

Toxicity Studies Of Traditional Siddha Polyherbal Formulation *Kadukkai Nei* In Adult Albino Wistar Rats

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Abstract

Introduction: Siddha system is an ancient holistic system of medicine originated from south India now comes under AYUSH becoming popular all over the world. The principle of Siddha system of medicine is “Food is medicine; Medicine is food” that promotes the health of body, mind and soul. *Kadukkai Nei* an internal Siddha medicine contains eleven herbals and it also includes cow’s milk and ghee. *Kadukkai Nei* is indicated for obesity in classical Siddha text. Though this is a time tested traditional medicine, it lacks scientific validation. Hence it was planned to conduct acute and sub chronic toxicity studies for *Kadukkai Nei* in Adult Albino Wistar rats.

AIM: To evaluate Acute and Subchronic toxicity studies for Siddha Polyherbal formulation *Kadukkai Nei* among Adult Albino Wistar rats.

Materials And Methods: In acute toxicity study 3 animals for each step for a total of 2 steps (n= 6 animals) were used. 2000 mg/kg of body weight as starting dose with doses depleting to 300mg, 50mg, and 5mg that were prepared shortly prior to administration. In sub chronic toxicity study 10 animals of both the sexes were used in each group. Hence total of 60 animals were used in 3 groups. *Kadukkai Nei* (KN) in two doses 200mg, 400mg/kg body weight were administered orally.

Results And Conclusion: The results of acute toxicity study indicated that *Kadukkai Nei* via oral route did not produce any signs of toxicity and no mortality were found in animals during the 14 days of study period. Sub chronic toxicity study proven that the drug *Kadukkai Nei* was well tolerated for about 90days at two dose levels.

Keywords: *Kadukkai Nei*, Siddha Polyherbal medicine, Toxicity studies

INTRODUCTION

Siddha system is an ancient holistic system of medicine originated from south India now comes under AYUSH becoming popular all over the world. The principle of Siddha system of medicine is “Food is medicine; Medicine is food” that promotes the health of body, mind and soul. Siddha system is an Ancient Holistic system of medicine originated from south India. *Agathiar* the first and foremost *siddhar*, guru of all *Siddhars*, known as “Father of Siddha medicine” laid the foundation of this system. In *Siddha* concept, the healthy soul only can be attained through a healthy body and mind. Hence Siddha system of medicine constitutes internal and external medicines to strengthen the physical body and mind. *Kadukkai Nei* an internal Siddha medicine found to have 11 herbals and it also includes cow’s milk and ghee. *Kadukkai Nei* is indicated for obesity, anemia, anasarca and respiratory infections. Hence it was planned to conduct toxicity studies for *Kadukkai Nei* in adult albino wistar rats. Therefore, the study aimed to evaluate Acute and sub chronic toxicity studies for Siddha Polyherbal formulation *Kadukkai Nei* among adult Albino wistar rats.

MATERIALS AND METHODS

Toxicity studies

This animal study protocol was approved by Institutional Animal Ethics Committee (IAEC) that was conducted in KK College of Pharmacy, Gerugambakkam, Chennai- 122, according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi. CPCSEA Approval No. 135/PO/Re/S/10/CPCSEA) and Project approval No. KKCP /2018/42 and KKCP/2019/12.

Acute toxicity study

Acute toxicity study was conducted to determine the Maximum Lethal Dose after single oral administration of test substance according to Acute toxicity class method OECD guidelines 423.

Experimental animal

Healthy young adult female Wistar rats, 8–10 weeks old, weighing between 200–300 grams and nulliparous (non-

pregnant) rats were used for the study. During the overall period of toxicity study, the experimental animals were housed under optimal environmental conditions, maintained at a temperature of 25°C–30°C and relative

humidity of 30%–70%, with a 12:12-hours light and dark cycle. They were provided regular access to drinking water and a conventional laboratory diet. Animals were randomly selected and acclimatized to laboratory conditions for at least five days before the test. Approximately 10% of fur was removed from the dorsal area of the back using a hair remover cream to avoid any abrasion to the skin. A total of six animals were used, with three animals in each step of the two-step procedure. The dose level began with a starting dose of 2000 mg/kg of body weight, with subsequent doses decreasing to 300 mg, 50 mg, and 5 mg, all of which were prepared shortly before administration. Prior to dosing, animals were fasted and then weighed to ensure accurate dosing.

