Original Research Paper

Volume-9 | Issue-4 | April-2019 | PRINT ISSN No 2249-555X

Ayurveda

ACUTE AND CHRONIC TOXICITY STUDY OF SIDDHA HERBAL FORMULATION NELLI KUDINEER SAMULAM

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ABSTRACT

BACKGROUND: The Plant (Herb) is still considered among the important source of bioactive compound, especially in Siddha medicine that has been used for long peroids. The Nelli Kudineer (NK) has been mentioned in classical Siddha literature *GunapadamMooligaiVaguppu(Murugesu Mudaliyar C.S,2013)(1)* for the management of R*athamoolam (Shanmugavelu.K.N.,2003)(2)* and it has been correlated symptoms in modern medicine is Bleeding Haemorrhoids.

OBJECTIVE: The objective of this study was to investigate the acute and Chronic toxicity of Siddha herbal formulation Nelli Kudineer (Samulam).

METHOD: Acute toxicity and chronic toxicity of Nelli Kudineer (NK) is carried out as per the OECD-423 guidelines. In the acute toxicity study were used in female albino Wister rats, single and multiple enteral dose (60,300,2000 mg/kg) for 14 days administered all group of treated animals. At the end of the study, the trail animals are sacrificed and results were recorded.

RESULTS: The results are assessed for the effect of Nelli Kudineer. Animals body weight, relative organ changes, haematological, biochemical and histopathological parameters showed good progress. In the acute and chronic toxicity studies no mortality or behavioural changes were observed in treated rats used in Nelli Kudineer (2000 mg/kg) indicating that the LD 50 was less than Value is P 0.05.

CONCLUSION: These results exhibit the absence of acute and chronic toxicity after treatment of Nelli Kudineer was observed. So, all the results were revealed NK is safer and high therapeutic uses in long period.

KEYWORDS : Nelli Kudineer, Amla, Siddha Medicine, Toxicity studies.

INTRODUCTION

In clinical practice, Nelli Kudineer was used in ano-rectal disorders, especially in rathamoolam(Haemorrhoids). Moolanoi is a common problem in a modern word, because diet and life style is more prevalence and incidence of disease. NK is basically astringent in nature and reduces the dilated blood veins. The "*Rathamoolam*" is in *YugiVaidyaChinthamani*-800.(*Ramachandran.S.P,.2013)(3)* it can be correlated in Modern Medicine as Bleeding Haemorrhoids. All the veins are lined with valves that permit blood to flow in only one direction (back to the heart). Excess pressure on these valves can cause them to weaken and fail, allowing blood to flow in the wrong direction or to stagnate, it causes haemorrhoids.

The Nelli Kudineer, was majorly composed of Phyllanthus Emblica (Linn.) Phyllanthaceae family. The tree is small to medium in size, reaching 1–8 m (3 ft 3 in–26 ft 3 in) in height. The branchlets are not glabrous or finely pubescent, 10–20 cm (3.9–7.9 in) long**.**The fruit is nearly spherical, light greenish yellow, quite smooth and hard on appearance, with six vertical stripes or furrows (Yoganarasinhan 2000). It is Sour, Astringent and Sweet in taste, Cold potency, sweet in division as per Siddha Literature (Murugesan 2013). Amla is an extremely rich source of vitamin C. It also balances the both Pitham and Vaatham by virtue of sweet taste. The Kapham is balanced primarily due to its drying action. So it is essential to evaluate the safety and toxicity of the Nelli Kudineer, before their uses in human health. Preclinical toxicity studies are necessitate for determining a safety profile.

MATERIALS AND METHODS

MATERIALS

Collection and Authentication

The parts of Nelli were freshly collected from Tenkasi, Tamilnadu area and identified by the Gunapadam department experts at Government Siddha Medical College and Hospital, Palayamkottai. Whole part of amla used in this study.

PURIFICATION AND PREPARATION

The adulterants from the raw drugs were removed, cleaned and dried in shade. The purified raw drugs were coarsely powdered and taken as a Kudineer Chooranam form.

EXPERIMENTALANIMALS

The female Wister albino rats, weighing 180-200g±20 were taken in this study. All animals were maintained under standard laboratory conditions of temperature (22±2 °C) and humidity 50±15% with 12 h day 12 h night cycle. Rats had free access to water and rodent pellet diet (Hindustan Lever Ltd, Bangalore, India). Animals were acclimatized to laboratory conditions one week prior initiation to the experiments.

