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NEPHROPROTECTIVE ACTIVITY OF DECOCTION OF INDIGOFERA TINCTORIA (*AVURI KUDINEER*) AGAINST CISPLATIN-INDUCED NEPHROPATHY IN RATS

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ABSTRACT

Cisplatin is widely used chemotherapeutic agent for the treatment of several human malignancies. The efficacy is dose dependent, but the significant risk of nephrotoxicity frequently inhibits the use of higher doses to maximize its anti-neoplastic effects. In the present investigation, the *Avuri kudineer* [Decoction of Indigofera tinctoria] made of indigo leaves AKL, the *Avuri kudineer* made of indigo root and leaves AKRL was evaluated for nephroprotective activity in Cisplatin induced renal damage in rats. Nephrotoxicity was induced in wistar albino rats by intra-peritoneal administration of Cisplatin 5mg/kg. Effect of concurrent administration of AKL and AKRL *Avuri kudineer* at a dose of 500 mg/kg and 1000mg/kg were given for respective animal groups by oral route was determined using serum creatinine and blood urea and change in body weight as indicators of kidney damage. The decoctions significantly decreased the cisplatin induced nephrotoxicity. Remarkable changes were observed in body weight, serum creatinine and urea levels. It was observed that the *Avuri kudineer* [AKRL] significantly protected the kidneys from injury than the *Avuri kudineer* [AKL]. Current study results showed that the *Avuri kudineer* [Decoction of Indigofera tinctoria] possess significant nephroprotective activity against cisplatin induced renal damage.

Keywords: Cisplatin, *Avuri kudineer,*Nephroprotective, Indigofera tinctoria

1. INTRODUCTION

Renal failure is a common clinical syndrome. It is defined as a rapid decline in renal function resulting in abnormal retention of serum creatinine and blood urea, which must be excreted. Kidney disease is the ninth leading cause of death. Approximately, 19 million adults have chronic kidney disease and an estimated 80,000 persons have chronic kidney failure diagnosed annually in India. Recent literature, have shown a prevalence of chronic renal failure of 0.16% and 0.79% in India. Till date for End Stage Renal Failure, renal replacement is the only therapy. In case, of non-availability of kidney, dialysis is the only alternative, which unfortunately is severely limited by several constraints including a good amount of expenditure. No exclusive drug has been reported so far, as such in any category of medical treatment. Nephrotoxicity is the third most common problem of the renal system with an estimated lifetime risk of 2-5% in Asia, 8-15% in Europe and America and around 20% in the Middle East. Cisplatin is extensively used for the treatment of several cancers like testicular and lungs cancer. Unfortunately, the gracious drug cisplatin is conjoined with a brutal side effect since it induces nephrotoxicity.The present study is

undertaken in an attempt to characterize the pathophysiological events associated with recovery from this model of acute renal failure in the rat, and to evaluate any possible role of tubular obstruction or disruption, which might relate to the characteristic slowness of the recovery process.There is a growing interest of public in traditional medicine, particularly in the treatment of nephrotoxicity partly because of limited choice in the pharmacotherapy.Certain Indian Medicinal plants have been reported to exhibit protective effect of renal tissues against injuries. Since there are only few researches made on this field of nephroprotection, this present study of nephroprotective activity of *Avuri kudineer* [Decoction of *Indigofera tinctoria,Linn*] will satisfy the research for better and cost effective nephroprotection. Indigofera tinctoria, Linn, Family-Fabaceae, Indigo, *Tamil:Avuri*was one of the original sources of indigo dye.The plant has been extensively used in *siddha* for its bitter taste and possess antidote,anti-oxidant,[rejuvenative property]*sobhanasini*[diuretic], anthelmintic, anti periodic activities. Roots used for anti poison, giddiness, colic, gonorrhoea. Juice of leaves is used for liver and spleen enlargement, epilepsy and other nervous affections asthma, whooping cough, heart palpitations, various lung and renal problems, and oedema. Decoction of the leaves used in bites and stings of venomous insects and reptiles, to relieve the pain and also burn and scalds. Leaves give complexion to the body. The whole plant of Indigo contains glycoside indican, indigotine, indirubin, and flavanoids and it is a rich source of potash, the ash 4.4% containing as much as 9.5% of soluble potassium salts. The pharmacological activities of indigo, hepatoprotective, anti-dyslipidemic, anti-neoplastic, anti-nociceptive, anti-HIV, anti-microbial, anti-diabetic were reported. In the present study, an effort has been made to establish the scientific validation for the nephroprotective property of *Avuri kudineer* using Cisplatin induced nephritic injury model in rats.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant material