Grouping of Animal

It is a step down procedure. Absence or presence of mortality if seen in animals dosed at one step will determine the next step. Dosing of another 3 animals can be at next higher or lower level. The advantage of this procedure is minimum usage of animals. *Kadukkai Nei* (Test substance) was administered in a single dose by gauge using a stomach tube. Food was withheld for 3 – 4 hrs after administration of test substance.

Observations

After dosing animals were observed keenly for first 30 minutes and special attention was given during first 4 hrs, intermittently for next 6 hrs, later 24 hrs – 48 hrs, so observed for a total of 14 days for the signs of toxicity. Noted for the time of signs of appearance and disappearance of toxicity. Observations like changes in skin colour, fur, eyes, respiratory, circulatory, ANS, behavioral pattern, tremors, convulsions, diarrhea, salivation, lethargy etc. Individual weight of the animal should be determined shortly prior to test substance administered and at least weekly thereafter weight changes were recorded. At the end of the test surviving animals will be weighed and will be sacrificed as per CPCSEA norms.²

Sub chronic toxicity study- OECD guidelines 408

Sub chronic toxicity study was conducted in male and female adult Albino Wistar rats, 8-10 weeks old according to OECD guidelines 408.

Experimental animals

Healthy young adult male and female nulliparous (non-pregnant) adult Albino Wistar rats, 8–10 weeks old, weighing between 200–300 grams were used in this study. During the overall toxicity study, the experimental animals were housed under optimal environmental conditions, maintained at a temperature of 25°C–30°C and relative humidity of 30%–70%, with a 12:12-hours of light and dark cycle. They were provided with a regular supply of drinking water and a conventional laboratory diet. The animals were randomly selected and acclimatized to laboratory conditions for at least five days prior to the test. Approximately 10% of fur was removed from the dorsal area of the back using a hair remover cream to avoid any abrasion to the skin. Ten animals of both sexes were used in each group, making a total of 60 animals across three groups. *Kadukkai Nei* (KN) was administered orally in two dose levels, 200 mg/kg body weight and 400 mg/kg body weight, using a stomach tube. The dose was prepared shortly prior to administration, and animals were fasted before dosing.

GROUPING OF ANIMALS

Table 1.1 Animals for Sub chronic toxicity study

Groups	Dose level	Animals
Group I	Normal Control	10 male +10 female rats
Group II	Low dose KN 200 mg/ Kg	10 male +10 female rats
Group III	High dose KN 400 mg/ Kg	10 male +10 female rats

Test animals were divided into 3 groups. Group I animals were given normal lab diet and drinking water. Group II and Group III animals were treated with *Kadukkai Nei* in the doses of (200mg, 400mg) given by oral gauge daily for 90 days respectively. Food was withheld for 3–4hrs after administration of test substance. Twice daily all the animals were observed for morbidity and mortality. All the animals were weighed at least a week. Measurements of food / water consumption were made at least weekly. Signs of changes in skin, eyes, mucous membrane, any secretions and excretions, autonomic activity eg: lacrimation, Piloerection, pupil size, unusual respiratory pattern, changes in gait, posture, clonic or tonic movements, excessive grooming, repetitive circling, bizarre behavior eg: self-mutilation, walking backwards were recorded. Hematology examination, bio chemical examinations were done. All the gross pathological lesions were examined for heart, liver, brain, spleen and kidneys.³

RESULTS

Acute toxicity study

Table1.2: Observations on signs of acute toxicity for 14 days

Alertness	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aggressiveness	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rearing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Grooming	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Touch response	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alopecia	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tremors	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Muscle spasm	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catatonia	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hypnosis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Exophthalmos	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eyeclosure on touch	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Diarrhoea	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Writhing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oedema	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abnormal respiration	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Circling	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pinna reflex	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Straub tail	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Seizures	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urine stains	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Skin colour	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-	-	-	-	-	-

In Acute toxicity study, the dose of KN 2000 mg/ kg b.w in a single and as a starting dose was administrated to the female adult albino Wistar rats which were fasted overnight. Food with held for further 3-4hrs to observe the signs of toxicity. On hourly observation there was alertness, grooming, touch response ,eye closure on touch were present for 4hrs, 6hrs and 24-48 hrs totally for 14 days.