TOXICITY STUDY METHOD:

Acute and chronic toxicity of Nelli Kudineer is carried out as per the guidelines (OECD) 423. After the animal ethical clearance from Institutional Animal Ethics Committee (KMCP/29/1.5.18).

ACUTE ORAL TOXICITY

The female Wister albino rat are fasted over night and provided only water, after which the Nelli Kudineer is administered by gastric intubation to Group1 animals orally administered the dose of 50 $mg.kg^{-1}$ body weight in *Nelli Kudineer*. The animals are then observed for 14 days and maintained with normal food. No mortality rate were observed after 14 days, all the animals are noted, no toxic effects were observed in this study., then the same dose is repeated again for confirmation. However. The procedure is repeated for further higher doses such as 300 and 2,000 $mg.kg^{-1}$ body weight. No mortality of animal is noted. Toxic symptoms are observed for 72 hrs including behavioral changes, locomotion, convulsions and mortality (Shetty Akhila et al 2007 & Shah Ayub et al.1997, Bürger et al.2005)(4,5,6)

Cage Side/Histopathology Observations:

All the animals were observed, including the changes in skin, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems, and somatomotor activities are noted. End of experiment no tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted. Body weight, food and water intake are recorded at two-day intervals. Surviving animals are fasted overnight, weighed and humanely killed on the 15^{th} day using anesthetic ether. All test animals are subjected to gross necropsy.

Chronic toxicity for Nelli Kudineer

The Male and female Wister albino rats weighing 180-200 ± 20 g are used for the present study. The animals are divided into five groups of six animals in each group. The animals in Group I are administered 0.5 ml Tween orally for 90 days. In Group II are administered with 50 $mg.kg^{-1}$ b.w. of the Nelli Kudineer orally once daily for 90 days. The animals in Group III are administered with 100 $mg.kg^{-1}$ b.w. of the Nelli Kudineer orally once daily for 90 days. The animals in Group IV and V are administered once daily with 200 and 400 $mg.kg^{-1}$ b.w. of the Nelli Kudineer for 90 days orally (Pieme,*et al* 2006, Joshi, *et al* 2007,

Mythilypriya, *et al.,* 2007).(7,8,9) The animals are then weighed first and every five days and recorded the weight variations. At the end of the treatment, blood samples are collected by puncturing retro orbital plexus after mild anesthesia for biochemical analysis. which is analyzed for total cholesterol, total triglyceride, HDL-cholesterol levels, LDL-cholesterol, plasma glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), Urea and creatinine level.

RESULTS

Acute toxicity study with Nelli Kudineer

There was no mortality or morbidity were observed in three group of animals, during 14-days period in single 50,300,2000 mg/kg/bw, oral administration all selected animals(**Table-1**). The animals did not show any changes in the general appearance during the observation period. Morphological characteristics such skin, eyes and nose appeared in normal, found no tremors, convulsion, salivation, diarrhea, lethargy or unusual behaviors were observed. Gait and posture, reactivity to handling or sensory stimuli, grip strength was also normal.

Table.1.Acute toxicity study of NelliKudineer on experimental rat

	Dose(mg.kg^{-1})	Sign of Toxicity (ST.NB^{-1})	Mortality (D.S^{-1})
Group I	0	0/3	0/3
Group II	300	0/3	0/3
Group III	2000	0/3	0/3

ST- sign of toxicity; NB- normal behaviour; D- died; S- survive. Values are expressed as number of animals (n=3).

Chronic Toxicity of Nelli Kudineer:

Table .2.representatd the effect of Nelli Kudineer (NK) was observed after 20 days, the body weight changes was noted ($p<0.05$). Where, group I animals (GPI) were treated with normal saline (5 ml.kg^{-1}), group II animals (GPII) with 50 mg.kg^{-1} of **Nelli Kudineer**, group III animals (GPIII) with 100 mg.kg^{-1} of **Nelli Kudineer**, group IV animals (GPIV) with 200 mg.kg^{-1} of **Nelli Kudineer**,group V animals (GPV) with 400 mg.kg^{-1}**Nelli Kudineer**. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV, and V. The statistical analysis was carried out using one way ANOVA method, where **P<0.01 *P<0.05.

