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RENAL PROTECTIVE ACTIVITY OF *ORTHOSIPHON STAMINEUS* LEAF EXTRACT AGAINST CISPLATIN INDUCED RENALTOXICITY

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ABSTRACT

This study has been initiated to determine whether orthosiphon stamineus methanol leaf extract can protect against cisplatin induced nephrotoxicity in rats. Cisplatin is one of the most widely used and most potent chemotherapy drugs. However, side effects in normal tissues and organs, notably nephrotoxicity in the kidneys, limit the use of cisplatin and related platinum-based therapeutics. A single dose of cisplatin (16mg/kg bwt) injected i.p. caused a significant increase in blood urea, serum creatinine and urinary protein levels with a significant decrease in reduced glutathione (GSH) content of kidney tissue as compared to control group. On the other hand, administration of orthosiphon stamineus methanol leaf extracts (100mg/kg and 200mg/kg) orally 1h before cisplatin (16 mg/kg bwt, i.p) protected the kidney as indicated by restoration of Blood urea, creatinine, urinary protein and GSH levels. Co-administration of the orthosiphon leaf extract with cisplatin significantly prevented renal toxicity both functionally & histologically. These results suggested that the methanol leaf extract of *Orthosiphon stamineus* had a protective effect against cisplatin induced renal injury.

KEYWORDS: *Orthosiphon stamineus*, leaves, Cisplatin, Renal toxicity.

INTRODUCTION:

Orthosiphon stamineus Misaikucing(OS) is well-known over the years because its capabalities to Flush the kidneys and urinary tract as it has mild diuretic property, act as a filteration in order to remove the commonest waste products such as urea, or stone in the kidney, replace the potassium lost from the body during urination as it

has enough potassium contents, act as the remedy for other diseases such as diabetes, gout, arthritis, rheumatic and kidney stone disease, besides can prevent the formation of kidney stone and infection in urinary tract, arteriosclerosis (capillary and circulatory disorders),reduces the cholesterol level in blood, lower the high blood pressure and relieves spasms of the smooth muscle in the walls of the internal organs, makes it valuable for gallbladder problem.

Orthosiphon stamineus have been reported to possess anti-hypertensive[2], hypoglycemic activity[1] and diuretic effect[3] Almost all parts of the plant can be used. The healing power of misai kucing plant is because of its great chemical contents for instance cirsimaritin, myoinositol, orthosiphon, pillion, rhamnasin, bmlt oioioldsalvigenin, kerotin, kerotinoid, minyak pati, flavanoid, glukosid, glikoprotein, saponin dan terpenoid**.**

Nephrotoxicity is one of the main side effect caused by cisplatin (CP)[16] a widely used anti neoplastic agent administered to treat a variety of cancers such as ovarian, testicular,bladder head and neck and uterine cervix carcinomas [4,5] Various data indicate that cisplatin induces oxidative stress [6,7] lipid peroxides[8] and DNA damage [9]

Earlier studies reveals that the cisplatin has been shown to cause nephrotoxicity in human beings, mice, dog and rats. Several investigators have suggested different mechanisms by which cisplatin selectively kills the proximal tubule cells.It was hypothesized that cisplatin is activated in the kidney to toxic metabolite through a platinum-glutathione conjugate, then to a cysteinyl glycine platinum conjugate,which is further processed to a cysteine conjugate which is a metabolically reactive thiol.

The leaves of *Orthosiphon stamineus* were used by the tribal people of Malaysia to treat kidney disorders.Hence present study was focused on the effect of methanol extracts of leaves of Orthosiphon stamineus on the renal damage induced by cisplatin.

Materials and methods:

Plant Material: The leaves of *Orthosiphon Stamineus* plant were Collected from Siddha research institute, Arumbakkam, Chennai. The material was dried in shade; they were powdered and extracted with methanol. The extract was evaporated under low pressure by using buchi type evaporators.

Animals:

Male wister albino rats weighing (150-200g) were obtained from Raja Muthaiah Medical College, Annamalai University, Chidambaram, Tamilnadu. They were maintained at standard housing conditions and fed with commercial diet and provided with water ad libitum during the experiment. The Institutional animal ethics committee (Reg No.160/1999/CPCSEA) permitted the study.

Evaluation of Reno protective activity:

Cisplatin induced renal toxicity:

Four groups of six animals were used to study the effect of OS methanol leaf extract on cisplatin induced renal toxicity and changes in renal function.