Table1.3: Observation of mortality in animals of acute toxicity study

Study drug	Dose level	No. of animals used/ Gender	Mortality			Toxicity profile
			After 24 hours	After 7days	After 14 days	
<i>Kadukkai Nei</i>	2000 mg/Kg	3/ Female	0	0	0	No adverse effects

For acute toxicity study, the step down procedure had been followed and in the first step 3 animals were taken. Depending upon the toxicity of the study drug the next step with 3 animals could be included in the study. From the above table it was evident that the test drug KN at a single dose of 2000 mg /kg produced no toxic signs for 24 hours, 7days and totally for 14 days.No mortality was found during the 14 days of study period after administration of *Kadukkai Nei* at the maximum dose of 2000mg/kg of body weight.

Table1.4: Biochemical profile of acute toxicity study

Biochemical tests	Control	KadukkaiNei
Blood glucose(mg/dl)	106.83±6.22	100.2±11.21
SGOT(AST)U/L	110.53±8.10	103.43±8.90
SGPT(ALT)U/L	58.47±5.40	53.30±3.59
ALP(U/L)	45.03±1.52	40.83±3.36
Total Protein(g/L)	5.73±0.19	6.17±0.99
Total Bilirubin(mg/dl)	1.37±0.34	1.27±0.19
Urea(mg/dl)	39.33±1.37	39.67±4.03
Creatinine(mg/dl)	0.56±0.01	0.58±0.03
Uric acid	5.30±0.11	5.45±0.12
Total Cholesterol(mg/dl)	52.70±0.78	53.57±1.66
LDL(mg/dl)	72.28±0.81	72.39±0.59

Values were expressed as mean ±SD (n=6). Values of HDL significant when compared with control. **p<0.01(one way ANOVA followed by Post hoc t-test).

TGL(mg/dl)	104.97±3.78	105.53±3.77
HDL(mg/dl)	26.73±0.01	31.60±1.14**

Biochemical profile of acute toxicity study of KN showed no abnormal values of Liver profile (SGPT, SGOT, ALT, total bilirubin, total protein, blood glucose), Renal profile (Urea, Creatinine, Uric acid) and Lipid profile (TC, TGL, LDL, HDL) when compared with control. Values were expressed as mean ± SD (n=3).

Table 1.5. Hematological profile of acute toxicity study

Hematological tests	Control	KadukkaiNei
Hemoglobin(g/dl)	15.27±0.45	14.22±0.52
RBC(10 ⁶ cells/μL)	8.85±0.20	8.78±0.09
WBC(10 ³ cells/μL)	10.92±0.03	11.29±0.04***
Neutrophils(%)	14.49±0.94	14.69±0.41
Eosinophils(%)	0.67±0.02	0.69±0.41
Lymphocytes(%)	81.70±0.50	82.53±1.04
MCV	55.10±0.96	56.02±1.57
MCH	18.57±0.51	18.97±0.58
MCHC	36.30±0.80	35.47±2.90
Platelets(10 ³ cells/μL)	820.23±53.20	801.13±46.08

Values of WBC were significant when compared with Control. *** P<0.001(one way ANOVA followed by Post hoc t-test). Hematological profile of acute toxicity study of KN showed increased lymphocytes there by promotes the immune level.

Table 1.6. Functional behavior of animals in acute toxicity study

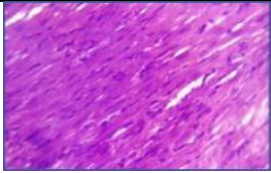
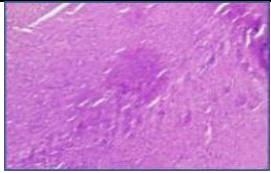

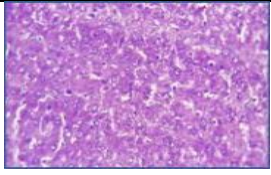
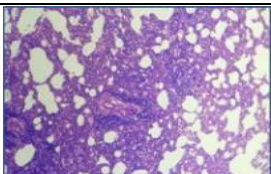
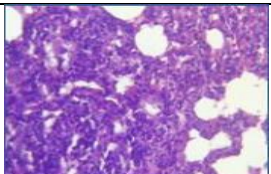
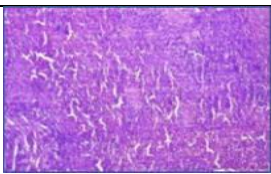
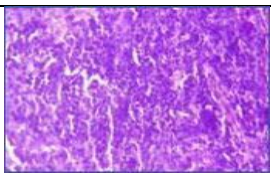
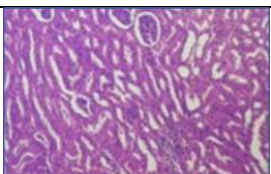
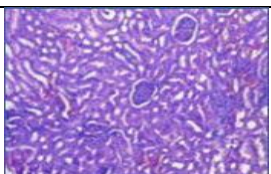
General appearance	Observations
Skin colour	Normal
Animal appearance	Normal
Behaviour	Normal
Motor activity	Normal
Convulsions	No
Tremor	No
Aggressiveness	Not aggressive
Writhing reflex	No