Table.2.Effect of Nelli Kudineer in Chronic Toxicity

Gp	Treatment	Day 1	Day 30	Day 60	Day 90
I	**Control** Normal Saline	188.15 ±6.8	188.45±6.20	197.15±6.35	197.7±6.58
II	NelliKudineer **50 mg.kg^{-1}**	195.30 ±6.4	194.30±6.30	199.25±6.70	1990.30 ±6.72*
III	NelliKudineer **100 mg.kg^{-1}**	187.35 ±5.7	190.30±6.40	197.55±7.10	198.36 ±6.30*
IV	NelliKudineer **200 mg.kg^{-1}**	196.30 ±7.2	199.15±6.50	199.90±7.20**	207.45 ±7.26**
V	NelliKudineer **400 mg.kg^{-1}**	188.65±6.05	193.15±5.60	196.60±6.35**	208.66 ±7.38**

Effect of Nelli Kudineer on internal organs

The effects of Nelli Kudineer on the kidney, heart, liver and brain of the rats were observed. The final study revealed, no specific toxic changes noted in internal organs. It was compared with the control group animals (**Table.3).**The group I animals (GPI) treated with normal saline (5 ml.kg^{-1}), group II animals (GPII) with 50 mg.kg^{-1} of **Nelli Kudineer**, group III animals (GPIII) with 100 mg.kg^{-1} of **Nelli Kudineer**, group IV animals (GPIV) with 200 mg.kg^{-1} of **Nelli Kudineer**, group V animals (GPV) with 400 mg.kg^{-1}**Nelli Kudineer**. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV, and V. The statistical analysis was carried out using one way ANOVA method, where **P<0.01.

Table.3.Toxic effects of internal organs:

Gp	Treatment	Heart (gms)	Kidney (gms)	Liver (gms)	Brain (gms)
I	**Control** Normal Saline	0.35 ± 0.05	0.65± 0.03	3.36± 0.05	0.68± 0.05
II	Nelli Kudineer **50 mg.kg^{-1}**	0.36± 0.02	0.81± 0.03	3.48± 0.03	0.72± 0.3
III	Nelli Kudineer **100 mg.kg^{-1}**	0.37± 0.06	0.79± 0.04	3.42±0.02	0.69± 0.2
IV	Nelli Kudineer **200 mg.kg^{-1}**	0.36± 0.04	0.74± 0.02	3.38± 0.02	0.76± 0.05
V	Nelli Kudineer **400 mg.kg^{-1}**	0.35± 0.03	0.75± 0.03	3.41± 0.03	0.78± 0.05

Effect of Nelli Kudineer on biochemical profiles of rats

Table 4. Showed the effect of **Nelli Kudineer**was significant decrease ($p<0.05$) in the plasma glucose level in treated rats especially at higher dose (400 mg.kg^{-1}) compared with control groups. The control group I animals (GPI) treated with normal saline (5 ml.kg^{-1}), group II animals (GPII) with 50 mg.kg^{-1} of **Nelli Kudineer**, group III animals (GPIII) with 100 mg.kg^{-1} of **Nelli Kudineer**, group IV animals (GPIV) with 200 mg.kg^{-1} of, group V animals (GPV) with 400 mg.kg^{-1}**Nelli Kudineer**. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV, and V. The statistical analysis was carried out using one way ANOVA method, where **P<0.01 *P<0.05. Significant decrease ($p<0.05$) in the plasma total cholesterol (TC), triglyceride (TG) and LDL-cholesterol levels. So, there is no evidence of severe toxicity associated with the administration of higher concentration of **Nk**.

Table.4.Effect of Nelli Kudineer on biochemical profiles of rats

Gp	Treatment	Glucose (mg.dl-1)	Cholesterol (mg.dl-1)	Triglyceride (mg.dl-1)	HDL (mg.dl-1)	LDL (mg.dl-1)
I	**Control Normal Saline**	97.65±0.62	41.62±0.56	30.25±0.45	138.25± 0.55	84.15±1.72
II	**Nelli Kudineer 50 mg.kg-1**	95.50±0.56	27.85±0.25*	15.22±0.23*	178.28± 0.65*	72.59±1.28
III	**Nelli Kudineer 100 mg.kg-1**	92.45±0.47	29.74±0.26*	17.42±0.28*	168.18±0.78*	69.84±1.10
IV	**Nelli Kudineer 200 mg.kg-1**	93.25±0.55**	35.18±0.30	19.84±0.38*	187.30± 0.84*	48.60±1.30
V	**Nelli Kudineer 400 mg.kg-1**	87.25±0.45**	34.78±0.28	20.28±0.34*	185.2± 0.85*	46.50±0.84

Effect of Nelli Kudineer on biochemical parameters AST, ALT, ALP, TP and Albumin in results

Table.5 showed the biochemical variation in AST, ALT, ALP, TP and albumin in female wister albino rats found, group I animals were compared with other groups such as II, III, IV, and V. The statistical analysis was carried out using one way ANOVA method, where **P<0.01 *P<0.05.

