



Nephroprotective potential of Neermulli/Nerunjil Kudineer on Cisplatin induced nephrotoxicity in Wistar albino rats

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ABSTRACT

Nerunjil kudineer/Neermulli Kudineer is an official Siddha polyherbal formulation used for the various ailments related to the kidney. Cisplatin is an alkylating agent used in chemotherapy for the treatment of various cancers. Its use is limited due to their nephrotoxicity. In this study nephroprotective effect of Nerunjil kudineer (NK) on cisplatin-induced nephrotoxicity in Wistar albino Rats was studied. Male Wistar albino rats were used for this study. Animals were divided into 5 groups (n=6) as control, Cisplatin control (Single dose 7 mg/Kg i.p on the 7th day), Cisplatin with Cystone (p.o), NK200 and NK400 mg/Kg (p.o) for 10 days. At the end of the study, animals were weighed and sacrificed to estimate the relative kidney weight, serum creatinine and Urea. Kidney tissue was estimated for oxidant-antioxidant parameters (MDA, GSH & SOD) and histology study was carried out. Cisplatin reduced body weight and increased kidney weight significantly (P<0.0001). It deprived the renal function by elevating serum creatinine & urea, MDA and reduction in endogenous antioxidants GSH and SOD significantly (P<0.0001). Cisplatin group exhibited focal tubular necrosis and congested blood vessels in histology study. The standard drug Cystone and NK400 significantly increased the body weight, reduced the kidney weight, normalized the kidney function parameters (Serum Cr and Urea), bolstered antioxidant status and showed a trend towards the recovery of histological alterations. NK showed a dose-dependent activity and higher dose, NK400mg /Kg possessed strong nephroprotective activity, which may be due to the efficient antioxidant potential, which reduces lipid peroxidation and oxidative stress induced by cisplatin.

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INTRODUCTION

Nephrotoxicity or renal toxicity is one of the most common disorder in the kidney and occurs when the kidney is exposed to a drug or toxin that causes damage to the kidneys. Drugs cause approximately 20 percent of community and hospital-acquired incidents of acute renal failure (Nash *et al.*, 2002; Kaufman *et al.*, 1991). Among older adults, the incidence of drug-induced nephrotoxicity may be as high as 66 percent (Kohli *et al.*, 2000). Cisplatin (cisdiaminedichloroplatinum II, CDDP) is a co-ordinate metal complex with significant anti-

neoplastic activity and the side effects including acute and chronic renal insufficiency, renal magnesium wasting, and electrolyte disturbances like hypomagnesemia, hypocalcaemia, hypophosphatemia and hypokalemia are common with cisplatin treatment (Blachley and Hill, 1981). The Electrolyte disturbances and renal damage may be associated with focal necrosis at major parts of nephron (Benoehr et al., 2005). The occurrence of toxicities in patients with cancer chemotherapy is found to be a grave concern, the elimination of side effects and possible toxicities become a major problem during chemotherapy (Rebecca et al., 2006).

Cisplatin induces nephrotoxicity by generating oxygen free radicals like superoxide anion and hydroxyl radicals and stimulates lipid peroxidation in renal tissue (Masuda et al., 1994; Kruidering et al., 1997). Superoxide dismutase (SOD) and o-(beta-hydroxyethyl)-rutoside were acting as a free radical scavenger and reported to be a partial protective agent against cisplatin-induced nephrotoxicity (Dobyan et al., 1986; McGinness et al., 1978). Therefore, the generation of free radicals may play a crucial role in cisplatin-induced nephrotoxicity. So, there is a continuous search for the agents which can reduce or ameliorate the nephrotoxicity induced by the Cisplatin for which there is no proper remedy in allopathy. Therefore, the present work mainly focuses on the Indian system of medicine for using naturally available antioxidants, which can effectively scavenge the reactive oxygen species to ameliorate the nephrotoxicity.

