight

All animals involved in the study gained comparable body weight throughout the study period. But no significant changes in the body weight (fig) and body weight gained (fig) or lost in the treaded test groups were observed compared with control group during the study.

Table 6.3.4: Effect of Naaval Kottai Mathirai total body weight in male Wistar Rats – 28 day repeated oral toxicity study

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial day (g)</th>
<th>On 7th day (g)</th>
<th>On 14th day (g)</th>
<th>On 21st day (g)</th>
<th>On 28th day (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>143.6±3.08</td>
<td>150±3.30</td>
<td>155.24±2.72</td>
<td>160.32±2.81</td>
<td>165.44±2.70</td>
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<tr>
<td>NKM 90 mg/kg</td>
<td>140.82±2.17</td>
<td>146.62±2.11</td>
<td>151.8±2.09</td>
<td>157.1±1.99</td>
<td>162.5±2.17</td>
</tr>
<tr>
<td>NKM 450 mg/kg</td>
<td>141.86±1.32</td>
<td>148.36±1.37</td>
<td>153.7±1.30</td>
<td>159.3±1.35</td>
<td>164.54±1.21</td>
</tr>
<tr>
<td>NKM 900 mg/kg</td>
<td>144.98±3.21</td>
<td>151.32±2.05</td>
<td>156.7±1.38</td>
<td>163.04±1.24</td>
<td>168.3±1.06</td>
</tr>
</tbody>
</table>

Table no: 6.3.5. Effect of Naaval Kottai Mathirai on body weight in female Wistar albino rats - 28 day repeated oral toxicity study

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial day (g)</th>
<th>On 7th day (g)</th>
<th>On 14th day (g)</th>
<th>On 21st day (g)</th>
<th>On 28th day (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>140.86±1.71</td>
<td>146.72±1.39</td>
<td>151.88±1.99</td>
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<tr>
<td>NKM 90 mg/kg</td>
<td>141.7±2.56</td>
<td>147.34±1.89</td>
<td>153.1±1.78</td>
<td>158.24±2.91</td>
<td>163.22±2.68</td>
</tr>
<tr>
<td>NKM 450 mg/kg</td>
<td>142.22±1.66</td>
<td>148.5±2.06</td>
<td>153.9±2.12</td>
<td>161.04±2.07</td>
<td>167.52±2.10</td>
</tr>
<tr>
<td>NKM 900 mg/kg</td>
<td>142.42±1.78</td>
<td>150.14±1.57</td>
<td>156.22±2.13</td>
<td>162.4±2.25</td>
<td>170.2±2.06</td>
</tr>
</tbody>
</table>

Food consumption

The amount of pellets consumed by Wistar albino rats from different test dose groups during the period of 28 days was found to be comparable with that by control group. The data were not shown since no significant difference was observed.

Clinical observations

All male and female rats of Group I – IV were free from abnormal clinical signs throughout the period of test drug administration period of 28 days.

Mortality and morbidity

All male and female rats of Group I – IV were survived and had good health throughout the period of test drug administration.
Haematology

The results of analyzes of haematological parameters were shown in the figures 6.3.3 to 6.3.15. The results obtained reflected some significant changes in the values of various parameters assayed when compared with those of corresponding controls. Never the less, the decrease or increase in the values obtained was within normal physiological limits and the effect was not observed to be dose dependent\(^{(45)}\).

Male Wistar albino rats

- Increased values of Granulocyte were obtained for rats in the dose group administered 900 mg/kg b.wt of NKM sacrificed on day 29 \( (P=0.0234) \).

Female Wistar albino rats

- Decreased values of Lymphocyte were obtained for rats in the dose group administered 90 mg/kg b.wt \( (P=0.0001) \) and 450 mg/kg b.wt \( (P=0.0243) \) of NKM sacrificed on day 29.
- Decreased values of RDW were obtained for rats in the dose group administered 90mg/kg b.wt \( (P=0.0344) \) and 900 mg/kg b.wt \( (P=0.0172) \) of NKM sacrificed on day 29.
- Increased values of MCV were obtained for rats in the dose groups administered 90mg/kg b.wt \( (P=0.0163) \), 450 mg/kg b.wt \( (P=0.0192) \) and 900 mg/kg b.wt \( (P=0.0117) \) of NKM sacrificed on day 29.
- Increased values of MCH were obtained for rats in the dose groups administered 450 mg/kg b.wt of NKM sacrificed on day 29 \( (P=0.0158) \)
- Increased values of Platelet crit were obtained for rats in the dose group administered 90 mg/kg b.wt of NKM sacrificed on day 29 \( (P=0.5047) \).
- Decreased values of PDW were obtained for rats in the dose group administered 90 mg/kg b.wt of NKM sacrificed on day 29 \( (P=0.4337) \).
Figure: 6.3.3: Effect of *Naaval Kottai Mathirai* on White blood Corpuscles in male Albino Wistar rats

Figure: 6.3.4: Effect of *Naaval Kottai Mathirai* on White blood Corpuscles in female Albino Wistar rats
Figure: 6.3.5: Effect of *Naaval Kottai Mathirai* on Haemoglobin in male and female Albino Wistar rats

![Image of Haemoglobin graph]

Figure: 6.3.6: Effect of *Naaval Kottai Mathirai* on Red blood Corpuscles in male and female Albino Wistar rats

![Image of Total RBC graph]
Figure: 6.3.7: Effect of Naaval Kottai Mathirai on Red cell distribution width in male and female Albino Wistar rats

Figure: 6.3.8: Effect of Naaval Kottai Mathirai on Hematocrit value in male and female Albino Wistar rats
Figure: 6.3.9: Effect of Naaval Kottai Mathirai on mean corpuscular volume in male and female Albino Wistar rats.

Figure: 6.3.10: Effect of Naaval Kottai Mathirai on mean corpuscular hemoglobin in male and female Albino Wistar rats.
Figure: 6.3.11: Effect of *Naaval Kottai Mathirai* on mean corpuscular haemoglobin concentration in male and female Albino Wistar rats

Figure: 6.3.12: Effect of *Naaval Kottai Mathirai* on platelet count in male and female Albino Wistar rats
Figure: 6.3.13: Effect of Naaval Kottai Mathirai on platelet crit in male and female Albino Wistar rats

Figure: 6.3.14: Effect of Naaval Kottai Mathirai on platelet distribution width in male and female Albino Wistar rats
Figure: 6.3.15: Effect of Naaval Kottai Mathirai on mean platelet volume in male and female Albino Wistar rats

Serum Biochemistry

The results of analyzes of biochemical parameters were shown in the figures 6.3.16 to 6.3.27. The results obtained reflected some significant changes in the values of various parameters assayed when compared with those of corresponding controls. Nevertheless, the decrease or increase in the values obtained was within normal physiological limits and the effect was not observed to be dose dependent.

**Male Wistar albino rats**
- Decreased values of Urea were obtained for rats in the dose group administered 450 mg/kg b.wt ($P=0.0160$) and 900 mg/kg b.wt ($P=0.0334$) of NKM sacrificed on day 29.