The plant *Indigofera tinctoria,* Linnwas collected at *Siddha* Medicinal Plants and Garden [SMPG], Mettur, Tamilnadu, Weighing about 5 kgs. The Plant material was then authenticated at SMPG and at Government *Siddha* Medical College, Chennai, by the Professors of P.G.*Gunapaadam* [Pharmacology] Department and voucher specimen has been deposited at the herbarium for further reference.

2.2 Preparation of Avuri Kudineer [Decoction of Indigo]

As per the literature the '*Kudineer* was made with Indigo leaves as per *siddha* literature.25gms of coarse powder of indigo leaves was taken. To this 400ml of water [16 parts to indigo leaves] is taken in a container and both are mixed well and kept in a stove and heated at a slow flame. This is continued till the liquid is reduced to $^1/_8$ of the total volume. This is about 50ml of the net decoction ['*Kudineer'*].The decoction is then filtered and the plant residue is discarded.This is named as AKL [*Avuri kudineer* of indigo leaves].12.5gms of coarse powder of root and 12.5gms of coarse powder of leaves of indigo was taken and mixed well with 400ml of water and made decoction as mentioned above. This is named as AKRL [*Avuri kudineer* of indigo root and leaves].

2.4 Animals

Mice of either sex weighing 25-30g and male Wistar rats weighing 150-200g were obtained from the animal house of Vels University. Animals were fed on conventional diets and water *ad libitum* and they were maintained under standard conditions of humidity, temperature (20-24°C) and light (12-h light: 12-h dark cycle). The rats were randomly assigned to control and different treatment groups, six animals per group. The Institutional Animal Ethics Committee approved the experimental protocol and the conditions in the animal house approved by Committee for Supervision on Experiments on Animals. The study was conducted in accordance with IAEC guidelines (Registration no.- III/VELS/COL/13/CPCSEA/IAEC/23.09.11). The animals were acclimatized for one week under laboratory conditions.

2.5 Acute toxicity studies

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD-425) received from Committee for the Purpose of Control and Supervision of Experiments on

Animals (CPCSEA). Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. All observations are systematically recorded.

2.6 Experimental design

The dose limits were selected on the basis of oral acute toxicity studies in mice, in accordance with the OECD guidelines. Total forty two wistar rats were divided randomly into seven groups of six animals each. Group I received oral dose of normal saline only for 14 days served as normal control. Group II received single dose of cisplatin (5 mg/kg of body weight; i.p.) on day 1 treated as control. Group III and IV received *Avuri kudineer* leaf extract at the dose levels of 500 and 1000mg/kg b.w. once in a day for 14 days after single dose of cisplatin on day 1. Group V and VI received *Avuri kudineer* root and leaf extract at the dose levels of 500 and 1000mg/kg b.w. once in a day for 14 days respectively along with the single dose of cisplatin as earlier considered as test groups respectively. Group VII served as standard received cystone Syrup (5 ml/kg; p.o.). After Cisplatin treatment, the animals were weighed, and 0.1-ml blood samples were taken for blood chemistry analysis of blood urea nitrogen and creatinine. Changes in blood urea nitrogen and creatinine were used to indicate differences in Cisplatin-induced nephrotoxicity between the drugs and vehicle treated rats.

2.7 Urine analysis

Urine was collected over 24 hrs on 14th day by keeping the test animals in individual metabolic cages. The volume of collected urine samples was measured followed by estimation of biochemical parameters, namely urine creatinine and urine albumin.

2.8 Biochemical assays

Blood samples were collected from the test animals under anaesthesia by retro-orbital vein puncture using a fine capillary before sacrifice under ether anaesthesia and centrifuged using the table top centrifuge (REMI) at 3000 rpm to get serum and parameters including creatinine, urea, albumin, and total protein were estimated. The biochemical estimations were done in a Biochemical-semi-auto analyzer by standard procedures using commercial kits for assessment of renal toxicity.