Nerunjil kudineer also called as Neermulli kudineer is a classical and official Siddha formulation. It is a polyherbal formulation used by Siddha practitioners for various kidney disorders. As per (Govt. of India and Family Welfare, 2008; of AYUSH, 2011), it is comprised of an equal proportion of 11 different ingredients from different plants, as in Table 1 (Mudaliar, 2002). In Siddha system of medicine, it is prescribed for various renal disorders and clinical conditions like Inflammation, Oedema, Ascites, Absolute urinary retention or suppression of urine and renal calculi. (Mudaliar and Utthamarayan, 1998; of Health and Welfare, 1991; Mudaliar, 2012). From the scientific evidence collected from the literatures, it was understood that the ingredients of polyherbal formulation Nerunjil/Neermulli Kudineer possessed predominantly diuretic, antiurolithiatic, antimicrobial, anti-inflammatory and antioxidant activities, moderately immunomodulatory, hepato and nephroprotective activity (Kumar et al., 2019). Till date, there is no study to authenticate its nephroprotective activity. In this current study, it was planned to explore the

nephroprotective activity against Cisplatin induced nephrotoxicity in Wistar albino rats.

MATERIALS AND METHODS

Chemicals

The nerunjil kudineer powder was obtained from the IMPCOPS (Batch No: SPT-027), Chennai. Cisplatin was obtained from Celon Laboratories Pvt. Ltd., Hyderabad. Cystone syrup was obtained from Himalaya drugs, Bangalore. Kits for the estimation of urea and creatinine were procured from Reckon Diagnostics P. Ltd. All other chemicals, reagents and solvents used in this study and analysis were of analytical grade.

Preparation of Nerunjil kudineer decoction

The nerunjil kudineer powder was weighed and packed in a whatman filter paper (Size:1), and extraction was done by decoction method with Millipore water as the menstruum. Extraction was stopped when the menstruum reduced to one fourth from its initial volume. The further extract was concentrated by evaporation and freeze-dried to powder and named as Nerunjil Kudineer extract powder (NK). Further NK was used by dispersing in Millipore water.

Experimental Animals

Male Wistar Albino rats of 8-10 weeks old with 200-250 g weight were used for the study. For this study, Institutional Animal Ethics Committee (IAEC/CPCSEA) approval was obtained (IAEC No: APCP/IAEC/2018-2019/1). During the study phase, animals were kept in polypropylene cages under ambient conditions of temperature ($25 \pm 2^\circ\text{C}$), relative humidity ($60 \pm 5\%$) and 12 hrs of light and dark cycle. Animals were fed with normal mouse chow and water *ad libitum* throughout the study period.

Acute toxicity study

Since the Nerunjil kudineer is an official Siddha formulation and relatively non-toxic in clinical practice the highest dose of 2000 mg/kg/p.o was used in the acute toxicity study as per OECD 423 guidelines (Acute oral toxicity- Acute oral toxic class method, 2000).

Nephroprotective activity study design

To evaluate the nephroprotective activity of NK, 24 male Wistar rats were randomly divided into 5 groups of 6 animals each. Group I (Normal control): Treated with 0.9% Normal Saline for ten days. Group II (Cisplatin control): Administered with a single dose of cisplatin (7 mg/kg, i.p.) on 7th day alone. Group III (Cp+Cystone): Treated with

Table 1: Nerunjil kudineer ingredients with reference to Siddha literatures

S. No	Siddha Ingredients	Vernacular/Tamil name	Botanical Name	Parts used
1	Nerunjil samoolam	Small Caltrops (Nerunjil)	<i>Tribulus terrestris</i> L.	Whole plant
2	Nelli vattral	Emblica myrobalan (Nellikai)	<i>Phyllanthus emblica</i> L.	Dried fruit
3	Neermulli samoolam	Long leaved barleria (Neermulli)	<i>Asteracantha longifolia</i> L. (Nees)	Whole plant
4	Parangipattai	China root (Parankisakkai)	<i>Smilax china</i> L.	Root tuber
5	Manathakkali vattral	Black Night shade (Manathakkali)	<i>Solanum nigrum</i> L.	Dried fruit
6	Sarakkondrai puli	Indian Laburnum, Purging Cassia (Sarakkondrai)	<i>Cassia fistula</i> L.	Fruit pulp
7	Sombu	Fennel (Sombu)	<i>Foeniculum vulgare</i> Mill.	Dried fruit
8	Vellari vithai	Cucumber (Vellari)	<i>Cucumis sativus</i> L.	Seeds
9	Surai kodi	Bottle gourd (Surai)	<i>Lagenaria siceraria</i> (Mol) Standl	Stem climber
10	Kadukkai thol	Myrobalan (Kadukkai)	<i>Terminalia chebula</i> Retz.	Dried fruit (Pericarp)
11	Thandrikkai thol	Beleric Myrobalan (Thandrikkai)	<i>Terminalia bellerica</i> (Gaertn.) Roxb.	Dried fruit (Pericarp)