**Female Wistar albino rats**
- Increased values of Urea were obtained for rats in the dose group administered 450 mg/kg b.wt of NKM sacrificed on day 29 ($P=0.0058$)
Figure: 6.3.16: Effect of *Naaval Kottai Mathirai* on Glucose value in male and female Albino Wistar rats

![Glucose - Male & Female](image)

Figure: 6.3.17: Effect of *Naaval Kottai Mathirai* on Cholesterol value male and female Albino Wistar rats

![Cholesterol - Male & Female](image)
Figure: 6.3.18: Effect of *Naaval Kottai Mathirai* on Triglyceride value male and female Albino Wistar rats

![Triglyceride - Male & Female Rats](image1)

Figure: 6.3.19: Effect of *Naaval Kottai Mathirai* on Urea value male and female Albino Wistar rats

![Urea - Male & Female Rats](image2)
Figure: 6.3.20: Effect of *Naaval Kottai Mathirai* on Creatinine value male and female Albino Wistar rats

Figure: 6.3.21: Effect of *Naaval Kottai Mathirai* on Bilirubin value male and female Albino Wistar rats
Figure: 6.3.22: Effect of *Naaval Kottai Mathirai* on Protein value in male and female Albino Wistar rats

![Protein - Male & Female Rats](image)

<table>
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<tr>
<th>Treatment</th>
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<th>Female Mean</th>
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</thead>
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<td>6.18614</td>
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<tr>
<td>900 mg/kg</td>
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<td>6.24618</td>
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</tbody>
</table>

Figure: 6.3.23: Effect of *Naaval Kottai Mathirai* on Alkaline phosphatase value male and female Albino Wistar Rats

![Alkaline phosphatase - Male & Female Rats](image)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Male Mean</th>
<th>Female Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<td>90 mg/kg</td>
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<tr>
<td>900 mg/kg</td>
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</tr>
</tbody>
</table>
Figure: 6.3.24: Effect of Naaval Kottai Mathirai on SGOT value male and female Albino Wistar rats

Figure: 6.3.25: Effect of Naaval Kottai Mathirai on SGPT value male and female Albino Wistar rats
Figure: 6.3.26: Effect of *Naaval Kottai Mathirai* on Sodium value male and female Albino Wistar rats.

Figure: 6.3.27: Effect of *Naaval Kottai Mathirai* on Potassium value male and female Albino Wistar rats.
Histo-pathological study report of 28 days repeated dose oral toxicity study in male rats

Plate 6.3.3: Histology of Liver of 28 days repeated oral toxicity study of Naaval Kottai Mathirai. CMLi - H&E sliced Liver of male Wistar rat in control group showing normal architecture (10X), NKMMLi - H&E sliced Liver of male Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),

Plate 6.3.4: - Histology of Kidney of 28 days repeated oral toxicity study of Naaval Kottai Mathirai. CMKi-H&E sliced kidney of Male Wistar rat in control group showing normal architecture (10X), NKMMKi - H&E sliced Kidney of male Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),
Plate 6.3.5: - Histology of Lungs of 28 days repeated oral toxicity study of *Naaval Kottai Mathirai*. CMLu - H&E sliced Lungs of male Wistar rat in control group showing normal architecture (10X), NKMMLu - H&E sliced Lungs of male Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),

Plate 6.3.6:- Histology of Heart of 28 days repeated oral toxicity study of *Naaval Kottai Mathirai*. CMHe - H&E sliced Heart of male Wistar rat in control group showing normal architecture (10X), NKMMHe - H&E sliced Heart of male Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),
Plate 6.3.7:- Histology of Spleen of 28 days repeated oral toxicity study of Naaval Kottai Mathirai. CMSp - H&E sliced Spleen of male Wistar rat in control group showing normal architecture (10X), NKMMSp - H&E sliced Spleen of male Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),

Plate 6.3.8:- Histology of Stomach of 28 days repeated oral toxicity study of Naaval Kottai Mathirai. CMSt - H&E sliced Stomach of Male Wistar rat in control group showing normal architecture (10X), NKMMSSt - H&E sliced Stomach of male Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),
Plate 6.3.9:- Histology of Brain of 28 days repeated oral toxicity study of *Naaval Kottai Mathirai*. CMBr - H&E sliced Brain of male Wistar rat in control group showing normal architecture (10X), NKMBBr - H&E sliced Cerebrum of male Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),

Histo-pathological study report of 28 days repeated dose oral toxicity study in Female rats

Plate 6.3.10:- Histology of Liver of 28 days repeated oral toxicity study of *Naaval Kottai Mathirai*. CFLi - H&E sliced Liver of female Wistar rat in control group showing normal architecture (10X), NKMFLi - H&E sliced Liver of female Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),
Plate 6.3.11: Histology of Kidney of 28 days repeated oral toxicity study of *Naaval Kottai Mathirai*. CFKi-H&E sliced kidney of Female Wistar rat in control group showing normal architecture (10X), NKMFKi - H&E sliced Kidney of female Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),

Plate 6.3.12: Histology of Lungs of 28 days repeated oral toxicity study of *Naaval Kottai Mathirai*. CFLu - H&E sliced Lungs of Female Wistar rat in control group showing normal architecture (10X), NKMFLu - H&E sliced Lungs of female Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),
Plate 6.3.13: Histology of Heart of 28 days repeated oral toxicity study of *Naaval Kottai Mathirai*. CFHe - H&E sliced Heart of female Wistar rat in control group showing normal architecture (10X), NKMFHe - H&E sliced Heart of female Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),

Plate 6.3.14: Histology of Spleen of 28 days repeated oral toxicity study of *Naaval Kottai Mathirai*. CFSp - H&E sliced Spleen of female Wistar rat in control group showing normal architecture (10X), NKMFSp - H&E sliced Spleen of female Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),
Plate 6.3.15: Histology of Stomach of 28 days repeated oral toxicity study of *Naaval Kottai Mathirai*. CFSt - H&E sliced Stomach of Female Wistar rat in control group showing normal architecture (10X), NKMFS - H&E sliced Stomach of female Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),

Plate 6.3.16: Histology of Brain of 28 days repeated oral toxicity study of *Naaval Kottai Mathirai*. CFBr - H&E sliced Brain of female Wistar rat in control group showing normal architecture (10X), NKMFB - H&E sliced Cerebrum of female Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),
Randomization, Numbering and Grouping of Wistar Rats

The rats allotted to different groups are noted in the Table 6.3.6.

Table 6.3.6: Allocation of Animal Identification code for 90 day Repeated oral toxicity study

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I – Control</td>
<td>Vehicle – distilled water 1mL</td>
</tr>
<tr>
<td>II – Test group at low dose level</td>
<td>NKM at 90 mg/kg b. wt suspended in 2 mL distilled water</td>
</tr>
<tr>
<td>III – Test group at intermittent dose level</td>
<td>NKM at 450 mg/kg b. wt suspended in 2 mL distilled water</td>
</tr>
<tr>
<td>IV – Test group at high dose level</td>
<td>NKM at 900 mg/kg b. wt suspended in 2 mL distilled water</td>
</tr>
</tbody>
</table>

Body weight

All animals involved in the study gained comparable body weight throughout the study period. But no significant change in the body weight and body weight gained or lost in the treated test groups were observed compared with control group during the study.