2.9 Histopathology

The kidneys were removed from the rats and organs were fixed using a formaldehyde solution (10% v/v of formaldehyde in normal saline), embedded with paraffin wax followed by preparation of tissue sections using a microtome for histopathological study.

2.10Statistics

Data obtained in the experiment were expressed in terms of mean ± SEM. Statistical significance of data was assessed by analysis of variance (one-way ANOVA) followed by a comparison between different groups using "Dunnet" test. The significance level was set at $P<0.05$. The treatment group was compared with the control group

3. RESULTS AND DISCUSSION

The acute toxicity of *Avuri kudineer* was not occurred at 2000mg/kg (as per the OECD - 425) on mice but toxic symptoms like aggressiveness, tremors, mild diarrhoea, dyspnoea and abdominal writhing were observed after 48 hours of oral drug treatment at the dose level of 5000 mg/kg and total duration of study was 14 days. Hence, one-tenth and one twentieth dose was selected as therapeutic dose from maximum tolerable dose from toxicity study. Cisplatin is a common, highly toxic chemotherapeutic agent.Cisplatin is a widely used and effective chemotherapeutic agent that binds to and alkylates DNA and triggers transcription inhibition, cell cycle arrest, and apoptosis. In addition, Cisplatin generates reactive oxygen species, which are known as one of the pathogenic intermediates following chemotherapy. Cisplatin is dose-limited by a high incidence of toxicities, including progressive and irreversible nephrotoxicity. *Avuri kudineer* with Cisplatin treated rats had normal BUN after the i.p. model of Cisplatin administration, whereas the Cisplatin with 2% CMC treated rats had abnormally high BUN. Also, data indicate that the *Avuri kudineer* prior to Cisplatin animals had normal creatinine

levels, whereas the creatinine levels in the Cisplatin with 2% CMC-treated rats were abnormally elevated (P<0.01). The Cystone-treated rats were provided with consistent protection against renal toxicity, as indicated by moderate BUN, showing nephroprotection. In present study, the rats treated with single dose of cisplatin shown marked reduction of body weight as compared to normal group also caused a mark reduction of glomerular filtration rate, which is accompanied by increase in serum creatinine level indicating induction of acute renal failure. The *Avuri kudineer* showed remarkable elevation in body weight with a significant (P<0.01) increase in urine volume output. There is no presence of albumin content, any cast/deposits in the urine output.

Table 1
Measurement of Body weight changes after Avuri kudineer treatment

Drug treatment	***Periodical Weight changes after Avuri kudineer treatment***				
	Day1	***Day4***	***Day7***	***Day10***	***Day14***
Normal (Saline)	220.12±2.45	224.2±3.50	230.15±3.13	233.16±2.50	235.11±3.10
Control (Cisplatin alone)	214.14±2.19	212.81±4.91	216.10±3.21	210.10±3.87	206.45±3.41
AKL 500mg/kg + Cisplatin	212.13±2.46	208.38±2.66	214.52±3.40	218.60±3.12	233.78±3.78
AKL 1000mg/kg+ Cisplatin	217.20±3.14	216.40±4.03	231.16±4.12	228.18±3.60	229.13±3.00
AKRL 500mg/kg + Cisplatin	215.10±2.88	221.46±2.66	230.12±3.30	236.15±3.68	240.26±4.34
AKRL 1000mg/kg + Cisplatin	219.52±4.12	224.00±4.11	228.55±4.32	234.02±5.19	239.38±5.13
Standard Cystone treated	216.21±3.48	219.48±4.14	221.18±4.26	224.10±3.88	228.52±4.18

Values are the mean ± S.E.M. of six rats/treatment.

Creatinine, is mostly derived from endogenous sources by tissue creatinine breakdown. Thus serum urea and uric acid concentration is often considered a more reliable renal function predictor than serum creatinine. However, the urine creatinine, urea and uric acid decreased significantly (P<0.01) as compared with the control group.