the standard drug Cystone (Rao and Rao, 1998) (5 ml/kg, p.o) for ten days. Group IV(Cp+NK200) & V (Cp+NK400): Group IV and V were given with test drug NK 200 mg/kg p.o and 400 mg/kg p.o. Respectively for all ten days. Group III, IV and V were administered with a single dose of cisplatin (7 mg/kg,i.p.) on the 7th day along with group II, after one hour from administering the standard/test drug. Body weight was recorded, and blood was collected at the initial period to estimate the serum creatinine & Urea. At the end of the study period after taking the body weight, all the animals were sacrificed by anaesthetizing with pentobarbitone sodium (60mg/kg; i.p.) to collect blood samples and dissection of the kidney. Blood samples were collected via cardiac puncture, kept aside for 1 hr at 4°C and centrifuged to separate serum. The serum samples were taken for the estimation of creatinine and urea. The dissected kidneys samples were weighed individually, and the tissue homogenates were prepared for estimation of Oxidant–Antioxidant Parameters and section of the kidney was taken for histopathology studies.

Estimation of Body and kidney weight

Body weights of all the groups were recorded at the first and final day of the study period, to read the change in body weight. After sacrificing the animals, dissected kidneys were weighed individ-

ually. Change in body weight and Relative kidney weight for each group was calculated to study the nephroprotective activity of Nerunjil kudineer. It is reported that body weight loss has a good concordance with the onset of Cisplatin side effects (Bulacio and Torres, 2015). Relative kidney weight was calculated by the following formula and expressed in percentage.

$$\text{Relative kidney weight} = (\text{Kidney Wt}/\text{Body Wt}) \times 100$$

Estimation of Kidney function parameters

Serum Creatinine is determined by the alkaline picric acid method by estimating the colored creatinine-alkaline picrate complex at 510 nm (modified Jaffe's kinetic method) and expressed as mg/dl (Peake and Whiting, 2006). Serum urea was determined by estimating the colored compound diacetyl monoxime at 578 nm (modified Berthelot methodology) and expressed as mg/dl (Henry, 1968; Chaney and Marbach, 1962). Both creatinine and urea were estimated using the commercial diagnostic kit (Reckon Diagnostics P. Ltd.).

Assessment of Renal Oxidant–Antioxidant Parameters

Kidney samples were removed from -8°C, thawed, weighed and 10% homogenate was prepared in tissue homogenizer with 0.025M Tris-HCl of pH 7.4.

This homogenate was taken for the estimation of MDA, GSH and SOD.

Estimation of malondialdehyde (MDA)

Malondialdehyde (MDA) concentration in tissues was measured as it is the major product of membrane lipid peroxidation as per the method by (Ohkawa *et al.*, 1979). The principle of this method depends on the formation of pink color resulted in a reaction between MDA and thiobarbituric acid. This reaction producing a thiobarbituric acid reactive substance (TBARS), pink color, measured spectrophotometrically at 532 nm. To 0.2 ml of tissue homogenate, 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% thiobarbituric acid was added. The mixture was made up to 5 ml with distilled water and then heated in an oil bath at 95°C for 60 min. After cooling, 5 ml of n-butanol: pyridine (15:1 v/v) mixture was added and shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was taken, and its absorbance was measured at 532 nm. The tissue MDA levels were measured from the standard curve. The level of MDA is expressed as nmoles of mg of tissue.

Estimation of Reduced glutathione (GSH)

Reduced glutathione (GSH) was measured by reaction with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) to give a yellow compound that absorbs at 412 nm (Ellman's method) (Moron *et al.*, 1979). Homogenate was centrifuged with an equal volume of 10% trichloroacetic acid. To the 0.2 ml of supernatant 2 ml of Na₂HPO₄ (0.4 M; pH 8.0) and 2 ml of freshly prepared DTNB reagent prepared in 1% trisodium citrate was added and mixed thoroughly. The intensity of yellow colour developed is measured in a spectrophotometer at 412 nm. The tissue GSH levels were measured from the standard curve. The values are expressed as μ moles/g wet tissue.

