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Toxicological Evaluation of Anti- Diabetic Siddha Medicine Naaval Kottai Mathirai in Rat Model

Sivakkumar. S[1]*, Juliet. L[2], Ganapathy. G[1]
[1]*National Institute of Siddha, Tambaram Sanatorium, Chennai, India- 60047.*
[2]*Sri Sairam Siddha Medical College and Research Centre, Chennai, India -600044*

***Abstract**: Naaval Kottai Mathirai is a herbal Siddha preparation prepared from the seed of Syzygium cumini and leaf juice of Aristolochia bracheolata which is widely used in South India as traditional Siddha medicine for the treatment of diabetes mellitus (Madhumegam). The present study is aimed to investigate the acute and sub chronic toxicity of anti diabetic Siddha medicine Naaval Kottai Mathirai in healthy Wistar rats. In acute study, NKM (2000mg /kg) was administered to the wistar albino rats and observed for 14 days. For Sub chronic toxicity study the drug NKM was administered with the dose of LD 90 mgm, MD 450 mgm, HD 900 mgm for 90 days. The results of this study, the drug NKM neither produced significant changes in the consumption of food and intake of water , nor affected biochemical parameters, hematological parameters (full blood count) and histopathology studies . This study concluded that, the results revealed that the drug NKM at a dose of 2 g/kg was found to be toxicologically safe as a potent anti-hyperglycemic agent in Wistar rats.*

***Keywords:** Siddha- Acute Toxicity- Sub chronic Toxicity- Naaval Kottai Mathirai*

I. Introduction

The use of herbal medicines particularly Ayurvedha, Siddha and Unani medicines for healing purpose has been increasing in which plants are mostly used for medicine preparation. Medicinal plants have long been considered as valuable sources of medicine for treating variety of diseases and ailments. The increase in the indiscriminate use of plant extracts is further aggravated by the belief that plants are safe simply because they are natural in origin [1]. The use of medicinal plants for healing purposes has been increasingly popular as they are believed as beneficial and free of side effects [2].

Since the beginning of the human race plants were the preferable source of medicines. Medicinal plants are often used without satisfactory demonstration of their pharmacological activities. Moreover, many people believe that traditional medicines have no adverse effects. During the past few years it is observed that, the adverse effects of phytomedicines, as well as its adulteration, toxicity, and drug interaction are common problems related to public health [3]. The reorganization and acceptability of herbal medicines has been limited due to lack of defined chemical characterization, dose regimen and adequate toxicity data to determine their safety [4]. The consumption of medicinal plants as conventional medications or/and as curatives may cause adverse toxicological effects to human health.

However, the rationale for the utilization of medicinal plants has rested largely on long- term clinical experience with little or no scientific data on safety. With the upsurge in the use of herbal medicines for various diseases, toxicological investigation of medicinal plants is imperative based on the need to validate their folkloric usage [5].

In Siddha system of medicine the Naaval Kottai Mathirai [6] has been used for the management of Diabetes mellitus but there is no scientific safety data on it, at the same time there is sufficient safety data are available for the ingredients of NKM i.e seeds of Syzygium cumini (L) skeels and leaves of Aristolochia bracteolata Lam. NKM is an anti diabetic Siddha preparation. In which the seed powder of Syzygium cumini was grounded by adding the leaf juice of Aristolochia bracteata.

In South India, Siddha traditional healers are using this NKM for the management of *Madhumegam* (Diabetes Mellitus) , clinically this drug is working well. This drug helps to control the blood glucose level and reduce the clinical symptoms particularly increased frequency of urination and peripheral neuropathy and also prevent the complication of DM. For justifying this anti diabetic effect and safety of this drug, there is no evidence of pharmacological and toxicological data. So the present study aimed to evaluate the toxicological effect of NKM through acute and sub chronic toxicity studies.