Touch response	Normal
Tail Pinch response	Normal
Palpebral closure	Normal
Breathing	Normal
Urination	Normal
Salivation	No
Lacrimation	No
Diarrhoea	No

No abnormalities were detected from the functional behavior of animals in acute toxicity study inferred that CNS, ANS, respiratory system and digestive system were not affected totally for 14 days.

HISTOPATHOLOGICAL CHANGES IN ACUTE TOXICITY STUDY OF KN TREATED GROUPS

Figure-1. Histopathological changes on the effect of KN in acute toxicity study

S.NO	ORGANS	CONTROL GROUPS	DRUG TREATED GROUPS
1	HEART		
2	LIVER		
3	LUNGS		
4	SPLEEN		
5	KIDNEYS		

Histopathological study of acute toxicity study, normal architecture was observed in both the control and drug treated groups. Hence no organ damage found in acute toxicity study of KN.

Figure -2. Effect of KN towards food intake in sub chronic toxicity study

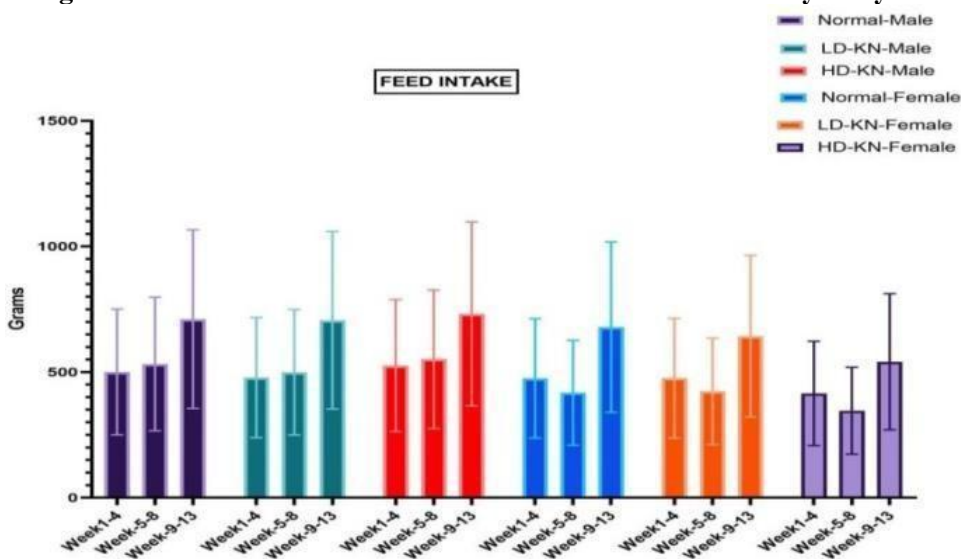
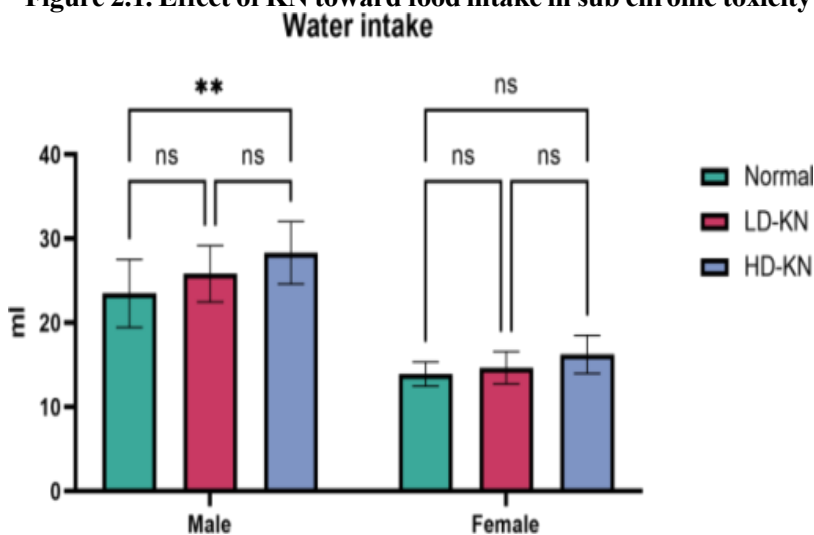