Group I

Received 0.5 ml saline (i.p)

Served as a control.

Group II

Received a single dose of cisplatin (16mg/kg body wt i.p)

Groups III&IV

Received methanol extract of *Orthosiphon Stamineus* (100mg/kg and 200mg/kg body wt P.O) 1 hr before the cisplatin injection (16mg/kg body wt i.p.)

Rats in all groups were sacrificed 72 hrs after treatment Blood samples were collected and Bio- chemical estimations were done.

Bio- Chemical study:

Animals were sacrificed by cervical dislocation. The blood samples were collected via retro orbital puncture and the serum was used for the assay of marker enzymes viz: blood urea, serum creatinine were estimated by the method of Brod and Sirota[12] and Marshell *et al*[13] respectively and levels of reduced glutathione by the method of Moron *et al*[15]the protein content was estimated by the method of Lowry et al[14] using bovine serum albumin as

standard and Changes in the body weight were recorded. Three rats per group were sacrificed and both kidneys were isolated from each rat. The kidneys were processed for histopathological examination**.**

Histopathology

The kidneys were sectioned longitudinally in two halves and were kept in 10% neutral formalin solution. Both kidneys were processed and embedded in paraffin wax and sections were taken using a microtome. The sections were stained with hematoxylin and eosin and were observed under a computerized light microsope.

Statistical analysis:

The results were expressed as mean ± sem of six animals from each group. The statistical analysis was carried out by one way analysis of variance (ANOVA) P value < 0.05 were considered significant**.**

Results:

Reno protective activity:

Cisplatin induced renal toxicity:

The administration of cisplatin to the animals resulted in marked increased in blood urea, urinary protein and serum creatinine. However glutathione level was decreased. The Kidney weights of the rats treated with cisplatin were decreased as compared to control group and *Orthosiphon stamineus* treated rats. At a dose of 100mg/kg the effect was only marginal whereas at 200mg./kg the drugs effectively prevented cisplatin induced renal toxicity**.**

Histopathology:

Control rats showed normal glomerular and tubular histology (Fig 1) whereas cisplatin was found to cause glomerular, peritubular and blood vessel congestion (Fig 2) and result in the presence of inflammatory cells in kidney sections from the cisplatin treated group. Treatment with the OS extract 200mg/kg (Fig 3) was found to reduce such changes in kidney histology induced by cisplatin.

Discussion and conclusion:

Previous reports on phytochemical studies on leaves of *Orthosiphon stamineus* reveal the presence of Beta- caryophyllene,Alpha-humulene, Caryophyllene epoxide, Eupatorin, Sinensetin, Scutellarine tetramethyl

ethers, Salvigenin, 7,3',4'-tri-O-methylluteolin, 5-hydroxy-6, 7',3',4'-tetramethoxyflavone, Ladanein, 6-hydroxy-5, 7',4'-trimethoxyflavone, 2,3-dicoffeoyltartrate, Rosmaric acid, 2-caffeoyltartrate, Terpenoids ,Diterpene ester, Orthosiphole A to E (diterpene dibenzoyl diacetyl ester of primarane type), Neoorthosiphol A and B, Orthosiphole F to J, Staminol A and B, Norstaminol A, Orthochromene A (benzochromene, Aglycone hederagenin (Triterpene saponins), Alpha-carotene, Beta-carotene, Neo-beta-carotene, Alpha-carotene oxide ,Vomifoliol, Aurantiamide acetate, Oleanolic acid.

Effects of methanol extracts of *Orthosiphon stamineus* leaves was tested at two dose levels i.e, 100 and 200 mg/kg body weight p.o against cisplatin induced nephrotoxicity. Nephroprotective activity was assessed by determination of serum marker levels and urinary functional parameters, determination of Glutathione activity and histopathological studies. This study shows that single injection of cisplatin in rats resulted in deterioration of renal function as indicated by elevation in creatinine, blood urea and urinary protein. These results are consistent with the previous studies on cisplatin-induced nephrotoxicity in experimental animals.[18,19] The results reveal that serum creatinine, blood urea and urinary protein returned approximately to the normal control levels when animals were injected with methanol extract of *Orthosiphon stamineus* l 1h before cisplatin. This indicates that methanol extract has a protective potential on cisplatin-induced nephrotoxicity Reduced glutathione has a multiple role as an antioxidant agent. It functions as a scavenger of reactive oxygen species, including hydroxyl radicals, singlet oxygen, nitric oxide and peroxynitrite.[17,20,21] Data of our study indicate that GSH increased when animals were injected with methanol extract before cisplatin administration.