II. Materials And Methods

2.1 Plant material and Naaval Kottai Mathirai preparation:

The seeds of *Syzygium cumini* (L.) skeels and *Aristolochia bracteolata* Lam. were collected from Seeganendal village , Pudukkottai district, Tamilnadu and duly authenticated by Prof. P. Jayaraman , Director , Plant Anatomy Research Centre , Chennai-45. The authentication number is **PARC/2016/3255 and 3256**. The

collected seeds of *S.cumini* were powdered and grounded by adding the leaf juice of *A. bracteolata* until its waxy consistency and made into 500mgm pills (*pattani size*). It was stored in an airtight container.

2.2 Experimental Animals

Healthy out bred Wistar Albino Rats of either sex weighing about 120 – 160 g were obtained from the Animal house of Tamil Nadu Veterinary and Animal Sciences University, Madhavaram, Chennai and maintained in the Animal house of National Institute of Siddha, Chennai. The female rats obtained were nulliparous and non pregnant. All the animals were properly maintained and strictly following the "Guidelines on care and maintenance of laboratory animals" that have been framed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forests and Climate Changes, Government of India. The animals received RO water ad libitum and fed with Rodent pellet which was purchased from Shri Venkateshwara Traders, Bengaluru. Before the induction of toxicity study, all the animals were acclimatized for seven days. The study protocol has got approval from Institutional Animal Ethical Committee of National Institute of Siddha, Chennai **(1248/Ac/09/CPCSEA-9 /Dec/2013/17).**

2.3 Acute toxicity Study: [7]

This study was carried out by following the procedure with the starting dose of 2000 mg/kg body weight of test drug mentioned in OECD 423 guideline; six female rats were randomly selected and acclimatized prior to the study. Each selected animal was kept in separate poly propylene cage and marked with picric acid on the fur for identification. The rats were fasted overnight before the administering of test drug. After the administration of test drug, the rats were deprived of feed for 16 h and water was not allowed for initial 3 h. The study was conducted initially with the starting dose of 2000 mg/kg administering in three rats and observed for mortality. As there was no mortality, three more rats were subjected to the study with the same dosage of test drug. The test drug NKM was administered through oral gavage suspended in the distilled water as single dose. The rats were observed for mortality, behavioural changes and clinical signs of toxicity for half an hour once in first four hours after dosing and thereafter periodically up to 14 days on same time of each day.

2.4 Sub-chronic toxicity study: [8]

This study was carried out by following OECD guidelines adopted for the testing of chemicals – 408. In the literature *Kannusamiyam ennum Pathartha Guna Vilakkam, Mooligai vakuppu,* the human intended dosage for NKM was recommended as *pattani* size 500 mg twice a day (1000 mg/day) . On the basis of body surface area conversion against human dose, 1000mg/kg/day dosage of NKM was calculated for rat (Paget and Barnes, 1964). In the present study, three doses of NKM of 90 mg/kg/day (Low dose), 450 mg/kg/day (Intermittent dose) and 900 mg/kg/day (High dose) were selected for administration. Wistar Albino rats of both sex were randomized into four groups of ten animals each (10 males, 10 females). Group I received a vehicle (distilled water) and served as control group. Group II, III and IV served as low, intermittent and high doses of NKM respectively. All the test substances were administered once daily via oral route through gastric gavage for 90 days. All the rats were observed daily for mortality, morbidity and abnormal clinical signs on each day for the same time. The body weight change, water and food consumption of each rat was monitored once a week. At the end of 90 days treatment, live rats were fasted over night and on the 91 st day under light chloroform anaesthesia, blood were drawn using capillary tube from the retro orbital sinus and added into a tube with potassium EDTA and a tube without anticoagulant. The haematological parameter tests such as Haemoglobin (Hb), Red Blood Cell count (RBC), White Blood Cell count (WBC), WBC Differential count - Lymphocyte, Monocyte and Granulocyte, Red Cell Distribution Width (RDW), Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelet (Plt), Platelet Crit (PCT), Platelet Distribution Width (PDW) and Mean Platelet Volume (MPV) were done in the EDTA mixed blood samples using Erba Mannhein® haematology analyser. The blood samples without anticoagulant were used for estimating biochemical parameters such as Glucose, Cholesterol, Triglyceride (TG), Protein, Urea, Creatinine, Bilirubin, Serum glutamic-oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT) and Alkaline Phosphatase (ALP) using Erba system Pack kits in Fully Automated Biochemistry analyzer (Transasia EM 360). Sodium, Potassium and Chloride content were estimated by using electrolyte analyser from Roche®. After withdrawal of blood, all the rats were sacrificed for gross necropsy and histopathological study. Organs including brain, lungs, heart, liver, kidney, spleen, testis and ovaries were studied for gross necropsy and weighed for calculating relative organ weight. Histopathological studies on liver, kidney, lungs, stomach, heart, spleen, brain, and femoro tibial joints were carried out for control and high dose group. The tissues of collected organs were fixed in 10% Neutral buffered formalin for 24 h. The tissues were trimmed, embedded in molten paraffin wax and sectioned (4-5 microns thickness) using rotary microtome. The sections were floated in hot water and placed in the glass slide. The