Table 6.3.7: Effect of Naaval Kottai Mathirai total body weight in male Wistar Rats – 90 day repeated oral toxicity study

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial day (g)</th>
<th>On 30th day (g)</th>
<th>On 60th day (g)</th>
<th>On 90th day (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>142.32±1.34</td>
<td>163.9±1.25</td>
<td>185.38±1.16</td>
<td>206.73±1.96</td>
</tr>
<tr>
<td>NKM 90 mg/kg</td>
<td>142.8±2.74</td>
<td>164.82±1.87</td>
<td>186.75±1.70</td>
<td>208.54±2.34</td>
</tr>
<tr>
<td>NKM 450 mg/kg</td>
<td>142.4±2.59</td>
<td>165.94±2.2</td>
<td>185.7±6.43</td>
<td>208.85±1.53</td>
</tr>
<tr>
<td>NKM 900 mg/kg</td>
<td>142.4±2.71</td>
<td>164.42±3.12</td>
<td>184.89±6.44</td>
<td>209.21±1.94</td>
</tr>
</tbody>
</table>
Table no: 6.3.8 Effect of *Naaval Kottai Mathirai* on body weight in female Wistar albino rats - 90 day repeated oral toxicity study

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial day (g)</th>
<th>On 30th day (g)</th>
<th>On 60th day (g)</th>
<th>On 90th day (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>141.1 ±2.33</td>
<td>163.99±2.26</td>
<td>186.4±1.91</td>
<td>208.26±2.11</td>
</tr>
<tr>
<td>NKM 90 mg/kg</td>
<td>141.2±2.09</td>
<td>164.88±1.91</td>
<td>187.26±1.95</td>
<td>208.16 ±2.42</td>
</tr>
<tr>
<td>NKM 450 mg/kg</td>
<td>141.4±2.36</td>
<td>163.98±2.23</td>
<td>186.57±2.04</td>
<td>207.84±2.89</td>
</tr>
<tr>
<td>NKM 900 mg/kg</td>
<td>141.59±2.22</td>
<td>164.11±2.07</td>
<td>186.31±1.65</td>
<td>208.71±2.06</td>
</tr>
</tbody>
</table>

**Food consumption**

The amount of pellets consumed by Wistar albino rats from different test dose groups during the period of 90 days was found to be comparable with that by control group. The data were not shown since no significant difference was observed.

**Clinical observations**

All male and female rats of Group I – IV were free from abnormal clinical signs throughout the period of test drug administration period of 90 days.

**Mortality and morbidity**

All male and female rats of Group I – IV were survived and had good health throughout the period of test drug administration period of 90 days.

**Urine analysis**

The glucose, bilirubin, ketones, blood and urobilinogen were found nil in the urine samples of three groups. The data of urinalysis of control and high dose groups were not shown since any specific abnormality related to the treatment was not observed.

**Haematology**

The results of analyzes of haematological parameters were shown in the figures 6.3.28 to 6.3.40. The results obtained reflected some significant changes in the values of various parameters assayed when compared with those of corresponding controls. The decrease or increase in the values obtained was within normal physiological limits and the effect was not observed to be dose dependent(45).
**Male Wistar albino rats**

- Decreased values of MCV were obtained for rats in the dose group administered 90mg/kgbw.t \((P=0.0018)\), 450 mg/kg b.wt \((P=0.0023)\) and 900 mg/kg b.wt \((P=0.0333)\) of NKM sacrificed on day 91.

- Decreased values of MCH were obtained for rats in the dose groups administered 450 mg/kg b.wt \((P=0.0023)\) and 900 mg/kg b.wt \((P=0.0214)\) of NKM sacrificed on day 91.

- Decreased values of PDW were obtained for rats in the dose group administered 90 mg/kg b.wt of NKM sacrificed on day 91 \((P=0.0005)\).

- Decreased values of MPV were obtained for rats in the dose groups administered 900 mg/kg b.wt of NKM sacrificed on day 91 \((P=0.0386)\).

**Female Wistar albino rats**

- Decreased values of Monocyte were obtained for rats in the dose group administered 90mg/kgb.wt \((P=0.0540)\), 450 mg/kg b.wt \((P=0.0540)\) and 900 mg/kg b.wt \((P=0.0495)\) of NKM sacrificed on day 91.

- Decreased values of RDW were obtained for rats in the dose group administered 450 mg/kg b.wt of NKM sacrificed on day 91 \((P=0.0108)\).

- Increased values of MCHC were obtained for rats in the dose groups administered 90 mg/kg b.wt \((P=0.0099)\) and 900 mg/kg b.wt \((P=0.0039)\) of NKM sacrificed on day 91.
Figure: 6.3.28: Effect of Naaval Kottai Mathirai on White blood Corpuscles in male Albino Wistar rats
Figure: 6.3.29: Effect of *Naaval Kottai Mathirai* on White blood Corpuscles in female Albino Wistar rats

Figure: 6.3.30: Effect of *Naaval Kottai Mathirai* on Haemoglobin in male and female Albino Wistar rats
Figure: 6.3.31: Effect of Naaval Kottai Mathirai on Red blood Corpuscles in male and female Albino Wistar rats

Figure: 6.3.32: Effect of Naaval Kottai Mathirai on Red cell distribution width in male and female Albino Wistar rats
Figure: 6.3.33: Effect of *Naaval Kottai Mathirai* on Hematocrit value in male and female Albino Wistar rats.

Figure: 6.3.34: Effect of *Naaval Kottai Mathirai* on mean corpuscular volume in male and female Albino Wistar rats.
Figure: 6.3.35: Effect of Naaval Kottai Mathirai on mean corpuscular hemoglobin in male and female Albino Wistar rats

Figure: 6.3.36: Effect of Naaval Kottai Mathirai on mean corpuscular haemoglobin concentration in male and female Albino Wistar rats
Figure: 6.3.37: Effect of *Naaval Kottai Mathirai* on platelet count in male and female Albino Wistar rats

Figure: 6.3.38: Effect of *Naaval Kottai Mathirai* on platelet crit in male and female Albino Wistar rats
Figure: 6.3.39: Effect of *Naaval Kottai Mathirai* on platelet distribution width in male and female Albino Wistar rats.

![Platelet Distribution Width - Male & Female](image)

Figure: 6.3.40: Effect of *Naaval Kottai Mathirai* on mean platelet volume in male and female Albino Wistar rats.

![Mean Platelet Volume - Male & Female](image)
Serum Biochemistry

The results of analyzes of biochemical parameters were shown in the figures 6.3.41 to 6.3.52. The results obtained reflected some significant changes in the values of various parameters assayed when compared with those of corresponding controls. Nevertheless, the decrease or increase in the values obtained was within normal physiological limits and the effect was not observed to be dose dependent.