Figure 1.2: Feed intake–Sub chronic toxicity study

Less feed intake were seen in HDKN female wistar rats when compared with HDKN male and compared with LDKN male and female rats during the experimental period of 90 days of toxicity study.⁴ Feed intake will be reduced while taking anti-obesity drugs.⁵ Thus KN might be more sensitive to female rats and hence the reduction of feed was observed in female rats during 90 days of study period and found that the anti obesity activity of KN was more pronounced in adult female albino wistar rats.

Figure 2.1. Effect of KN toward food intake in sub chronic toxicity study



Less water consumption seen in female rats compared with male rats during the experimental period of 90 days of toxicity study. The more feed and the reason for more water consumption. The food and water consumptions of the drug treated rats, which were measured throughout the study, were not statistically significant compared to the control group of rats.

Effect of KN towards functional behavior in sub chronic toxicity study

Physical observation of the drug treated rats showed no signs of toxicity in their skin, fur, eyes, mucus membrane, or behavioral changes, diarrhea, tremors, salivation, sleep, and coma. Hence functional behavior of Sub chronic toxicity study produced no abnormal findings.

Table2.1: Effect of KN towards biochemical profile in sub chronic toxicity study

Test/Parameters	Male			Female		
	Control	LD-KN	HD-KN	Control	LD-KN	HD-KN
Glucose (mg/dl)	109.30±4.99	101.10±7.41	129.6±6.55	97.12±5.16	106.40±13.46	108.14±9.71
SGOT (AST) U/L	122.37±7.01	111.78±5.01	107.76±5.22	94.73±4.14	103.52±6.27	103.78±6.03
SGPT (ALT) U/L	59.07±2.20	67.14±6.03	62.92±4.08	53.47±2.57	55±3.27	51.02±1.57
ALP (U/L)	151.56±15	158.54±227.22	155.12±9.71	119.22±29.99	109.21±12.20	128.20±6.16
Total Protein (g/L)	6.83±0.09	6.16±0.08	6.35±0.25	6.69±0.27	6.45±0.45	6.59±0.12
Total Bilirubin (mg/dl)	0.62±0.10	0.52±0.18	0.78±0.18	0.42±0.17	0.44±0.16	0.46±0.07
Urea (mg/dl)	19.15±1.11	18.88±0.88	18.68±1.41	22.23±0.42	22.22±0.95	27.15±4.92
Creatinine (mg/dl)	0.53±0.03	0.47±0.04	0.45±0.11	0.56±0.03	0.50±0.04	0.51±0.03
Uric acid (mg/dl)	5.34±0.55	5.52±0.42	5.32±0.59	5.42±0.64	5.58±0.45	5.30±0.93
Total Cholesterol(mg/dl)	60.48±4.81	65.82±3.41	71.60±16.11	65.79±3.98	80±10.04	91.8±10.1
LDL (mg/dl)	20.75±2.50	25.36±2.34	28.78±3.24	26.90±4.16	27.26±1.45	34±3.63
TGL (mg/dl)	50.17±3.84	72.80±12.12	84.80±7.87	57.40±2.72	67.40±19.51	102.6±21.59
HDL (mg/dl)	21.22±2.90	26.55±2.31	43.78±3.06	25.17±2.85	27.92±1.03	42.60±7.07

From the blood samples of all the animals the results of sub chronic toxicity study Biochemical,haematological parameters were measured using Digital Siemens kits. Data were expressed as mean ±SD. (n=10). (One way ANOVA followed by (Post hoc t-test), when compared with control of both male and female rats.

Lymphocytes values were increased both in acute and Sub chronic toxicity study. Hence the KN administered group of rats showed increased lymphocytes revealed that KN has influence over immune system. No statistically significant differences were recorded in hematological parameters, inferred that KN didn't produce any abnormalities in the blood cells.