Estimation of Superoxide dismutase (SOD)

Superoxide dismutase enzyme activity was determined by the method described by (Marklund and Marklund, 1974). Tissue homogenate was centrifuged at 5000 rpm for 10 minutes, and to the 0.1 ml supernatant, 2.95 ml of phosphate buffer (0.1M; pH 8.4) and 0.05 ml of pyrogallol (7.5mM) was added, and the change in absorbance was recorded at an interval of 60 s for 2 min at 420 nm. One unit of enzyme activity was defined as the amount of enzyme required to produce 50% inhibition of pyrogallol autoxidation under assay conditions and expressed as U/mg protein.

Histopathology examination

The kidneys harvested from each group were

fixed in 10% neutral buffered formaldehyde. Tissues dehydration, clearing in xylene and paraffin embedding was done according to the standard method (Sood, 2002). Sections were cut by a rotary microtome at 5–7 μ m thick, and were stained by haematoxylin-eosin and mounted with DPX. Sections were examined under a light microscope and findings documented by the certified histopathologists (Ogeturk *et al.*, 2005).

Statistical analysis

All the Data were expressed as Mean \pm SEM. GraphPad Prism 7 was used for statistical analysis. One way analysis of variance followed by posthoc turkeys test was used to find the significant difference in means of various groups. Values of P < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Acute toxicity study

NK at the dose of 2000mg/kg/po did not exhibit any abnormalities or mortality in rats. As per OECD 423 guidelines the dose is categorised to be "Unclassified" under the toxicity scale. Hence further study with higher doses was not executed. Based on this report, the dose was fixed for nephroprotective study.

Effect of NK on Body and Kidney weight

At the beginning of the study, body weight was not significantly different (Table 2). Control group showed normal weight gain during the study period, but cisplatin control group showed a significant decrease in body weight (P<0.0001). Whereas standard drug cysteine and test drugs NK200 & NK 400 showed an increase in body weight but significantly less (P<0.0001) than the control group at the same time it showed a significant increase (P<0.0001) in weight when compared to cisplatin group. The reduction in body weight following cisplatin administration may be caused by polyuria resulting from tubular injury (Humanes *et al.*, 2012), which in turn leads to dehydration. Moreover, gastrointestinal toxicity may also contribute to its weight reduction. (Ali *et al.*, 2007; Atessahin *et al.*, 2005). Relative kidney weight was significantly high (P<0.0001) for cisplatin group but all other groups showed no significant difference when compared to the control group (Table 2). The weight gain of kidney in Cisplatin group is due to the increase in the kidney damage by Cisplatin (Nematbakhsh *et al.*, 2013). The reduction in relative kidney weight compared to cisplatin group indicates the reduction in the tissue damage.

Effect of NK on Serum Creatinine and Urea

Table 2: Effect of NK on Body and kidney weight in Cisplatin induced nephrotoxicrats

Treatment Groups	Initial Body-weight	Final Body-weight	Δ Body Wt (g)	Relative kidney Wt (%)
I - Control	235.67 \pm 3.15	252.67 \pm 2.33	17.00 \pm 2.35	0.81 \pm 0.03
II - Cisplatin Control	232.33 \pm 4.90	221.50 \pm 3.68	-10.83 \pm 6.24 ^a	1.19 \pm 0.07 ^a
III - Cp + Cystone	225.00 \pm 5.22	227.17 \pm 6.25	2.17 \pm 5.33 ^{a,b}	0.83 \pm 0.02 ^c
IV - Cp+NK200	230.67 \pm 3.50	233.00 \pm 3.71	2.33 \pm 1.69 ^{a,b}	0.90 \pm 0.01 ^c
V - Cp+NK400	224.33 \pm 6.74	229.67 \pm 5.44	5.33 \pm 1.65 ^{a,b}	0.87 \pm 0.03 ^c

Values expressed as Mean \pm SEM; n=6. a - P<0.0001 Vs Control group. b -P<0.0001 Vs Cisplatin control group. c- No significant difference with Control group

Table 3: Effect of NK on kidney function parameters (Serum Creatinine and Urea)in Cisplatin-induced nephrotoxic rats