Male Wistar albino rats

- Increased values of Triglyceride were obtained for rats in the dose group administered 90 mg/kg b.wt of NKM sacrificed on day 91 (P=0.0039).
- Increased values of Cholesterol were obtained for rats in the dose groups administered 450 mg/kg (P=0.0001) and 900 mg/kg b.wt (P=0.0011) of NKM sacrificed on day 91.
- Increased values of Urea were obtained for rats in the dose group administered 90 mg/kg b.wt of NKM sacrificed on day 91 (P=0.0075).
- Increased values of Creatinine were obtained for rats in the dose group administered 90 mg/kg b.wt of NKM sacrificed on day 91 (P=0.0249).
- Decreased values of SGPT were obtained for rats in the dose group administered 90 mg/kg b.wt of NKM sacrificed on day 91 (P=0.0061)

Female Wistar albino rats

- Increased values of Cholesterol were obtained for rats in the dose group administered 90 mg/kg b.wt (P=0.0008) and 450 mg/kg b.wt (P=0.0001) of NKM sacrificed on day 91
- Increased values of Urea were obtained for rats in the dose group administered 450 mg/kg b.wt of NKM sacrificed on day 91 (P=0.0062)
Figure: 6.3.41: Effect of *Naaval Kottai Mathirai* on Glucose value in male and female Albino Wistar rats

![Glucose - Male & Female](image)

Figure: 6.3.42: Effect of *Naaval Kottai Mathirai* on Cholesterol value male and female Albino Wistar rats

![Cholesterol - Male & Female](image)
Figure: 6.3.43: Effect of Naaval Kottai Mathirai on Triglyceride value male and female Albino Wistar rats

Figure: 6.3.44: Effect of Naaval Kottai Mathirai on Urea value male and female Albino Wistar rats
Figure: 6.3.45: Effect of Naaval Kottai Mathirai on Creatinine value male and female Albino Wistar rats

Figure: 6.3.46: Effect of Naaval Kottai Mathirai on Bilirubin value male and female Albino Wistar rats
Figure: 6.3.47: Effect of *Naaval Kottai Mathirai* on Protein value in male and female Albino Wistar rats

![Protein - Male & Female Rats](image)

Figure: 6.3.48: Effect of *Naaval Kottai Mathirai* on Alkaline phosphatase value male and female Albino Wistar Rats

![Alkaline phosphatase - Male & Female Rats](image)
Figure: 6.3.49: Effect of *Naaval Kottai Mathirai* on SGOT value male and female Albino Wistar rats

![Graph showing the effect of Naaval Kottai Mathirai on SGOT value for male and female rats.](image)

Figure: 6.3.50: Effect of *Naaval Kottai Mathirai* on SGPT value male and female Albino Wistar rats

![Graph showing the effect of Naaval Kottai Mathirai on SGPT value for male and female rats.](image)
Figure: 6.3.51: Effect of *Naaval Kottai Mathirai* on Sodium value male and female Albino Wistar rats.

Figure: 6.3.52: Effect of *Naaval Kottai Mathirai* on Potassium value male and female Albino Wistar rats.
Histo-pathological study report of 90 days repeated dose oral toxicity study in male rats

Plate 6.3.17: Histology of Liver of 90 days repeated oral toxicity study of *Naaval Kottai Mathirai*. CMLi - H&E sliced Liver of male Wistar rat in control group showing normal architecture (10X), NKMMLi - H&E sliced Liver of male Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),

Plate 6.3.18: Histology of Kidney of 90 days repeated oral toxicity study of *Naaval Kottai Mathirai*. CMKi-H&E sliced kidney of male Wistar rat in control group showing normal architecture (10X), NKMMKi - H&E sliced Kidney of male Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),
Plate 6.3.19: - Histology of Lungs of 90 days repeated oral toxicity study of Naaval Kottai Mathirai. CMLu - H&E sliced Lungs of male Wistar rat in control group showing normal architecture (10X), NKMMLu - H&E sliced Lungs of male Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),

Plate 6.3.20:- Histology of Heart of 90 days repeated oral toxicity study of Naaval Kottai Mathirai. CMHe - H&E sliced Heart of male Wistar rat in control group showing normal architecture (10X), NKMMHe - H&E sliced Heart of male Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),
Plate 6.3.21: Histology of Spleen of 90 days repeated oral toxicity study of Naaval Kottai Mathirai. CMSp - H&E sliced Spleen of male Wistar rat in control group showing normal architecture (10X), NKMMSp - H&E sliced Spleen of male Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),

Plate 6.3.22: Histology of Stomach of 90 days repeated oral toxicity study of Naaval Kottai Mathirai. CMS - H&E sliced Stomach of Male Wistar rat in control group showing normal architecture (10X), NKMMS - H&E sliced Stomach of male Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),
Plate 6.3.23: Histology of Brain of 90 days repeated oral toxicity study of *Naaval Kottai Mathirai*. CMBr - H&E sliced Brain of male Wistar rat in control group showing normal architecture (10X), NKMMBr - H&E sliced Cerebrum of male Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),

Histo-pathological study report of 90 days repeated dose oral toxicity study in Female rats

Plate 6.3.24: Histology of Liver of 90 days repeated oral toxicity study of *Naaval Kottai Mathirai*. CFLi - H&E sliced Liver of female Wistar rat in control group showing normal architecture (10X), NKMFLi - H&E sliced Liver of female Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),
Plate 6.3.25: - Histology of Kidney of 90 days repeated oral toxicity study of *Naaval Kottai Mathirai*. CFKi-H&E sliced kidney of female Wistar rat in control group showing normal architecture (10X), NKMFKi - H&E sliced Kidney of female Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),

Plate 6.3.26: - Histology of Lungs of 90 days repeated oral toxicity study of *Naaval Kottai Mathirai*. CFLu - H&E sliced Lungs of female Wistar rat in control group showing normal architecture (10X), NKMFLu - H&E sliced Lungs of female Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),
Plate 6.3.27: Histology of Spleen of 90 days repeated oral toxicity study of *Naaval Kottai Mathirai*. CFSp - H&E sliced Spleen of female Wistar rat in control group showing normal architecture (10X), NKMFSp - H&E sliced Spleen of female Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),

Plate 6.3.28: Histology of Heart of 90 days repeated oral toxicity study of *Naaval Kottai Mathirai*. CFHe - H&E sliced Heart of female Wistar rat in control group showing normal architecture (10X), NKMHe - H&E sliced Heart of female Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),
Plate 6.3.29.- Histology of Stomach of 90 days repeated oral toxicity study of Naaval Kottai Mathirai. CFSt - H&E sliced Stomach of female Wistar rat in control group showing normal architecture (10X), NKMFS - H&E sliced Stomach of female Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),

Plate 6.3.30:- Histology of Brain of 90 days repeated oral toxicity study of Naaval Kottai Mathirai. CFBr - H&E sliced Brain of female Wistar rat in control group showing normal architecture (10X), NKMFB - H&E sliced Cerebrum of female Wistar rat in high dose test group treated with NKM at 900 mg/kg showing normal architecture (10X),
6.4. Anti bacterial activity:
The Anti bacterial activity has been done for the study drug *Naaval Kottai Mathirai*.

