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Research article

Toxicity study on Siddha formulation *Munnai Illai Kudineer* in Albino Rat

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Abstract

Munnailai kudineer(Premna corymbosa) is the Siddha formulation used in the treatment of vatha diseases and in pain magement. The aim of this present study is to evaluate the acute and sub acute toxicity of Munnaiilai kudineer(MK). Toxicity of Munnaiilai kudineer is carried out as per the guidelines Organization of Economic Co-operation and Development (OECD) -423 guidelines. The result of toxicity study shows that there was no significant change in animal behavior due to the absence of toxicity. The animals treated with MK showed normal growth pattern and body weight compared with control rats treated with normal saline. The overall results suggest that Munnaiilaikudineer(MK) are non-toxic to the haaematopoietic and leucopoietic system.

Keywords: Siddha medicine, Munnailai kudineer, Rat models.

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Introduction

Siddha system is unique among the Indian system of medicine. It is believed to have been developed by the siddhar's the ancient supernatural spiritual saints of India.In Siddha system of medicine, the raw materials like plant, mineral, and animal resources are acquired from the natural surroundings. They have been used extensively for many centuries after thorough evaluation of the drug by traditional way. Siddha system emphasizes the dose regimen and pertinent vehicle for every medicine intake.

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Natural Munnai (Premnacorymbosa) is a potent medicinal plant in the Siddha system of medicine. Traditionally the leaves are used in the treatment of vatha diseases, giddiness, loss of appetite and in pain management. Determination of acute oral toxicity is usually the initial screening step in the assessment and evaluation of the toxic characteristics of all compounds. The types of toxicity tests which are routinely performed by pharmaceutical manufacturers in the investigation of a new drug involve acute and subacute and toxicity. Acute toxicity is involved in estimation of LD_{50} (the dose which has proved to be lethal (causing death) to 50% of the tested group of animals. (ShettyAkhila, *et al.,* 2007).

MATERIALS AND METHODS

Collection of Plant Material:

The munnai illai(Premna corymbos will be freshly collected in and around palayamkottai and Tirunelveli district. It will be identified and authenticated by the Medicinal Botanist and Gunapadam experts at Government Siddha Medical College and Hospital.

Prepation of MunnaiillaiKudineer:

Fresh leaves of Munnai is cleaned and made drying in shade. Then it is made into coarse powder. The Kudineer is prepared by boiling 7.5gms of powderin 200ml of water

Acute oral toxicity of Munnaiilaikudineer:

Acute oral toxicity of Munnaiilaikudineer is carried out as per the guidelines Organization of Economic Co-operation and Development (OECD) -423 guidelines after the animal ethical clearance from Institutional Animal Ethics Committee.(KMCP/20/2018)

The albino are fasted over night and provided only water, after which the **Munnaiilaikudineer**is administered by gastric intubations to the relevant group of animals orally at the dose of 50 $mg.kg^{-1}$ body weight in Tween-80. The animals are then observed for 14 days and maintained with normal food. A mortality rate of 2 or 3 animals in 14 days is recorded

and the dose is said to be toxic dose. But when mortality of one animal is observed, then the same dose is repeated again for confirmation. However, if mortality is not observed, the procedure is repeated for further higher doses such as 300 and 2,000 $mg.kg^{-1}$ body weight. Toxic symptoms are observed for 72 hrs including behavioral changes, locomotion, convulsions and mortality (Shah Ayub, 1997, Bürger, 2005).

Cage Side Observations

Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern. Special attention is directed for the observation of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma.

Experimental animals

Acute toxicity study

Male and female Wister albino rat weighing 180 ± 10 g are used in acute toxicity study. The animals are divided into three groups of three animals, totally 18 animals were used in this study. The Group I animals are administered a single daily dose of 0.5 ml of Tween 80 orally for 15 days. In Group II are administered with (300 $mg.kg^{-1}$b.w.MK).once a day for 15 days. The Group III are fed 2000 $mg.kg^{-1}$b.w. once daily for 15 days for acute toxicity studies. On HPE revealed no acute toxic symptoms were observed after 15 th day All test animals were subjected to gross necropsy.

Sub acute toxicity study

Male and female Wistar rats weighing 180 ± 10 g are used for the present study. The animals are divided into five groups of six animals each. The dose of the preparation is calculated based on the body weight of the animal. The animals in Group I are administered with a single daily dose of 0.5 ml of Tween 80 orally for 20 days. The animals in Group II are administered with 50 $mg.kg^{-1}$b.w. of the MK orally once daily for 20 days. The animals in Group III are administered with 100 $mg.kg^{-1}$b.w. of the MK orally once daily for 20 days.

The animals in Group IV and V are administered once daily with 200 and 400 $mg.kg^{-1}$b.w. of the MK respectively for 20 days orally (Pieme,*et al* 2006, Joshi, *et al* 2007, Mythilypriya, *et al.,* 2007).The animals are then weighed every five days, from the start of the treatment, to record the weight variation. At the end of the treatment, blood samples are collected by puncturing retro orbital plexus after mild anesthesia for biochemical analysis.