Treatment Gro	Creatinine (mg/dl)		Urea (mg/dl)	
	Initial	Final	Initial	Final
I - Control	0.56 \pm 0.01	0.63 \pm 0.02	47.00 \pm 2.48	41.33 \pm 1.78
II - Cisplatin Control	0.53 \pm 0.03	3.34 \pm 0.06 ^a	47.67 \pm 2.96	116.67 \pm 3.82 ^a
III - Cp+Cystone	0.43 \pm 0.03	0.94 \pm 0.03 ^{a,b}	48.33 \pm 1.67	61.33 \pm 2.42 ^{d,b}
IV - Cp+NK200	0.53 \pm 0.04	1.43 \pm 0.03 ^{a,b}	45.00 \pm 1.59	94.00 \pm 2.35 ^{a,e}
V - Cp+NK400	0.50 \pm 0.02	0.86 \pm 0.04 ^{d,b}	47.33 \pm 2.25	56.83 \pm 4.77 ^c

Values expressed as Mean \pm SEM; n=6. a - P<0.0001 Vs Control group. b - P<0.0001 Vs Cisplatin control group. c- No significant difference with the control group. d- less significant difference (P<0.05) with Control group. e- less significant difference (P<0.05) with Cisplatin control group

Initial creatinine levels (Table 3) of all groups was not significantly different, except Cp+Cystone group, which showed very less significant difference with control group P<0.05. This difference may be due to the difference in body mass (Table 2). At the end of the study Control group showed a usual increase in creatinine. The cisplatin control group showed a significant increase with P<0.0001. All other treatment groups except NK400 showed a significant increase in creatinine (P<0.0001) with the control group at the same time it was significantly less (P<0.0001) when compared with the Cisplatin control group. Among all the treated group NK400 showed (0.86 \pm 0.04 mg/dl) less significant difference compared to control group (0.63 \pm 0.02 mg/dl) with P<0.05.

Urea level of different study groups was not significantly different in the initial period (Table 3). At the end of the study, Cisplatin control group showed a significant increase (116.67 \pm 3.82 mg/dl) in urea compared to control group (41.33 \pm 1.78 mg/dl)

with P<0.0001. Whereas Cp+Cystone group showed (61.33 \pm 2.42) a less significant difference with the control group with P<0.05, but it was significantly less when compared to Cisplatin control group with P<0.0001. Cp+NK 200 group showed an significant increase (94.00 \pm 2.35 mg/dl) in urea level with P<0.0001 compared to control group and also exhibits less significant difference compared to Cisplatin control group. Among test groups, Cp+NK400 exposed 56.83 \pm 4.77 mg/dl of urea, which was not significantly different from the control group.

Effect of NK on Renal Oxidant-Antioxidant Parameters

MDA, GSH and SOD values of various groups were depicted in Table 4. Compared to the control group, the Cisplatin control exhibited a significant increase (P<0.0001) in the level of lipid peroxidation marker, MDA level. And also decrease in the level of antioxidants GSH (P<0.0001) and SOD (P<0.0001) was noted when compared to the control group. This clearly predicts the influence of cisplatin in

Table 4: Effect of NK on Oxidant–Antioxidant Parameters in Cisplatin induced nephrotoxic

Treatment Groups	MDA (nmol/g Tissue)	GSH (μ mol/g Tissue)	SOD (U/mg Protein)
I - Control	67.5 \pm 2.00	0.785 \pm 0.045	5.31 \pm 0.14
II - Cisplatin Control	165.4 \pm 4.94 ^a	0.358 \pm 0.034 ^a	3.33 \pm 0.16 ^a
III - Cp + Cystone	72.8 \pm 4.86 ^c	0.657 \pm 0.018 ^c	5.14 \pm 0.10 ^c
IV - Cp + NK 200	76.5 \pm 2.41 ^c	0.632 \pm 0.027 ^{d,b}	4.37 \pm 0.16 ^{d,b}
V - Cp + NK 400	65.1 \pm 0.76 ^c	0.769 \pm 0.029 ^c	5.17 \pm 0.11 ^c

Values expressed as Mean \pm SEM; n=6. a - P<0.0001 Vs Control group. b - P<0.0001 Vs Cisplatin control group. c - No significant difference with the control group. d- less significant difference (P<0.05) with Control group

inducing the oxidative stress in the kidney (Yuce et al., 2007). In treatment groups, Cp+cystone and Cp+NK400 showed no significant difference in all the three parameters MDA, GSH and SOD when compared to Control group. The lower dose of Nerunjil kudineer Cp+NK200 group showed no significant difference in the MDA level compared to the control group. Whereas in GSH and SOD parameters concerned, Cp+NK200 showed a less significant decrease (P<0.05) compared to control group and significant increase (P<0.0001) compared to Cisplatin control group.