Based on the earlier reports leaves contain flavanoids as one of the phytochemical constituents, which posses broad spectrum of biological activities. Presence of high levels of flavonoids, triterpenoids derivatives in methanol extract may contribute to its nephroprotective activity via their antioxidant property.

In conclusion, the Methanol extract of *Orthosiphon stamineus* leaves protected cisplatin–induced renal damage in rats.

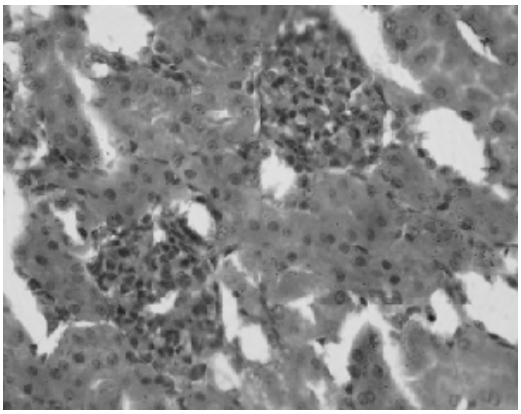

Figure-1

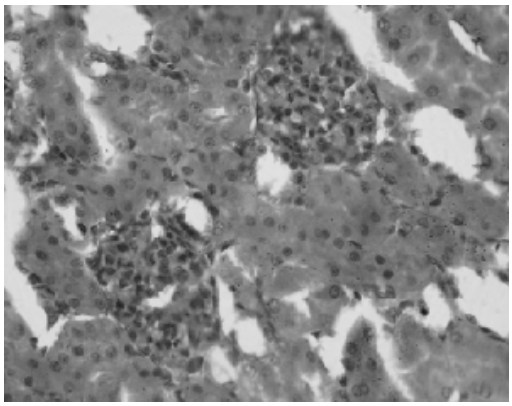

Figure-2

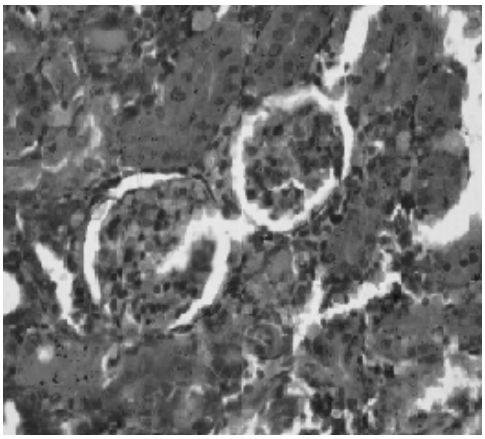

Figure-3

Effect of Methanol Leaf extract of Orthosiphon Stamineus on Cisplatin induced Renal Toxicity in Rats

Group	Weight of Kidney	Blood urea (mg/dl)	Serum Creatinine	Urinary Protein	Glutathione (ugm/dl)
I Control	5.33 ±0.33	26.69±1.56	0.95±0.99	3.40±0.277	3.735±0.078
II cisplatin	-3.83±0.31	60.55±6.02	4.23±0.288	5.98±0.063	0.9450±0.049
IIIcisplatin + 100mg/kg	2.17±0.31	39.43±1.57	2.08±0.158	4.77±0.124	1.943±0.072
IV cisplatin + 200mg/kg	4±0.37	28.08± 1.03	1.01 ±0.083	3.65 ±0.150	3.30 ±0.120

Values are mean ± sem of 6 animals in each groups. Group II com Pared with group I (P<0.001) Group III and IV compared with Group II (P<0.001) (ONE WAY ANOVA)