The collected blood sample is centrifuged within 5 min of collection at 4000 g for 10 min to obtain plasma, which is analyzed for total cholesterol, total triglyceride, HDL-cholesterol levels,LDL-cholesterol,plasma glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine and urea.

Table1 . Acute toxicity studyof Munnaiilai kudineer 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

. The acute toxicityof Munnaiilaikudineeron experimental mice was tested using OECD-423 guidelines, where ST- sign of toxicity; NB- normal behaviour; D- died; S- survive. Values are expressed as number of animals (n=3).

Effect of Munnaiilaikudineer on body weight changes in rats

The effect of MK was observed for their effect on the body weight changes from the study it was observed that, there was significant increase ($p<0.05$) in body weight in all the animals observed.

Table.2.The effects of Munnaiilai kudineer on body weight changes in rats.

Treatment	Day 1	Day 5	Day 10	Day 20
Control	**186.15±6.8**	**188.45 ±6.20**	**193.15 ±6.35**	**197.7±6.58**
MK 50 mg.kg^{-1}	**193.30 ±6.4**	**194.30 ±6.30**	**195.25 ±6.70**	**199.30±6.72***
MK 100 mg.kg^{-1}	**185.35 ±5.7**	**190.30 ±6.40**	**198.55 ±7.10**	**198.36±6.30***
MK 200 mg.kg^{-1}	**194.30 ±7.2**	**199.15±6.50**	**195.90 ±7.20****	**207.45±7.26****
MK 400 mg.kg^{-1}	190.65 ±6.05	193.15 ±5.60	192.60 ±6.35**	208.66±7.38**

Table: 3 The effects of Munnai ilai kudineer on kidney, heart, liver and brain of the rats

Treatment	Heart (gms)	Kidney (gms)	Liver (gms)	Brain (gms)
Control	**0.37 ± 0.05**	**0.66± 0.03**	**3.31± 0.05**	**0.64± 0.05**
MK 50 mg.kg^{-1}	**0.38± 0.02**	**0.82± 0.03**	**3.41± 0.03**	**0.67± 0.3**
MK 100 mg.kg^{-1}	**0.39± 0.06**	**0.80± 0.04**	**3.33±0.02**	**0.65± 0.2**
MK 200 mg.kg^{-1}	**0.38± 0.04**	**0.75± 0.02**	**3.31± 0.02**	**0.72± 0.05**
MK 400 mg.kg^{-1}	0.37± 0.03	0.76± 0.03	3.34± 0.03	0.76± 0.05

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.4.The effect of Munnai ilai kudineer on biochemical parameters

Treatment	Glucose ($mg.dl^{-1}$)	Cholesterol ($mg.dl^{-1}$)	Triglyceride ($mg.dl^{-1}$)	HDL ($mg.dl^{-1}$)	LDL ($mg.dl^{-1}$)
Control	96.65± 0.62	39.62± 0.56	28.25± 0.45	138.25± 0.55	87.15±1.72
MK 50 $mg.kg^{-1}$	94.50± 0.56	27.85± 0.25*	13.22± 0.23*	178.28± 0.65*	74.59±1.28
MK 100 $mg.kg^{-1}$	91.45± 0.47	28.74± 0.26*	15.42± 0.28*	168.18±0.78*	71.84±1.10
MK 200 $mg.kg^{-1}$	92.25± 0.55**	35.18± 0.30	17.84± 0.38*	187.30± 0.84*	50.60±1.30
MK 400 $mg.kg^{-1}$	88.25± 0.45**	34.78± 0.28	19.28± 0.34*	185.2± 0.85*	49.50±0.84

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.5.The effects of Munnai ilai kudineer on biochemical parameters

Treatment	AST ($IU.l^{-1}$)	ALT ($IU.l^{-1}$)	ALP ($IU.l^{-1}$)	TP ($g.l^{-1}$)	ALBUMIN ($g.l^{-1}$)
Control	320.5±12.40	75.5± 3.18	256.58± 8.80	74.85± 3.32	39.15±2.35
MK50 $mg.kg^{-1}$	309.0±9.50**	73.5± 2.20**	269.10±2.75**	74.30± 2.32	36.30±2.65
MK 100 $mg.kg^{-1}$	310.3±7.20**	71.1± 3.15**	263.18±6.70**	84.15± 2.82	38.30±3.05
MK 200 $mg.kg^{-1}$	305.4±7.95	66.4± 2.90	268.00± 5.20	69.25± 3.32	40.20±2.75
MK 400 $mg.kg^{-1}$	315.2±8.20	68.3± 3.52	272.40± 4.40	76.05± 2.58	39.48±2.70

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.6.The effect of MK on haematological parameters