![Graph](image)

**Figure: 6.4.1. Anti-bacterial Effect of *Naaval Kottai Mathirai* Aganist Bacillus subtilis**

![Graph](image)

**Figure: 6.4.2. Anti-bacterial Effect of *Naaval Kottai Mathirai* Aganist Staphylococcus aureus**
Figure: 6.4.3. Anti-bacterial Effect of *Naaval Kottai Mathirai* Aganist Enterococcus faecalis

Figure: 6.4.4. Anti-bacterial Effect of *Naaval Kottai Mathirai* Aganist Pseudomonas aeruginosa
Figure: 6.4.5. Anti-bacterial Effect of Naaval Kottai Mathirai Aganist Escherichia coli

Figure: 6.4.6. Anti-bacterial Effect of Naaval Kottai Mathirai Aganist Klebsiella pneumoniae
Anti-Microbial Effect of NKM against Bacillus subtilis

Anti-Microbial Effect of NKM against Staphylococcus aureus

Anti-Microbial Effect of NKM against Enterococcus faecalis

Anti-Microbial Effect of NKM against Escherichia coli

Anti-Microbial Effect of NKM against Pseudomonas aeruginosa

Anti-Microbial Effect of NKM against Klebsiella pneumonia

Plate: 6.4.1 - 6.4.6: Anti Bacterial effect of Naaval Kottai Mathirai
6.5 Anti oxidant effect of *Naaval Kottai Mathirai*

![DPPH radical scavenging assay](image)

**Figure: 6.5.1.** Percentage inhibition of DPPH radicals by Ascorbic acid and NKM

![Nitric Oxide radical scavenging assay](image)

**Figure: 6.5.2.** Percentage inhibition of Nitric oxide radicals by Gallic acid and NKM
Figure: 6.5.3. Percentage inhibition of Hydrogen peroxide radicals by BHA and NKM

Figure: 6.5.4. ABTS radical scavenging Effect of Naaval Kottai Mathrai
6.6. *In-vitro* Alpha amylase & Alpha Glucosidase inhibition effect of *NKM*

**Figure: 6.6.1.** Alpha amylase inhibition effect of *Naaval Kottai Mathirai*

**Figure: 6.6.2.** Alpha Glucosidase inhibition effect of *Naaval Kottai Mathirai*
6.7. Glucose uptake activity of *Naaval Kottai Mathirai*

![Graph showing glucose uptake activity](image)

**Figure: 6.7.1. In vitro cytotoxic effect of *Naaval Kottai Mathirai***

**Table: 6.7.1. In -vitro glucose uptake activity of *Naaval Kottai Mathirai* in L6 cell lines.**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of the Test substances</th>
<th>Test Conc. In mcg/ml</th>
<th>Glucose uptake percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>-</td>
<td>0.74±1.19</td>
</tr>
<tr>
<td>2</td>
<td>Rosiglitazone</td>
<td>100</td>
<td>113.26±7.72</td>
</tr>
<tr>
<td>3</td>
<td><em>Naaval Kottai Mathirai</em></td>
<td>100</td>
<td>32.67±4.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>18.73±6.36</td>
</tr>
</tbody>
</table>
6.8. Anti diabetic activity of *Naaval Kottai Mathirai*

Table No: 6.8.1. Effect of *Naaval Kottai Mathirai* on animal’s body weight and blood glucose

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Body weight (g)</th>
<th>Blood glucose (mg / 100ml)</th>
<th>Blood glucose (mg / 100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
</tr>
<tr>
<td>G1</td>
<td>235 ± 7.32</td>
<td>238 ± 7.35</td>
<td>84.60 ± 3.30</td>
</tr>
<tr>
<td>G2</td>
<td>224 ± 6.68</td>
<td>168 ±4.40**(a)**</td>
<td>84.75 ± 3.42</td>
</tr>
<tr>
<td>G3</td>
<td>230 ± 7.28</td>
<td>234 ± 7.32</td>
<td>86.65 ± 4.22</td>
</tr>
<tr>
<td>G4</td>
<td>228 ± 7.25</td>
<td>238 ± 7.34</td>
<td>85.80 ± 3.70</td>
</tr>
<tr>
<td>G5</td>
<td>230 ± 7.38</td>
<td>235 ± 7.42</td>
<td>86.45 ± 3.80</td>
</tr>
</tbody>
</table>

Table: 6.8.2. Effect of *Naaval Kottai Mathirai* on Plasma insulin, Hemoglobin & Glycosylated haemoglobin.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Haemoglobin (gm/100ml)</th>
<th>Glycosylated haemoglobin HbA₁ (%)</th>
<th>Plasma Insulin (µU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>11.85 ± 1.65</td>
<td>0.38 ±0.05</td>
<td>34.50 ± 2.98</td>
</tr>
<tr>
<td>G2</td>
<td>6.20 ± 0.70**(a)**</td>
<td>0.96 ±0.15**(a)**</td>
<td>12.72 ± 1.78**(a)**</td>
</tr>
<tr>
<td>G3</td>
<td>11.18 ± 1.35**(b)**</td>
<td>0.40 ±0.06**(b)**</td>
<td>28.40 ± 2.50**(b)**</td>
</tr>
<tr>
<td>G4</td>
<td>10.40 ± 0.95**(b)**</td>
<td>0.46 ±0.09**(b)**</td>
<td>24.68 ± 2.38**(b)**</td>
</tr>
<tr>
<td>G5</td>
<td>10.96 ± 1.20**(b)**</td>
<td>0.42 ±0.05**(b)**</td>
<td>27.92 ± 2.65**(b)**</td>
</tr>
</tbody>
</table>
Table: 6.8.3. Effect of Naaval Kottai Mathirai on Serum lipids

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Total Cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>Phospholipids (mg/dl)</th>
<th>LDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>84.95 ±2.65</td>
<td>88.65 ± 2.60</td>
<td>53.40 ±1.86</td>
<td>123.70 ± 2.55</td>
<td>15.44 ± 1.36</td>
</tr>
<tr>
<td>G2</td>
<td>224.35 ±6.78**(a)</td>
<td>155.62 ±4.60**(a)</td>
<td>30.70 ±1.35** (a)</td>
<td>212.45 ±6.35** (a)</td>
<td>38.66 ± 2.44**(a)</td>
</tr>
<tr>
<td>G3</td>
<td>114.88 ±3.35**(b)</td>
<td>94.95 ±2.65**(b)</td>
<td>42.92 ±1.45</td>
<td>149.46 ±3.94</td>
<td>22.36 ± 1.88**(b)</td>
</tr>
<tr>
<td>G4</td>
<td>125.55 ±3.60**(b)</td>
<td>115.85 ±2.92**(b)</td>
<td>38.45 ±1.40** (b)</td>
<td>157.58 ±4.08** (b)</td>
<td>28.30 ± 1.90**(b)</td>
</tr>
<tr>
<td>G5</td>
<td>118.45 ±3.38**(b)</td>
<td>98.50 ±2.65**(b)</td>
<td>41.50 ±1.60** (b)</td>
<td>150.42 ±3.94** (b)</td>
<td>24.36 ± 1.75**(b)</td>
</tr>
</tbody>
</table>