Histopathology assessment

Histological micrographs of a kidney from the five different groups were portrayed in Figure 8 (A-E). Control group 8(A) showed no abnormalities, whereas in Cisplatin control group 8(B) focal tubular necrosis and congested blood vessels were found. In Cp+Cystone group 8(c) minimal focal tubular degeneration and congested blood vessels were noted, as the trend towards the recovery. Cp+NK200 group 8(D) exhibited mild tubular necrosis and mild congested blood vessels and glomeruli. 8(E) represents the Cp+NK400 group, which showed a good trend towards the recovery and in which mild focal tubular Degeneration and congestion of blood vessels was noted. Histopathology study evidently confirms that there was a good sign of recovery from the Cisplatin-induced nephrotoxicity.

Summary

Cisplatin is an anticancer drug used in the chemotherapy treatments of various types of cancers. Side effects, especially Nephrotoxicity, is the dose-limiting problem of Cisplatin (Sastry and Kellie, 2005). In this study, the nephroprotective effect of Nerunjil/Neermulli kudineer was assessed. Wistar Albino rats were used as the *In-Vivo* model as the changes in renal function observed in the rat system correlates well with the nephrotoxic effects of cisplatin in man (Daugaard, 1988). Cisplatin tox-

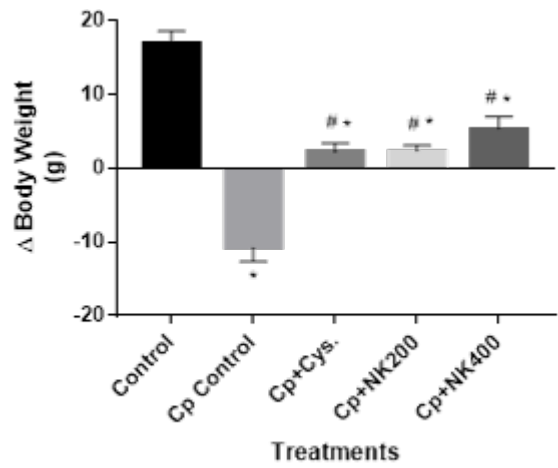


Figure 1: Difference in Body weight. * indicates significant difference with the control group and # indicates the significant difference with Cp control group with P<0.0001

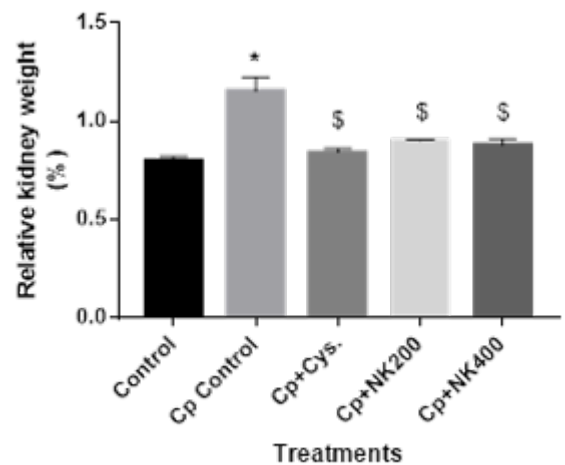


Figure 2: Relative kidney weight. * and \$ indicates significant difference with P<0.0001 and no significant difference with the control group, respectively

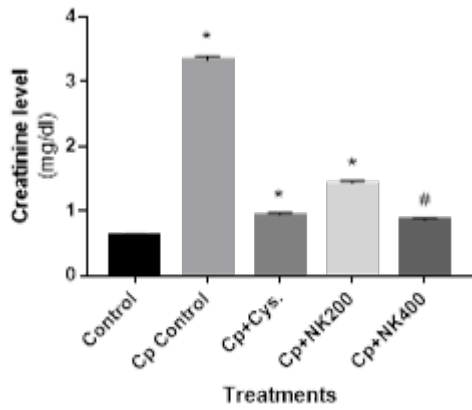


Figure 3: Creatinine level. * and # indicates significant difference ($P < 0.0001$) and less significant difference ($P < 0.05$) with the control group, respectively