Treatment	Haemoglobin ($mg.dl^{-1}$)	RBC (10^6 /mm^3)	WBC (10^6 /mm^3)	Calcium ($mg.dl^{-1}$)
Control	15.3± 0.25	9.15± 0.02	11.45± 0.05	9.45 ±0.02
MK 50 $mg.kg^{-1}$	16.5± 0.26*	9.50± 0.04*	9.55± 0.01*	9.21 ±0.02
MK100 $mg.kg^{-1}$	16.3± 0.15*	9.55± 0.02*	8.354± 0.32*	9.27 ±0.20
MK 200 $mg.kg^{-1}$	14.7± 0.20*	8.32± 0.12*	11.45± 0.03*	9.61 ±0.13
MK 400 $mg.kg^{-1}$	13.05± 0.35*	8516± 0.45*	10.55± 0.13*	9.75 ±0.02

Table.6.The effect of MK on haematological parameters such as HB, Calcium, RBC and WBC in rats. A study on the effect of MK on haematological parameters such as Hb, RBC, WBC, Calcium in rats was tested. 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<0.05.

Effect of Munnaiilaikudineeron internal organs in rats

The effects of Munnai ilai kudineer on kidney, heart, liver and brainof the rats were observed. From the study it was clear that, significant ($p<0.01$) changes in the weights of various organs of the animals occurred with higher doses of the extract (400 $mg.kg^{-1}$bwt), but macroscopic examinations did not show any changes in colour of the organs of the treated animals compared with the control.

Effect of Munnaiilaikudineer on biochemical profiles of rats

The effect of Munnai ilai kudineer on various biochemical parameters of the experimental animal 'rats' were tested.From the study it was evident that, there 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 rats. The control rats were administered only with 5 ml of normal saline. Significant decrease ($p<0.05$) in the plasma total cholesterol (TC), triglyceride (TG) and LDL-cholesterol levels were observed. But a significant increase ($p<0.05$) in HDL-cholesterol levels were observed in all the treated animals compared with the control animals. AST, ALT and ALP levels were also normal in the **MK** treated animals. From the results of biochemical study there was no evidence of severe toxicity associated with the administration of higher concentration of **MK**. The results are shown in Table.4.

Effect of Munnai ilai kudineer on haematological parameters in rats

The effects of Munnai ilai kudineer were observed for its effect on haematological parameters on the experimental rats. From the study it was evident that, a significant increase ($p<0.01$) were observed in the haemoglobin contents and RBC count in the group treated with 200 $mg.kg^{-1}$ body weight of **MK** and a significant decrease of the parameters occurred in the group treated with 400 $mg.kg^{-1}$ b.w.t compared with the control. There was no significant change in the calcium level in all the treated animals compared to the control.

DISCUSSION AND CONSLUSION

The evaluation of acute and sub acute dosing in experimental animals may be more relevant in determining the overall toxicity of the plant preparation.

The highest overall concordance of toxicity in animals in comparison with humans is with hematological, gastrointestinal, and cardiovascular adverse effects whiles certain adverse effects in humans, especially hypersensitivity and idiosyncratic reactions, are poorly correlated with toxicity observed in animals (Olson, *et al*., 2000).

In the present study, where the acute toxicity study of Munnaiilaikudineer 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 $mg.kg^{-1}$. Hence, $1/10^{th}$ of 2000 $mg.kg^{-1}$ i.e. 200 $mg.kg^{-1}$ of dose was selected as a minimum dose for sub-acute toxicity study (Abu TahaNael, *et al.,* 2008).

The results of sub-acute toxicity study shows that there was no significant change in animal behaviour due to the absence of toxicity. The animals treated with Munnaiilai kudineer showed normal growth pattern and body weight compared with control rats treated with normal saline. So the changes in body weight can be used as an indicator of adverse effects of drugs and chemicals (Tofovic and Jackson, 1999; Raza, *et al*., 2002; Teo, 2002).

The changes in enzymes like ALP, AST and ALT levels show liver impairment, due to toxicity (Hayes, 1989). Serum cholesterol and proteins mainly regulated via synthesis in the liver and increase or decrease in serum concentrations of constituents suggest liver toxicity. The results of the present study were assessed after 20 days of administration of Munnai ilai kudineer, and it was found that MK at all concentrations do not produce liver damage.

There was a slight decrease in plasma glucose level, when higher doses of Munnai ilai kudineer on (400 $mg.kg^{-1}$) were administered in the treated rats..

Analysis of blood parameters is likely to risk evaluation as the change in hematological system has a higher predictive value for human toxicity, when data are translated from animal studies (Olson, *et al*., 2000). After 28 days of treatment, there were no significant changes in the haematological parameters between control and treated groups. No significant changes in the levels of WBC, RBC were observed between control and test groups following repeated administration of MK. Interestingly, significant increase in the levels of hemoglobin was found in treatment with Munnai ilai kudineer with a higher dose of 400 $mg.kg^{-1}$. The possible reason could be that one of the constituents MK may increase absorption of iron.

The overall results suggest that MK are non toxic to the haaematopoietic and leucopoietic system. The haematopoietic and leucopoietic systems are the most sensitive targets for toxic compounds and an important index of physiological and pathological status in man and animal. (13) Therefore, it is possible to assume that the Munnai ilai kudineer is non haematotoxic.

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