Table: 6.8.4. Effect of Naaval Kottai Mathirai on glycogen content

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver Tissue Glycogen Content (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>41.28 ± 2.42</td>
</tr>
<tr>
<td>Group II</td>
<td>8.30 ± 0.60*&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III</td>
<td>32.52 ± 1.65*&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV</td>
<td>24.45 ± 1.15*&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V</td>
<td>26.60 ± 1.42*&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

- Values are expressed as mean ± SEM.
- Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests.
- ** (a) Values are significantly different from normal control G1 at P<0.001.
- ** (b) Values are significantly different from Diabetic control G2 at P<0.01.
Table: 6.8.5. Effect of *Naaval Kottai Mathirai* on enzymes involved in carbohydrate metabolism in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hexokinase (µg/mg)</th>
<th>Glucose-6-Phosphate (µg/mg)</th>
<th>Glucokinase (µg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0.216 ± 0.013</td>
<td>0.392 ± 0.010</td>
<td>23.40 ± 1.38</td>
</tr>
<tr>
<td>Group II</td>
<td>0.090 ± 0.004&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.128 ± 0.007&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.90 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III</td>
<td>0.126 ± 0.007&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.301 ± 0.010&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.20 ± 0.92&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV</td>
<td>0.120 ± 0.005&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.231 ± 0.007&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.12 ± 0.50&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V</td>
<td>0.141 ± 0.006&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.242 ± 0.008&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.16 ± 0.90&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

- Values are expressed as mean ± SEM.
- Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests.
- **(a)** Values are significantly different from normal control G1 at P<0.001.
- **(b)** Values are significantly different from Diabetic control G2 at P<0.01.
In siddha system of medicine, taste plays a major role in treating diseases. Each taste is due to the combination of two elements (Boothas). Medicines for certain disease are selected on the basis of taste and Pancha bootham (five elements). Astringent taste promotes the quality of blood. It decreases the excessive excretion of urine, feces and blood and also reduces the cholesterol level in blood. Likewise bitter taste has the functions of reducing excessive excretion of water due to hormonal imbalance and blood cholesterol level.\(^{(105)}\) Madhumegam is a disease caused due to vitiated kapha humour. The ingredients of the trial drug Naaval Kottai Mathirai are Aristolochia bracteolata having bitter taste and Syzygium cumini having astringent taste. According to Siddha literatures, bitterness and astringency smoothens kapha.\(^{(106)}\) As the trial drug possesses both bitterness and astringency it will be an effective medicine in the management of Madhumegam.

In recent studies, phytochemicals have received much attention in the treatment of diabetes for various reasons and many researchers have focused on isolation of hypoglycaemic agents from medicinal plants.\(^{(107)}\) Many herbal extracts has been reported for their anti diabetic activities and being used in Ayurveda for the treatment of diabetes, which are known to show an inhibitory effect on carbohydrate hydrolyzing enzyme inhibition, by their efficient capability to bind with proteins. This phenomenon contributes to lower postprandial hyperglycemia in diabetes.\(^{(108)}\)

Alpha amylase enzyme catalyses the hydrolysis of 1, 4 - glucosidic linkages of glycogen, starch and various oligosaccharides into monosaccharides, which are rapidly absorbed by the intestine. When alpha amylase enzyme in the digestive tract is inhibited, the absorption of glucose from starch also decreases and thus diabetes is controlled efficiently.\(^{(109)}\)

From the result of this study, the drug Naaval Kottai Mathirai (at a concentrations 10-100µg/ml) showed minimum alpha amylase inhibitory activity from 19.42 ± 5.92 to 60.92 ± 3.98 %, with an IC50 value 70.9 ± 12.37 µg/ml, compared to the standard, acarbose, which exhibited alpha amylase inhibitory activity from 39.83 ± 2.18 to 91.08 ± 5.43 with an IC50 value 13.29 ± 1.95 µg/ml. The results showed that it possess the effect of inhibition on alpha amylase activity. This may be due to the presence of potential alpha amylase inhibitors (alkaloids, Phenols, terpenoids or glycosides)
Inhibition of α-glucosidase enzyme activity results in the reduction of disaccharide hydrolysis which has beneficial effects on glycemic index control in diabetic patients. Several α-glucosidase inhibitors have been separated from medicinal plants for the development of new drugs with increased potency and lower adverse effects than the existing drugs. One of the strategies to control blood glucose level in type II diabetes mellitus is to either inhibit or reduce the production of glucose from the small intestine. Diet rich in carbohydrate causes sudden rise in the blood glucose level as the disaccharide in the food is rapidly absorbed in the intestine by the enzyme α-glucosidase which catalyses the breaks down of disaccharides into absorbable monosaccharides while α-glucosidase inhibitor inhibits the digestion of disaccharide and impedes the postprandial glucose excursion to enable overall smooth glucose profile.

NKM extracts possessed α-glucosidase inhibitory activity at 100 µg/ml concentration, the inhibitory activity is 54.53 ± 1.87 % and IC50 of 87.17 ± 0.35 µg/ml, compared to the standard acarbose, which exhibited 85.39 ± 1.05 % inhibition with IC50 of 34.48 ± 2.53 µg/ml. The present study indicated that the NKM could be useful in the management of postprandial hyperglycemia.

Urinary tract infections are more common, more severe, and carry worse out comes in patients with type 2 diabetes mellitus. They are more often caused by resistant pathogens. The factors contribute to the risk of urinary tract infections in diabetic patient are poor metabolic control, incomplete bladder emptying in autonomic neuropathy and impairments in the immune system. The extent of UTI varies from asymptomatic bacteriuria (ASB) to lower UTI (cystitis), pyelonephritis, and severe urosepsis. The serious complications of UTI such as emphysematous cystitis, pyelonephritis, renal abscesses and renal papillary necrosis are more frequently present in diabetic patients compared to general population. Escherichia coli, is the most common pathogens isolated from urine of diabetic patients with UTI. Other organisms includes Enterobacteriaceae such as Klebsiella spp., Proteus spp., Enterobacter spp., and Enterococci. Patients with diabetes are more prone to have resistant pathogens as the cause of their UTI, including extended-spectrum β-lactamase-positive Enterobacteriaceae. Over 90% of patients with diabetes mellitus has emphysematous pyelonephritis and 67% has emphysematous cystitis.

The management of UTI in diabetes patients is very difficult. The Patients are undergoing for antibiotic treatment for this issue in every occasion. The drug Naaval Kottai
Mathirai has been performed for anti bacterial effect against the selected human pathogens. In result of this study the drug NKM showed the significant anti bacterial effect against the Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia, Pseudomonas aeruginosa and Enterococcus faecalis.

The result of this study showed the significant anti bacterial effect that is a maximum zone of inhibition were obtained against Escherichia coli 15 mm, Staphylococcus aureus 17 mm, Bacillus subtilis 14 mm, Klebsiella pneumonia 12 mm, Pseudomonas aeruginosa 12 mm and Enterococcus faecalis 11 mm when compared to the standard, Streptomycin (20µg) which exhibited anti bacterial effect by the measurement of Zone of inhibition 25 mm, 18 mm, 23 mm, 19 mm, 14 mm and 18 mm respectively. From this study result the drug NKM can be used for Urinary tract infections of Diabetes patients also.