Table 2
Effect of treatment with Avuri kudineer on Serum urea, Uric acid and Creatinine levels.

S.No.	***Groups***	***Urea (mM/l)***	***Uric acid (µM/l)***	***Creatinine (µM/l)***
1.	Normal (2% CMC)	8.12± 0.20	128.11 ± 5.33	52.38 ± 2.16
2.	Control (Cisplatin alone)	12.61 ± 0.81	98.72 ± 4.15	82.76 ± 2.24
3.	AKL 500mg/kg + Cisplatin	10.88 ± 0.71*	112.15 ± 3.48**	77.61 ± 2.18*
4.	AKL 1000mg/kg + Cisplatin	9.04 ± 0.56**	120.06±5.21**	61.01 ± 3.24**
5.	AKRL 500mg/kg + Cisplatin	9.21 ± 0.62**	122.30 ± 4.00**	64.50 ± 2.38**
6.	AKRL 1000mg/kg + Cisplatin	8.65 ± 0.45**	124.28±4.36**	56.13 ± 2.21**
7.	Standard Cystone treated	8.22±0.32**	124.12±4.16**	50.12 ± 2.10**

Values are the mean ± S.E.M. of six rats/treatment. Significance *p <0.05, **p<0.01 Vs Control.

The serum creatinine and urea were found to be significantly (P<0.01) low when compared with the control group. The effect *Avuri kudineer* on cisplatin nephrotoxicity was evaluated mainly with the support of change of urine volume. After injected the single dose of cisplatin (10mg/kg) result increased urea and creatinine level as compare to control group and it was recovered significantly (P<0.01) in *Avuri kudineer* treatment but less Significant (P<0.05) on creatinine recovery in 500mg/kg treated group. Kidney homogenate analysis of AKL 1000mg/kg group for oxalate, calcium and Phosphate was 1.60, 3.33 and 3.08mg/g respectively. Similarly, the serum BUN was resulted in 38.22mg/dl. And for AKRL 1000mg/kg group 1.42, 3.03 and 2.91mg/g respectively and serum BUN was 35.41mg/dl. All these values were comparable with that of normal level. The change of renal function observed in the rat correlate well with the nephrotoxicity effect with man. The increased urea and creatinine level suggests the reduction of glomerular filtration rate. But protective treatment of *Avuri kudineer* with cisplatin significantly reduced the level of urea and creatinine that indicates increase glomerular filtration rate at all the higher dose treated in both AKL and AKRL. In our study, it was conformed that a single dose cisplatin significantly induced serum creatinine in wistar rats four days after administration. Result shown that significant reduction of serum creatinine level with protective treatment of 1000mg/kg but less significantly 500mg/kg dose of *Avuri kudineer* leaf extract

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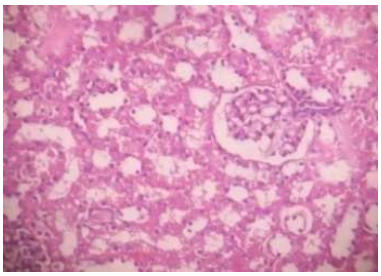
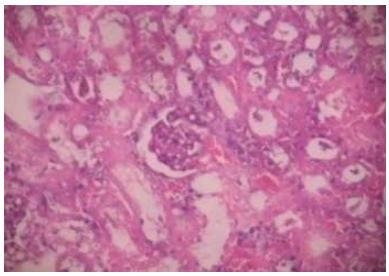
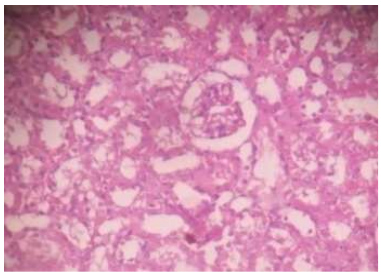
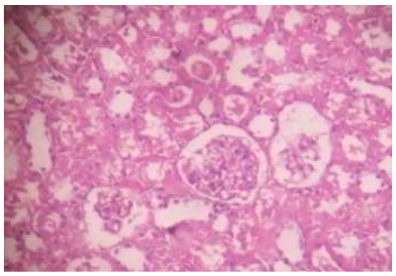
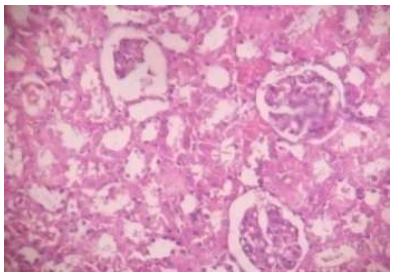
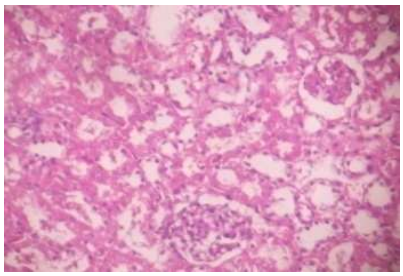
NORMAL **CISPLATIN ALONE**