slides were stained with Haematoxylin and Eosin (H&E), mounted in DPX and examined under light microscope (Singh and Sulochana, 1997).

2.5 Statistical analysis:

All data were expressed as mean ± standard deviation (SD). The test groups were compared with control group for testing significance and done by One-way Analysis of Variance (ANOVA) followed by Dunnett Multiple Comparisons Test using GRAPH PAD INSTAT version 3 software programs. Values of $p<0.05$ were considered significant.

III. Results And Discussion

The herbal preparations are administered without any standard dosage due to lack of adequate scientific data on their safety. This issue has raised concerns regarding the toxicity of herbal preparations. The popular perception that natural products do not present toxic effects might be explained within this context, since the recognition of product toxicity is only associated to its use when the effects do manifest immediately after administration. As the safety profile of this preparation NKM in acute and sub chronic tests are not determined yet, this research showed the safety of this anti diabetic Siddha medicine NKM in two models of toxicity assessment. It is deemed important to evaluate the toxicity effect of herbal preparation in order to increase the confidence in their safety to humans, particularly for use in the development of nutraceuticals and pharmaceuticals. To our best knowledge, this is the first study reported the toxicity effects of *Naaval Kottai Mathirai* in rats. The acute toxicity study does not show any toxic symptoms, changes in behaviour or mortality at 2000 mg/kg doses. Through-out the 14 day periods all animals were found to be healthy with no changes in their skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system and as well as somatomotor activity and behavioural patterns. No gross pathological abnormality in the organs was found even at this high dose. On the basis of above observations, this preparation has anticipated having an LD50 higher than 2000 mg/kg bodyweight which is not hazardous in acute doses. [9]

In a sub chronic toxicity study, it appeared that the *Naaval Kottai Mathirai* at the doses (90mg/kg, 450 mg/kg, & 900 mg/kg) did not produce any marked changes in both male and female rats, as evidenced by the absence of toxic symptoms, no changes in water/food ingestion, or weight gain. Normal organ weight revealed that the study drug did not produce organ swelling, atrophy or hypertrophy. 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 [10]. During the study, the treated test groups gained weight gradually in respective to the feed intake similar to the control group. The absolute and relative organs weight was also not altered by NKM treatments [Table- 1].

The changes observed in blood parameters analysed in laboratory animals provide the evidence of risk of toxic effects on haematological system (Olsan *et al.,* 2000). The haematological profile of the treated and control groups were presented in the (Table- 2). 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. However, the significant differences noted in the parameters were 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.