Free radicals are well known for their definite role in a wide variety of pathological manifestations of pain, inflammation, cancer, diabetes, Alzheimer disease, hepatic damage etc. Antioxidants fight against free radicals and defend us from various diseases. They apply their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms.

Scavenging activity of H₂O₂ by NKM may be attributed to their phenolics, which can donate electrons to H₂O₂, thus neutralizing it to water. The H₂O₂ scavenging activity was detected and compared with BHA. The result shows the % inhibition values of BHA and NKM was 78.63 ± 3.24 % and 69.15 ± 5.50 % respectively. The absorbance of the chromophore is measured at 230 nm in the presence of the fractions. The study drug NKM capable of scavenging H2O2 in a concentration dependent manner. The IC₅₀ value of BHA was found to be 48.61 ± 1.69 µg/ml, and NKM 67.19 ± 7.81 µg/ml.

NKM effectively reduced the generation of nitric oxide from sodium nitroprusside. Fig.6.7.2 shows the % inhibition values of Gallic acid and NKM was 88.9 % and 60.4 % respectively. Scavenging of nitric oxide radical is based on the formation of nitric oxide. Sodium nitroprusside, which reacts with oxygen in buffered saline to produce nitrite ions that can be measured by using Griess reagent. The absorbance of the chromophore is measured at 546 nm in the presence of the fractions. NKM decreases the amount of nitrite generated from the decomposition of sodium nitroprusside in vitro. The IC₅₀ values of Gallic acid was 23.02µg/ml, NKM was found to be 79.13 µg/ml.
The DPPH radical scavenging activity was detected and compared with Ascorbic acid. Fig 6.5.1 shows the % inhibition values of Ascorbic acid and NKM was 90.07 % and 73.64 % respectively. DPPH assay is one of the most extensively used methods for screening antioxidant activity of plant extracts. DPPH is a stable, nitrogen-centered free radical and in methanol solution it produces violet colour. Then it is reduced to diphenylpicryl hydrazine, a yellow colored product with the addition of the extract in a concentration-dependent manner. The IC\textsubscript{50} value of Ascorbic acid was 13.36 μg/ml, NKM was 53.39 μg/ml.

Polyphenols are the major plant compounds which possess high level of antioxidant activity. This property could be due to their ability to neutralize, adsorb and to quench free radicals (Duh et al., 1999). The observed result obtained showed that the drug NKM possesses antioxidant activity in a concentration dependent manner.

NKM was orally administered at higher dose 2 gm/kg to the Wistar Albino rats in acute toxicity study, during 28 days of repeated (sub acute) toxicity study and during 90 days of repeated (sub chronic) toxicity study, at daily doses of 90, 450 and 900 mg/kg of body weight to the Wistar Albino rats. The acute toxicity study showed no mortality of rats up to the dosage of 2000mg/kg. No behavioural changes or abnormal clinical signs were observed due to toxicity up to the above dosage throughout the end of 14 days study period. No gross pathological abnormality in the organs was found even at this high dose. LD50 value was found to be more than 2000 mg/kg body weight and therefore this test drug Naaval Kottai Mathirai falls under (Unclassified) category V with reference to Globally Harmonized classification System (116). It was clearly proved that the human therapeutic dose was absolutely free from acute toxicity.

For a period of 28 and 90 consecutive days of oral treatment of NKM at 90, 450 & 900 mg/kg/day in both sexes of rat, no treatment related toxicity signs or mortality were observed. Feed and water consumption of treated groups were found not to be significantly affected or changed in both sexes compared to the distilled water treated rats. Consumption of toxic substances effects at least a minimal reduction in body weight gain and internal organs weight (117) but no significant change in the body weight gained or lost in the treated test groups were observed compared with control group during the study. The absolute and relative organs weight was also not altered by NKM treatments. If NKM is a toxic substance, there will be a minimal reduction in internal organs weight and body weight. The changes observed in blood parameters analyzed in laboratory animals provide the evidence of risk of toxic effects on
haematological system. (118) White blood cell parameters tested such as Lymphocytes, Monocytes and Granulocytes in both male and female rats showed no significant differences in relation to the control group.

In 28 days (sub acute) toxicity study, in male groups, NKM at 900 mg/kg (high dose) induced to increase the values of Granulocyte, in relation to the control group. In female groups, NKM at 450 mg/kg (Intermittent dose) induced to increase the values of MCH and NKM at 90 mg/kg (low dose) induced to increase the values of Platelet crit, in relation to the control group. NKM at 90 mg/kg (low dose) induced to decrease the values of PDW, in relation to the control group. Lymphocyte value was decreased significantly in both NKM treated low dose (90 mg/kg) and intermittent dose (450 mg/kg). RDW value was decreased significantly in both NKM treated low dose (90 mg/kg) and high dose (900 mg/kg). MCV value was increased significantly in NKM treated all female groups (90, 450 and 900 mg/kg).

In 90 days (sub chronic) toxicity study, in male groups, NKM at 90 mg/kg (low dose) induced to decrease the values of PDW, and NKM at 900 mg/kg (high dose) induced to decrease the values of MPV in relation to the control group. MCV value was decreased significantly in all NKM treated low dose (90 mg/kg), intermittent dose (450 mg/kg) and high dose (900 mg/kg). MCH value was decreased significantly in both NKM treated intermittent dose (450 mg/kg) and high dose (900 mg / kg). In female groups, NKM at 450 mg/kg (intermittent dose) induced to decrease the values of RDW in relation to the control group. MCHC value was increased significantly in both NKM treated low dose (90 mg/kg) and high dose (900 mg/kg). Monocyte value was decreased significantly in all NKM treated low dose (90 mg/kg), intermittent dose (450 mg/kg) and high dose (900 mg/kg) groups.

Haematopoiesis is the process of formation of blood cellular components which includes leukopoiesis stage i.e. the formation of white blood cells in bone marrow (adults) and haematopoietic organs (foetus).(119) However, the significant differences noted in the parameters at low, intermittent and high dose groups lies within normal physiological limits indicated that NKM did not affect haematopoiesis or leukopoiesis in rats and that suggested NKM did not produce any toxicity in the blood forming organs affecting the haematopoietic indices.

Estimation of SGOT, SGPT, Bilirubin and ALP levels are the useful indicators of hepatic function and Protein, Urea, Creatinine and electrolytes such as Sodium, Potassium and
Chlorides are the useful indicators of renal function. The increased levels of both SGOT and SGPT in the blood are associated with injury of hepatocytes. \cite{120, 121}

In 28 days toxicity study, the renal parameters tested in male rats, urea level was significantly decreased treated at intermittent dose (450 mg/kg) and high dose (900 mg/kg) and in female rats, urea level was significantly increased treated at intermittent dose (450 mg/kg) in relation to the control group but creatinine showed no significant alteration in both sex at three dosages of NKM.