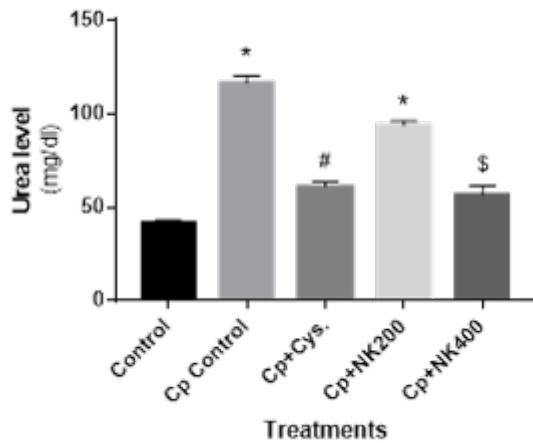


Figure 4: Urea level. * and # indicates significant difference ($P < 0.0001$) and less significant difference ($P < 0.05$) with the control group, respectively. \$ indicates no significant difference with the control group

icity was manifested by a decrease in body weight and increase in kidney weight, elevated serum creatinine and urea, altered oxidant-antioxidant parameters MDA, GSH, SOD and with histopathology abnormalities in the Cisplatin control group which received a single dose of Cisplatin 7 mg/Kg i.p. The body weight difference and relative kidney weight were found to get back to normal in the Standard Cp+Cystone, Cp+NK200 and Cp+NK400 compared to control group. Cp+NK400 showed good increase in body weight and reduced relative kidney weight than the other test groups. This indicates the reduced side effect and tissue damage (Atessahin *et al.*, 2005; Nematbakhsh *et al.*, 2013). Nephrotoxicity induced by Cisplatin is characterized by the diminution in renal function that is characterised by an increase in creatinine and

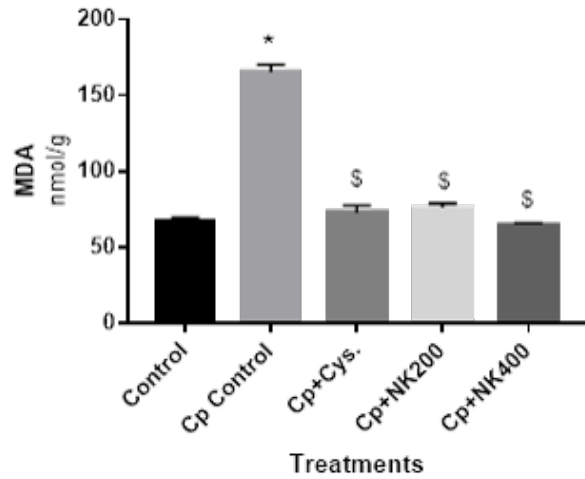


Figure 5: MDA level. * and \$ indicates significant difference ($P < 0.0001$) and no significant difference with the control group, respectively

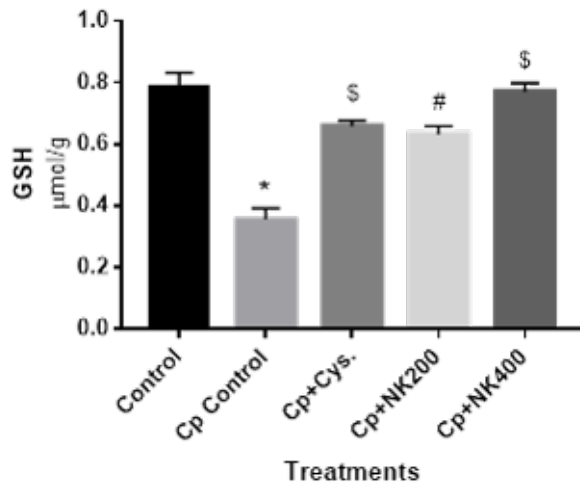


Figure 6: GSH level. * and # indicates significant difference ($P < 0.0001$) and very less significant difference ($P < 0.05$) with control group respectively. \$ indicates no significant difference with the control group

Urea level in serum (Farooqui *et al.*, 2017). Serum creatinine and urea were elevated very significantly in Cisplatin group, but it reversed towards normal in all other test groups. Cp+NK400 group showed the superior effect with a less significant difference in creatinine ($P < 0.05$) and No significant difference in Urea when compared to the control group.