The biochemical profiles of the treated group were presented in the (Table- 3). The biochemical parameters level indicates physiological condition. The increase and decrease of biochemical parameters can convey indications regarding toxicity of specific organs. In the present study, biochemical parameters were estimated particularly SGOT, SGPT, ALP, creatinine, total cholesterol and total protein were tested and the analysis showed that there was no significant differences in parameters level of the rats treated with NKM compared to the control. Although there is a slight decrease or increase in the level of biochemical parameters in treated rats compared to the control, these values were still within the normal range. The biochemical findings suggested that the administration of NKM did not cause any toxicological effect. Morphological examination on the vital organs, liver, kidney, heart and pancreas as well revealed no treatment-related changes due to the administration of NKM in the animals. The liver and kidney were studied extensively for histopathology observation because of their primary function to expel toxins that results from body's metabolism of food, drug or any other substances that was being consumed. In sub-chronic toxicity studies, the rats treated with the NKM showed normal architecture of the liver and kidney. There is no evidence of lesion due to toxic effect of NKM in the liver and kidney. (Figure no. 1 - 4) .

IV. Conclusion

The Naaval Kottai Mathirai was found to be nontoxic when oral acute and sub chronic 90-days toxicities in rats were performed. No signs of toxicity were observed in the histopathological studies. This study

data suggests that the oral administration of *Naaval Kottai Mathirai* did not induce any toxic effect at 900 mg/kg/day dose in rats, and this stands as an assurance of safe usage at its desirable human intended therapeutic dosage of 500 mgm two times a day in the practice of Siddha medicine.

Organ	Sex	Control	*Naaval Kottai Mathirai*		
			90 mg/kg	450 mg/kg	900 mg/kg
Brain (g%)	Male	1.00±0.02	1.00±0.04	1.01±0.03	0.99±0.03
	Female	0.98±0.03	0.99±0.01	1.0±0.02	1.0±0.02
Heart (g%)	Male	0.52±0.02	0.53±0.03	0.51±0.03	0.51±0.02
	Female	0.51±0.02	0.50±0.02	0.50±0.02	0.50±0.02
Liver (g%)	Male	4.63±0.19	4.65±0.19	4.69±0.24	4.68±0.17
	Female	4.49±0.13	4.62±0.19	4.77±0.18	4.70±0.16
Spleen (g%)	Male	0.50±0.03	0.50±0.02	0.49±0.02	0.49±0.01
	Female	0.47±0.02	0.49±0.02	0.49±0.01	0.50±0.02
Kidney (g%)	Male	1.37±0.06	1.42±0.06	1.43±0.04	1.47±0.09
	Female	1.37±0.05	1.40±0.03	1.40±0.02	1.39±0.02
Pancreas (g%)	Male	0.91±0.04	0.91±0.04	0.9±0.02	0.91±0.02
	Female	0.90±0.02	0.91±0.02	0.9±0.02	0.91±0.02
Testis (g%)	Male	1.95±0.06	1.89±0.12	1.89±0.09	1.90±0.10
Ovaries (g%)	Female	0.69±0.02	0.70±0.02	0.69±0.02	0.7±0.02

Table no: 1 Effect of *NKM* on relative organ weight in Male and Female Wistar albino rats - 90 day repeated oral toxicity study

Table no: 2 Effect of *NKM* on hematological parameters in male and Female Wistar albino rats – 90 day repeated oral toxicity study