In 90 days toxicity study male groups, the cholesterol level showed significant increase treated at the dose 450 mg/kg (Intermittent dose) and 900 mg/kg (high dose). The Triglyceride level was significantly increased in 90 mg/kg (low dose) group. Urea and creatinine level was significantly increased in low dose group (90 mg/kg). SGPT level was significantly decreased at low dose (90 mg/kg) group. Among female rats, the cholesterol level was increased in low dose (90 mg/kg) and intermittent dose (450 mg/kg) groups. Urea level was significantly increased in intermittent dose (450 mg/kg) group.

In liver disorders, the above indicated parameters would rise to more than one fold of their value and in renal disorders; the parameters would rise over their normal limits. \cite{122} High Cholesterol is an indirect marker to access the variations in liver function. \cite{123} In both male and female rats, there were no significant differences in biochemical parameters such as Triglycerides, Protein, Creatinine, SGPT, Sodium, Potassium and Chlorides were observed at three doses compared to the control group. Moreover, the statistically significant changes noted in the biochemical parameters lay within the normal physiological limits which favours NKM as a safer drug during metabolism and did not cause any major pathological lesions in vital organs.

This was further confirmed by the gross necropsy studies that had been on the organs which revealed no abnormal pathological morphology. In Histopathological Examination there is no abnormalities were found in all group rats of both sexes. Moreover, the oral administration of NKM for 28 and 90 consecutive days do not induce any biochemical, haematological, anatomical and histopathological signs of toxicity in both the sexes under the experimental conditions used.
Diabetes mellitus is a lifestyle systemic disorder which is increasing in rate of incidence day by day in the world. It is being a social and economical issue for the world’s population. Hence many researchers are interested to give a remedy for the disease. In that view, the researcher also had made an attempt to provide a new and permanent remedy for this disease.

In Siddha system of medicine the disease Madhumegam is correlated with the symptoms of Diabetes Mellitus. For treating Madhumegam, numerous formulations were illustrated in the Siddha literatures. Among them, a herbal formulation “Naaval Kottai Mathirai (NKM)” has been practiced a long time for treating Diabetes (Madhumegam). NKM has been chosen for the study to prove it is a safer and efficacious drug in the management of Diabetes (Madhumegam) in animal model as preclinical study.

As per the Siddha fundamental, the taste astringent improves the quality of blood and bitter taste helps to reduce the blood sugar. In this view, the ingredient seeds of Syzygium Cumini possess strong astringent taste and Aristolochia bracteolata has bitter taste. So this formulation may be effective on blood sugar control.

The trial drug Naaval Kottai Mathirai has been prepared as per the reference text in the Gunapadam lab, National Institute of Siddha after getting the proper authentication.

Three different batches of Naaval Kottai Mathirai has been prepared for the purpose of standardization. The following qualitative analytical parameters organoleptic characters, Loss on Drying, Total Ash, Water Soluble Ash, Acid insoluble Ash, Alcohol Soluble Extractive Value, Water soluble Extractive value, Preliminary Phytochemicals like Terpenoids, Flavonoids, Alkaloids, Saponins, Tannins, Steroids, Anthraquinone glycosides and Amino acids were analysed. In Phytochemicals analysis, the drug NKM shows the presence of Phenols, Terpenoids, Alkaloids, Saponins, Tannins, Quinones and Glycosides.

Heavy metals (lead, cadmium, mercury and arsenic) content of 3 samples of NKM were estimated by using atomic absorption spectroscopy (AAS). The content of Mercury, cadmium and arsenic were under below detectable levels and lead was within the permissible limits in all samples.
The microbial load, aflatoxins and pesticide levels were analyzed for the trial drug NKM and the results were found to be within the WHO permissible limits.

As per the AYUSH guidelines, the analytical parameters like physicochemical analysis, phytochemical analysis, and heavy metal analysis had been done for three samples of Naaval Kottai Mathirai. The results of the study show no difference between the three samples. Hence, one sample has been taken for further safety and efficacious studies.

The antibacterial activities of the sample NKM were carried out by disc diffusion method. From the results of this study, the drug NKM showed the significant antibacterial effect against the Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia, Pseudomonas aeruginosa and Enterococcus faecalis.

The anti-oxidant activity of test drug NKM was studied by DPPH, Nitric Oxide, Hydrogen Peroxide and ABTS radical scavenging assay. The results showed that it has significant anti-oxidant effect.

In Vitro Alpha Amylase Inhibition activity of Naaval Kottai Mathirai has been studied at different concentrations. It showed that, moderate inhibition effect against alpha amylase (60.9%) as compared with standard acarbose (91.8%). This proves the efficacy of NKM in controlling post prandial Hyperglycemia.

In Vitro Alpha glucosidase inhibition activity of Naaval Kottai Mathirai has been studied at different concentrations. It showed that, moderate inhibition effect against alpha glucosidase (54.53%) as compared with standard acarbose (85.39%).

In-vitro Glucose uptake assay was performed for the trial drug NKM. The result of this study showed that, the drug NKM possess minimum glucose uptake activity (32.67 % ) when compared with standard Rosiglitazone (113.26 % ).

To evaluate the safety of NKM, Wistar albino rats were used for performing acute, sub acute and sub chronic toxicity studies following OECD guidelines. 2g/kg of NKM was tested on six rats and observed nil mortality and morbidity. Median lethal dose was estimated as more than 2g/kg for the test drug. The sub-acute toxicity and sub chronic toxicity was observed on Wistar rats by giving NKM at three dose levels (90, 450 & 900 mg/kg) for 28 days and 90 days. The test drug dose was fixed from the human conversion dose (1000 mg/day) to rat. No mortality and abnormal clinical signs were observed during 28 days and 90 days in respective studies. All test dose treated animals gave comparable body weight and organ weight gain with that of control. Haematological, biochemical parameters and urinalysis were within the normal
limit. No significant abnormality was detected in gross necropsy study on organs and in H&E sliced organs.

The anti diabetic effect of NKM was carried out by high fat diet and low dose streptozotocin induced rat model. In this study, after 28days treatment of NKM, the blood sugar level and Glycosylated haemoglobin (HbA₁C) was reduced, and also increased the serum insulin and Liver Glycogen Content when compared with diabetic control. This study revealed that the drug *Naaval Kottai Mathirai* possesses good anti diabetic effect.
CONCLUSION

From the above preclinical analytical studies, toxicity studies an In-vitro and In-vivo efficacious studies, this drug Naaval Kottai Mathirai has been proved as a safe and effective Siddha formulation for diabetes mellitus.

Finally it is concluded that the drug Naaval Kottai Mathirai is a potent anti-diabetic drug which can be used for the management of diabetes mellitus (Madhumegam) in clinical practices.
The future studies are suggested on *Naaval Kottai Mathirai* on the following aspects:

- The results of the analytical parameters can be used as a standard in future to compare by comparing the qualitative and quantitative analyzes of different batch preparations of NKM.
- Based on the results proper clinical trials can be conducted.
- The anti-diabetic effect of *Naaval Kottai Mathirai* can screened for the use in Gestational and Juvenile diabetes.


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