Table2.2: Effect of KN towards organ weight of animals in Sub chronic toxicity study

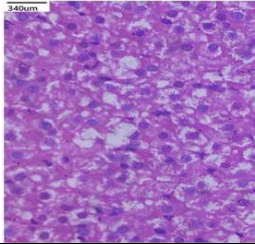
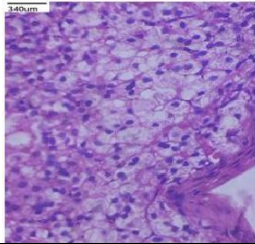
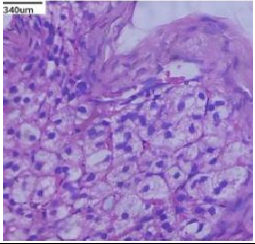
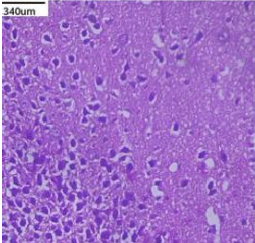
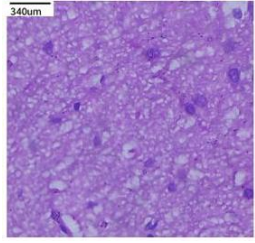
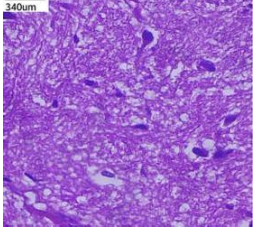
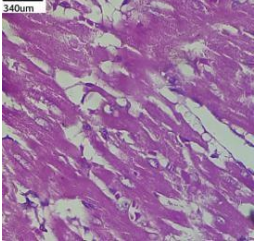
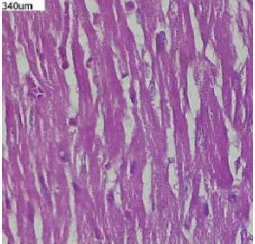
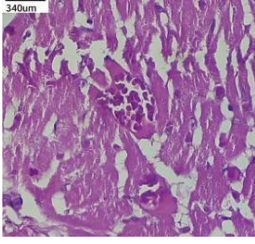
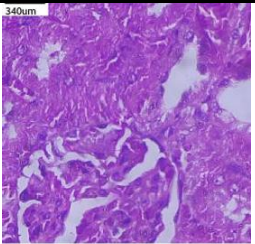
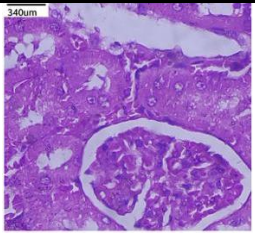
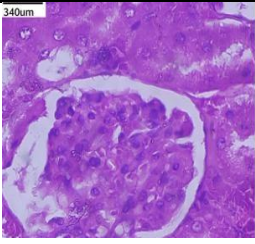
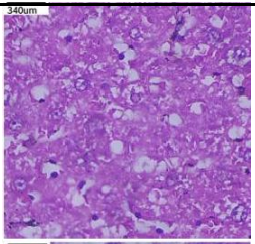
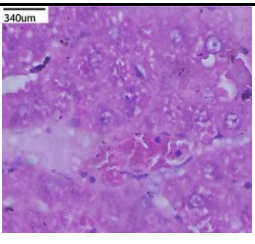
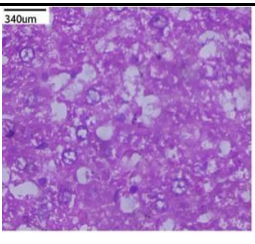
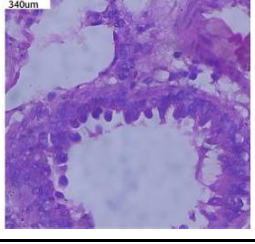
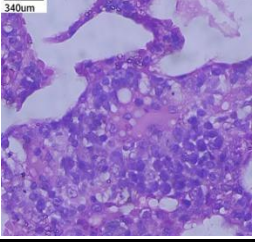
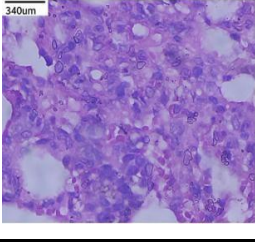
Organs	Normal Male	Male-LDKN	Male-HDKN	NormalFemale	Female LDKN	Female HDKN
Brain	2.18±0.08	1.97±0.11	1.98±0.13	1.98±0.13	1.98±0.05	1.98±0.08
Heart	1.31±0.23	1.25±0.14	1.29±0.28	1.29±0.28	1.12±0.06	1.07±0.02
Lungs	1.33±0.09	1.3±0.08	1.31±0.08	1.31±0.08	1.32±0.04	1.32±0.10
Liver	7.8±1.20	9.4±2.0	9.4±1.1	9.4±1.1	9.8±2.4	9.2±0.8
Spleen	0.71±0.18	0.71±0.01	0.694±0.03	0.694±0.03	0.738±0.08	0.7±0.02
Kidney	1.492±0.06	1.61±0.10	1.56±0.14	1.56±0.14	1.55±0.07	1.50±0.22
Stomach	1.66±0.10	1.68±0.14	1.64±0.39	1.64±0.39	1.65±0.18	1.70±0.35
Pancreas	0.114±0.014	0.13±0.012	0.126±0.011	0.126±0.011	0.146±0.047	0.122±0.012
Testes	0.69±0.3	0.65±0.10	0.66±0.2			
Ovary			0.39±0.03	0.38±0.04	0.43±0.03	