Several studies have suggested that oxidative stress is the major reason for nephrotoxicity caused by lipid peroxidation and free radicals. Cisplatin generates Reactive Oxygen Species (ROS) synthesis by inducing mitochondrial dysfunction and disrupting the electron transport chain. ROS reacts with mem-

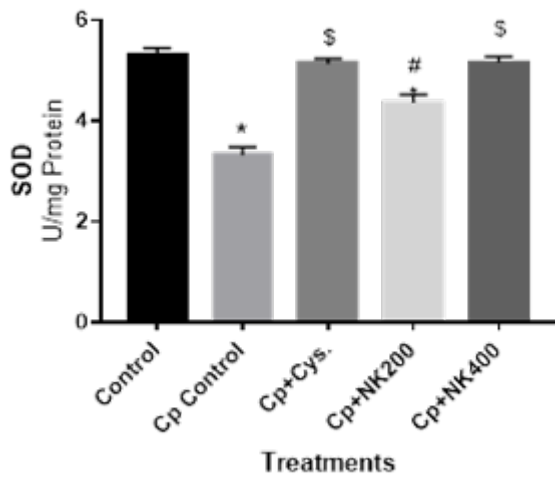


Figure 7: SOD level. * and # indicates significant difference ($P<0.0001$) and less significant difference ($P<0.05$) with the control group, respectively. \$ indicates no significant difference with the control group

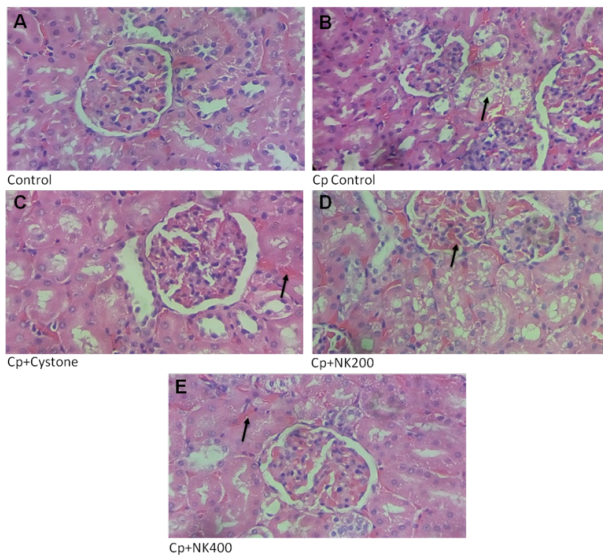


Figure 8: Light microscopy photo micrographs depicting sections from kidneys of rats various groups. A -Control; B- Cisplatin control; C- Cp+Cystone; D-Cp+NK200; Cp+NK400. Arrow mark indicates the abnormalities

brane lipids, cellular proteins and DNA resulting in their modification leading to cellular stress (Pabla and Dong, 2008). Lipid peroxidation is a consequence of excessive ROS production, which in turn leads to an increased level of lipid peroxidation marker MDA (Matsushima et al., 1998; Pabla and Dong, 2008). In the present study, the MDA level of Cisplatin group increased significantly ($P<0.0001$), and all other groups showed no significant difference with the control group. So both the stan-

ard Cystone and nerujil kudineer reduced the lipid peroxidation and oxidative stress. The excessive ROS produced is combated by endogenous antioxidants. However, when the synthesis of ROS override its destruction, there is over-consumption of these antioxidants, which was also evident in previous Cisplatin nephrotoxicity models (Al-Majed et al., 2006). The consumption of endogenous antioxidants glutathione and SOD will decrease their level in tissue. In this study, this same pattern was observed in the cisplatin-control group in which GSH and SOD level declined significantly compared to the control group. However, the nephroprotective treatment with Cystone and a higher dose (400 mg/Kg) of Nerunjil kudineer maintained GSH and SOD at near normal levels in the renal tissue with no significant difference compared to control group. This supports the antioxidant potential of Nerunjil kudineer. Histopathology study also substantiated the above result by showing mild focal tubular Degeneration and congestion of blood vessels, indicating a strong sign of recovery compared to the Cisplatin group, which exhibited focal tubular necrosis and congested blood vessels.

CONCLUSION

In conclusion, Nerunjil/Neermulli kudineer ameliorates the Cisplatin induced cytotoxicity in the kidney, and also it exhibited a dose-dependent nephroprotective activity. Higher dose, NK400mg/kg was found to be more potent and showed better activity than the standard drug Cystone. Nephroprotective activity may be due to efficient antioxidant potential, which reduces lipid peroxidation and oxidative stress. As a Siddha drug, the extract was taken as such for this study, in future antioxidant-rich fraction or isolated active components can be taken for investigation. Moreover, Nerunjil kudineer should be assessed its nephroprotective activity in Cisplatin treated tumor animal model, before stepping up to the clinical study.

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