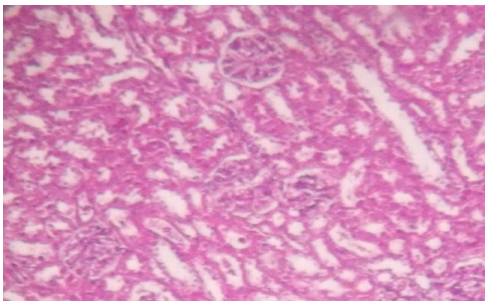
***AVURI KUDINEER*[AKL]:LOW DOSE:*AVURI KUDINEER*[AKL]:HIGH DOSE**

***AVURI KUDINEER*[AKRL]:LOW DOSE:*AVURI KUDINEER*[AKRL]:HIGH DOSE**

STANDARD CYSTONE TREATED



The histological features found from the tissue sections of different groups and the photomicrographs of tissue sections of kidney are presented. The histopathology of tissue sections suggest that the control group had encountered vast histological damages as evidenced by the glomerular and tubular congestion with abnormal Bowman's capsule, blood vessel congestion, epithelial cell desquamation, and presence of tubular cast. Inflammatory cells were also seen in kidney section from the Cisplatin-treated group. In Cisplatin group, mononuclear cells infiltrated mainly in the sub-capsular region and interstitial oedema was also noticed. Hyaline changes, vacuolization and necrosis in the proximal tubular epithelial cells were also seen. Concurrent treatment with the *Avuri kudineer* was found to reduce such changes in kidney histology induced by Cisplatin The histological features of the *Avuri kudineer* 500mg/kg treated group showed minimal cellular damage in contrast to the control group. The *Avuri kudineer* 1000group showed almost normal glomerular and tubular arrangements with minimal blood vessel congestion, epithelial cell desquamation, and presence of tubular cast with very few inflammatory cells.

4. CONCLUSION

The present study aimed to evaluate the protective effect of *Avuri kudineer* against cisplatin-induced nephropathy in rats. Cisplatin-administered rats (control group) had encountered acute kidney dysfunction as evidenced by elevation in serum urea and creatinine, decreased urine output and body weight with multiple histological damages. Treatment with the *Avuri kudineer* at the dose level of 500 and 1000mg/kg b.w., for 14 days significantly lowered the serum level of creatinine, urea and uric acid with a significant weight gain, and increased urine output when compared with the control group. The histological damages in the *Avuri kudineer* treated group were minimal in contrast to the toxic rats. The statistical significance of the nephroprotective activity of *Avuri kudineer* treated group and the Cystone treated group were compared against control were found almost equal as both groups gained significance ($P<0.01$) against the control group in most of the parameters including serum urea and creatinine. Out of two doses of *Avuri kudineer* both RL and L, the ARL higher doses showed striking nephrocurative activity than AL but both are showed significant nephroprotective activity. These biochemical results were supported by hisptopathological data. The results of our study suggest that the *Avuri kudineer* possesses nephroprotective potential on the dose dependant manner and substantiate the therapeutic utility in renal injury. Extensive further research is needed to elucidate the exact mechanism of nephroprotective action of the *Avuri kudineer*. According to the pathological result it can be inferred that *Avuri kudineer* had protective effect against degenerative injury caused by Cisplatin.

5. ACKNOWLEDGEMENTS

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