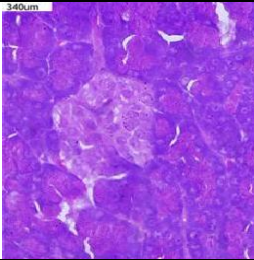
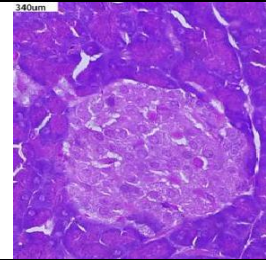
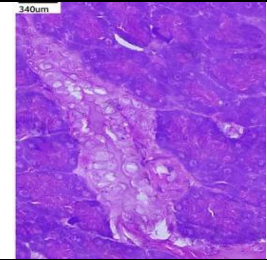
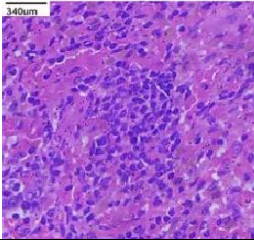
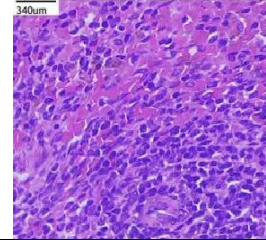
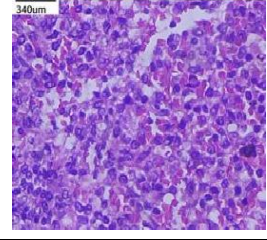
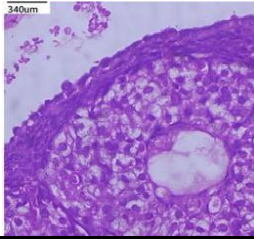
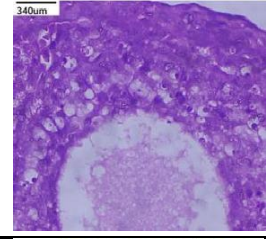
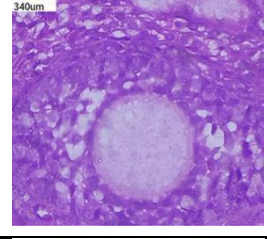
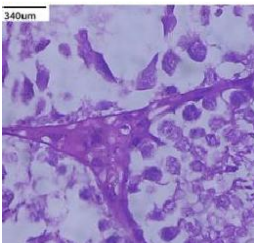
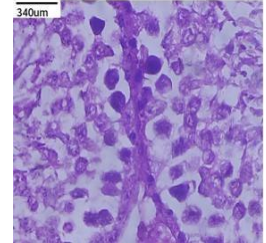
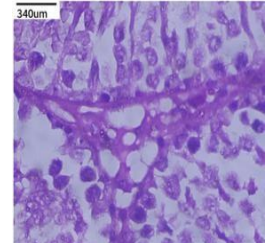
No significant changes seen with weight of organs of LDKN, HDKN male and female rats compared with Normal male and female adult albino wistar rats.

Histopathology reports of Sub chronic toxicity study

At the end of the study all the animals were euthanized and the organs were sent for histopathological analysis. At the final magnification of 100x tissues samples were photographed and analysed in an optical Microscope.