Parameter	Sex	Control	*Naaval Kottai Mathirai*		
			90 mg/kg	450 mg/kg	900 mg/kg
Total WBC (10^9/L)	Male	9.82±1.61	11.16±2.86	10.03±2.16	10.95±3.57
	Female	10.76±1.28	10.98±1.58	11.2±1.25	9.03±2.76
Lymphocyte (10^9/L)	Male	7.48±1.48	8.26±2.08	7.41±1.29	8.21±2.25
	Female	8.22±1.09	8. 36±1.32	8.99±1.20	6.86±2.00
Monocyte (10^9/L)	Male	0.25±0.09	0.37±0.16	0. 31±0.13	0. 37±0.15
	Female	0.41±0.19	0. 27±0.10* (p=0.0540)	0. 27±0.10* (p=0.0540)	0. 27±0.09* (p=0.0495)
Granulocyte (10^9/L)	Male	2.09±0.56	2.53±1.05	2.31±0.92	2. 37±1. 32
	Female	2.13±0.41	2.35±0.48	1.94±0.50	1.9±0.86
Haemoglobin (g/dL)	Male	10.88±1.71	10.46±1.54	11.04±0.83	10.03±1.63
	Female	11.01±1.99	11. 32±1.26	11.7±1.59	10.99±1.15
Total RBC (10^{12}/L)	Male	6.49±0.90	6.88±1.05	6.14±0.92	6.17±1.29
	Female	6.67±0.78	5.64±1.35	6.60±1.09	6.10±1.28
RDW (%)	Male	11.2±0.57	11.87±0.96	11.57±0.43	11.72±0.56
	Female	11.32±0.64	10.75±0.47	10.44±0.74* (p=0.0108)	11.19±1.02
Hematocrit (%)	Male	31.37±3.70	31.9 ±5.43	35.4±3.75	31.3±3.84
	Female	31.23±3.35	28.2±7.09	31.5±5.33	30.5±6.81
MCV (fL)	Male	49.07±2.10	46.2±1.32** (p=0.0018)	46.5±0.92** (p=0.0023)	47.3±1.22* (p=0.0333)
	Female	49.83±2.31	49.9±1.51	51.1±1.68	49.9±1.34
MCH (pg)	Male	15.89±0.68	15.2±0.4	14.9±0.56** (p=0.0023)	14.9±1.04** (p=0.0214)
	Female	15.61±1.03	16.2±0.54	15.65±1.78	16.4±0.76
MCHC (g/dL)	Male	31.66±1.70	32.92±1.13	32.18±0.69	31.73±1.97
	Female	31.06±1.52	32.54±0.57* (p=0.0099)	31. 31±1.79	32.93±0.94** (p=0.0039)
Platelet (10^9/L)	Male	249.5±58.47	273.3±58.44	225.6±31.4	219±52.47
	Female	259.2±54.57	251.7±57.24	272±62.3	265.1±75.18
Platelet crit (%)	Male	0.12±0.05	0.15±0.03	0.12±0.05	0.09±0.05
	Female	0.15±0.03	0.13±0.05	0.13±0.07	0.16±0.06
PDW (%)	Male	14.99±0.17	14.6±0.24** (p=0.0005)	14.9±0.29	14.73±0.37
	Female	15.19±0.69	15.06±0.28	15.17±0.44	15.3±0.26

MPV(fL)	Male	5.95±0.34	5.69±0.20	5.88±0.25	5.63±0.3* (p=0.0386)
	Female	5.96±0.48	5.91±0.22	5.96±0.39	6.08±0.32

WBC: White blood count; RBC: Red blood cell; RDW: Red cell distribution width; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PDW: Platelet distribution width; MPV: Mean platelet volume

Values are expressed as mean ± S.D. (n = 10). P value calculated using unpaired t test. Significance is indicated as *p < 0.05 and **p < 0.005 vs control group.

Table no: 3 Effect of Naaval Kottai Mathirai on biochemical parameters in Male and Female Wistar albino rats - 90 day repeated oral toxicity study