Table.5.The effects of biochemical variation in AST, ALT, ALP, TP and Albumin in results:

Gp	Treatment	AST (IU.l-1)	ALT (IU.l-1)	ALP (IU.l-1)	TP (g.l-1)	ALBUMIN (g.l-1)
I	**Control Normal Saline**	320.5 ±12.40	68.5 ± 3.18	253.58± 8.80	69.85 ± 3.32	39.15±2.35
II	**Nelli Kudineer 50 mg.kg-1**	309.0 ±9.50**	66.5 ± 2.20**	266.10 ± 2.75**	70.30 ± 2.32	36.30±2.65
III	**Nelli Kudineer 100 mg.kg-1**	310.3 ±7.20**	64.1 ±3.15**	260.18 ± 6.70**	80.15 ± 2.82	38.30±3.05
IV	**Nelli Kudineer 200 mg.kg-1**	305.4 ±7.95	59.4 ± 2.90	265.00 ± 5.20	69.25 ± 3.32	40.20±2.75
V	**Nelli Kudineer 400 mg.kg-1**	315.2 ± 8.20	61.3±3.52	269.40 ± 4.40	74.05 ± 2.58	39.48±2.70

Effect of Nelli Kudineer on Haemotolgical parameters

Table.6 showed on hematological test reveled significant increase ($p<0.01$) in Hb level. The calcium was increased in 100 to 400mg/kg in dose depending manner.The results of group I were compared with other groups such as II, III, IV and V. The statistical analysis was carried out using one way ANOVA method, where *P<0.05.

Table.6.Haemotolgical variations;

Gp	Treatment	Haemoglobin (g.dl-1)	RBC (106 /mm3)	WBC (106 /mm3)	Calcium (mg.dl-1)
I	**Control Normal Saline**	12.3± 0.25	9.15± 0.02	11.45± 0.05	9.45 ±0.02
II	**Nelli Kudineer 50 mg.kg-1**	13.5± 0.26*	9.50± 0.04*	9.55± 0.01*	9.21 ±0.02
III	**Nelli Kudineer 100 mg.kg-1**	13.3± 0.15*	9.55± 0.02*	8.35± 0.32*	9.27 ±0.20
IV	**Nelli Kudineer 200 mg.kg-1**	11.7± 0.20*	8.33± 0.12*	11.45± 0.03*	9.61 ±0.13
V	**Nelli Kudineer 400 mg.kg-1**	12.5± 0.35*	8.51± 0.45*	10.55± 0.13*	9.75 ±0.02

RESUTS AND DISCUSSION

The evaluation of acute and chronic toxicity in female wister albino rat was a highest overall concordance of toxicity in animals, It was compared with human hematological, gastrointestinal, and cardiovascular adverse, side effects. The hypersensitivity and idiosyncratic reactions, are poorly correlated with toxicity observed in animals (Olson H, et al 2000; Abu Taha Nael, et al 2008)(10,11)

The acute toxicity study of **Nelli Kudineer**was carried out as per OECD-423 guidelines, no mortality was observed in both the animals of control group as well as animals treated with a maximum dose of 2000 $mg.kg^{-1}$. The results of acute and chronic toxicity study showed, no significant changes were noted in all treated animals. The animals treated with **Nelli Kudineer** showed increased growth pattern and body weight compared with control rats treated with normal saline.(Tofobic, Jackson, 1999; Raza M, et al 2002; Teo S, et al 2002)(12,13,14) .

There significant changes in liver enzymes like ALP, AST and ALT levels, it was represent no significant liver impairment. (Hayes, 1989; Renuka chaphalkai, et al 2017).(15,16).The results of this study were assessed after 90 days of administration of **Nelli Kudineer**, and it was found that **Nelli Kudineer** at all concentrations do not produce liver damage. There was a slight decrease in plasma glucose level, when doses of Nellikudineer(100 mg.kg-1) were administered in the treated rats..After 90 days of treatment, there were no significant changes in the haematological parameters between control and treated groups. The overall results suggest that **Nelli Kudineer** are non toxic to the haaematopoietic and leucopoietic system and can be used for the mankind.

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