Figure-2.2 Effect of KN on Histopathological changes in KN treated groups and control groups in Sub chronic toxicity study

S.NO	Organs	Normal control	KN_LOW DOSE (200mg/kg)	KN_HIGH DOSE (400mg/kg)
1	ADRENAL			
2	BRAIN			
3	HEART			
4	KIDNEY			
5	LIVER			
6	LUNGS			

7	PANCREAS			
8	SPLEEN			
9	OVARY			
10	TESTIS			

No significant abnormality or cellular changes were observed in control groups and KN treatment groups of rat models.

DISCUSSION

There has been a rapid increase in the acceptance and popularity of traditional medicine all over developing world and the use is rapidly on the increase. Despite its global attention, traditional medicines face false accusations by its critics as it is being claimed to have hepatotoxicity, nephrotoxicity and very few of them have been clinically and preclinically proven.^{6,7}

Therefore the present work has been under taken to meet the safety of Siddha time tested formulation KN. Present study shows that the traditional Siddha formulation KN is safe at a dose of 2000mg/ kg b.w (Table -1.1) in a single oral dose indicating that the drug does not cause any acute toxicity even at high doses. The results sub chronic toxicity revealed no treatment related death or signs of toxicity in the treated animals in all the doses throughout the study. All hematological parameters were found to be within the normal physiological limits for rodents and no significant changes had been observed in treatment groups when compared with the control groups (Table 1.2 - Table 1.4)). Hence there were no serious toxicological implications such as destruction of Erythrocytes. Urea is the main product of protein metabolism and an increased level of urea in the blood is an indicator of renal impairment. But here in KN no significant changes pertained to renal parameters. In the biochemical parameters, serum markers and its chief enzymes such as Alanine amino Transaminase (ALT) and Aspar-tate amino transaminase (AST) were associated with health indices and are of diagnostic significance in routine clinical evaluation of the state of health and liver damage.⁸ The study drug KN did not cause any significant alteration in renal and liver parameters both at Low dose (200mg/Kg) and at high dose (400mg/Kg) in 90 days of Sub chronic toxicity study (Table-2.1 and 2.2) .

In acute toxicity study, there was no significant changes in the histopathology of vital organs that were tested. In sub chronic toxicity study of both low dose and high dose treated KN groups, the adrenal glands did not show any abnormality. In both the LDKN and HDKN groups of rats, the brain cells appeared normal in architecture, with no significant difference observed when compared to the normal group. Similarly, the kidney tissues did not show any noticeable structural abnormalities or significant differences across the groups. In the LDKN group, the heart exhibited normal histology, characterized by myofibrillar structures with striations and a branched appearance. In the Kidneys, normal cellularity were observed in the glomeruli, tubules, and interstitium between the tubular structures, with the capillary wall thickness also appearing normal. The liver sections also demonstrated distinct, normally shaped hepatocytes without lipid accumulation or other histological abnormalities. There was no significant difference between the normal groups and the treatment groups, and the lung cell architecture appeared normal. The pancreas was normal in appearance with no abnormalities such as infiltrations or inflammation, and the islets appeared normal in architecture. The spleen parenchyma cells showed no significant difference from the normal group. There was no infiltration or presence of lymphoid cells, indicating that the spleen architecture was normal. In Testis and ovaries, no pathological changes were observed, and showed normal architecture. When compared with normal tissues, histopathology of all the tissues of Low dose KN (200mg/kg) and High dose KN (400mg/kg) groups did not reveal any significant difference between animals. No abnormal gross findings were observed in the necropsies of any of the rats.

Kadukkai Nei was found to be safe which was evident from the acute toxicity study. Daily oral administration of KN for 90 days did not produce any symptoms of toxicity in rats of either sex, in the low and high doses tested at 200 and 400 mg/kg body weight. Hence from the results of sub chronic toxicity study it was proved that the drug Kadukkai Nei was well tolerated in repeated dose oral administration at the two (Low dose 200mg, High dose 400mg) dose levels in the study animals and there was no mortality and no abnormal signs were found in rats of group II and group III throughout the experimental period of 90 days.

CONCLUSION

The present acute and Sub-chronic toxicity results of *Kadukkai Nei* suggested that LD50 of developed formulation showed no mortality and no abnormal signs at the maximum dose of 2000mg/kg. Further studies on its target action at molecular levels, gene expression and clinical trials may be rational to substantiate the study results.

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