Parameter	**Sex**	**Control**	***Naaval Kottai Mathirai***		
			90 mg/kg	**450 mg/kg**	**900mg/kg**
Glucose (mg/dl)	Male	85.6±10.91	92.9±11.47	92.5±9.07	86.30±9.73
	Female	90.8±8.81	95.20±14.58	90.6±7.19	87.3±10.7
Cholesterol (mg/dl)	Male	55.2±4.41	60.6±4.83	64.9±4.20** (p=0.0001)	67.3±8.78** (p=0.0011)
	Female	55.3±4.47	65.9±7.06** (p=0.0008)	67.4±4.00** (p=0.0001)	56.4±6.73
Triglyceride (U/L)	Male	70.1±4.88	79.2±7.20** (p=0.0039)	72.1±5.54	72.2±5.05
	Female	69.4±4.69	71±6.32	70.2±7.16	69.7±7.98
Protein (g/dL)	Male	6. 51±0.55	6.13±0.38	6.14±0.58	6.19±0.58
	Female	6.35±0.67	6.01±0.46	6.07±0.62	6.06±0.57
Urea (mg/dl)	Male	29.6±5.31	36±4.13* (p=0.0075)	35.2±4.49	33.30±7.18
	Female	30.9±4.40	34±4.73	36.7±3.97* (p=0.0062)	33.3±6.21
Creatinine (mg/dl)	Male	0.78±0.22	1.01±0.20* (p=0.0249)	0.95±0.13	0.73±0.14
	Female	0.82±0.23	0.98±0.21	0.8±0.10	0.97±0.16
Bilirubin (mg/dl)	Male	0.5±0.2	0.65±0.29	0.51±0.16	0.53±0.12
	Female	0.54±0.18	0.55±0.16	0.49±0.11	0.48±0.12
SGOT (U/L)	Male	27.6±3.33	26.5±2.32	27.5±3.24	31.6±12.49
	Female	27.4±3.13	28.5±5.08	27.4±5.91	25.4±3.94
SGPT (U/L)	Male	56.4±4.11	51±3.65** (p=0.0061)	58±3.77	60.10±3.21
	Female	58.3±5.16	56.4±6.07	55.3±7.25	56.3±4.05
ALP(U/L)	Male	127.2±8.05	126.4±9.61	121.7±11.46	121±12.78
	Female	127±9.41	122.70±15.34	126.3±11.75	129.2±11.35
Sr. Cal	Male	7.93±1.08	7.57±1.02	6.65±0.73* (p=0.0001)	7.15±1.03
	Female	7.99±1.20	6.88±0.56* (p=0.0163)	8.75±1.30	7.68±0.61
Sr.Phos	Male	12.35±0.79	10.54±2.07	13.17±1.27	12.02±2.50
	Female	12.16±0.93	12.46±1.88	12.46±2.68	8.63±1.52* (p=0.0001)
Sodium (mmol/L)	Male	140.05±1.88	139.76±1.59	139.72±1.73	139.91±2.13
	Female	139.2±2.18	139.41±1.69	139.22±2.63	139.33±2.11
Potassium (mmol/L)	Male	4.84±0.24	4.85±0.21	4.89±0.21	4.76±0.26
	Female	4.88±0.22	4.87±0.22	4.83±0.24	4.79±0.22
Chloride (mmol/L)	Male	109.57±2.74	108.79±2.28	109.3±2.91	110.17±2.25
	Female	109.41±1.96	109.25±2.81	108.5±2.39	109.42±2.54

SGOT: Serum Glutamic Oxaloacetic Transaminase; SGPT: Serum Glutamic Pyruvic transaminase; ALP: Alkaline Phosphatase.

Values are expressed as mean ± S.D. (n = 10). P value calculated using unpaired t test. Significance is indicated as *p < 0.05 and **p < 0.005 vs control group.

Control, Liver-NAD

Figure-1

High Dose Liver-NAD

Figure-2

Control, Kidneys -NAD

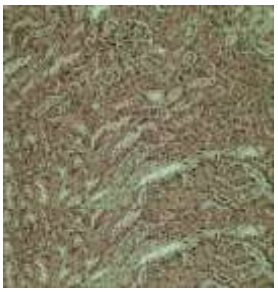

Figure-3

High Dose Kidneys-NAD

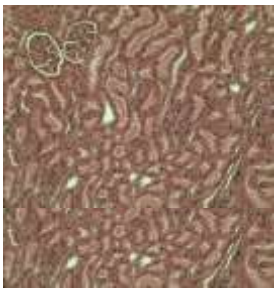

Figure-4

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