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REFERENCES

1. Mariyam, A., M.Z.Asmawi et al Hypoglycemic activity of the aqueous extract of Orthosiphon stamineus.*Fitoterapia* 1996;67(5):465-468.
2. Ohashi K, Bohgaki T,Shibuya H Antihypertensive Substances in the leaves of Kumis Kucing(orthosiphon stamineus) in java island. *Yakugaku zasshi.* 2000;120 ;(5):474-82.
3. Doan DD, Nguyen NH, Doan HK, et al. Studies on the individual and combined diuretic effects of four Vietnamese traditional Herbal remedied (Zeamays, Imperatacylindrical, plantago major and Orthosiphonstamineus) *JEthnopharmacol.*1992;36(3):222-5.
4. Sleijfer, D.T., S. Meijer, R.N.H. Mulder,.Cisplatin:A review of clinical applications and renal toxicity. *Pharmaceut. Weekblad Scientific* Edition 1985;7, 227-37.
5. Thigpen, T., R. Vance, L. Puneky, T. Khansurt, Chemotherapy in advanced ovarian carcinoma current standard of care based on randomized trials. *Gynecol. Oncol.*1994; 55,987-990.
6. Dobyan, D.C., J.M. Bull, F.R. Strebel, B.A. Sunderland, R.E. Bulger, Protective effect of 0 (beta-hydroxyethyl) rutosideon cis platinum-induced acute renal failure *Laboratory Investigation* 1986; 55, 557-563.
7. Meyer, K, N. Madias, Cisplatin nephrotoxicity. *Mineral and Electrolyte Metabolism* 1994; 20,201-13.
8. Matsushima, H., K. Yonemura, K. Ohishi, A.Hishida,. The role of oxygen free radicals in cisplatin-induced acute renal failure in rats. *J. of Laboratory and Clinical Medicine* 1998; 131, 518-526.

9. Yoshida, M., A. Khokhar, Y. Kido, F. Ali-Osman, Z. Siddik, Correlation of total & interstrand DNA adducts in tumor and kidney with antitumor efficacies anddifferential nephro toxicities of cisammine/cyclo hexyl amine dichloro platinum(II) and cisplatin. *Biochem. Pharmacol.* 1994;17, 793-9
10. Lieberthal, W., V. Triaca, J. Levine, Mechanisms of death induced by cisplatin in proximal tubular epithelial cells: apoptosis vs. necrosis. *American Journal of Physiology* 1996; 270, 700-708.
11. Somani, S. M., Husain, K. and Whitworth, C., Dose-dependent protection by lipoic acid against cisplatin induced nephrotoxicity in rats: Antioxidant defence system. *Pharmacol. Toxicol*., 2000;86, 234–241.
12. Brod, J. and Sirota, J. H. Practical Clinical Biochemistry (eds Varley, H. et al.), William Heinman Medical Books Ltd, London, vol. 1, pp. 1980; 456–460.
13. Marshell, M. H., Fingerhurt, B. and Miller, H., Practical Clinical Biochemistry (eds Varley, H. et al.), William Heinman Medical Books Ltd, London, 1980; vol. 1, pp. 478–480.
14. Lowry, H., Rosenberg, N. J., Farr, A. I. and Randa, R. J. Protein measurement with folin phenol reagent. *J. Biol. Chem.*, 1951; 193, 265–275.
15. Moron, M. A., Depierre. J. W. and Mannervik, B. (1979), Levels of glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochem. Biophys. Acta*, 1979; 582, 67–78.
16. Pratibha Ravindra, Dayanand A. Bhiwgade, Sameer Kulkarni, Padmanabh V. Rataboli and Chitra Y. Dhume et al, Cisplatin induced histological changes in renal tissue of rat. *journal of cell and animal biology* 2010;4(7), pp. 108-111
17. Halliwell B and Gutteridge JM Protection against oxidants in biological systems: the superoxide theory of oxygen toxicity. *In*: *Free Radicals in Biology andMedicine* (2nd ed.), Oxford, UK, Clarendon, 1989;pp.87-187
18. Behling EB, Sendao MC, Francescato HDC, AntunesLMG, Costa RS and Bianchi MP Comparative study of multiple dosage of quercetin against cisplatin induced nephrotoxicity and oxidative stress in rat kidneys. *Pharmacol. Rep*., 2006; **58**: 526-532.

19. Miyaji T, Kato A, Yasuda H, Fujigaki Y and Hishida A. Role of the increase in p21 in cisplatin-induced acute renal failure in rats. *J. Am. Soc. Nephrol.*,2001; **12**:900-908
20. Matsushima H, Yonemura K, Ohishi K and Hishida A The role of oxygen free radicals in cisplatin induced acute renal failure in rats. *J. Lab. Clin. Med.*,1998;**131**: 518-526
21. Leibbrandt ME, Wolfgang GH, Metz AL, Ozobia AA andHaskins JR Critical subcellular targets of cisplatin and related platinum analogs in rat renal proximal tubule cells. *Kidney Int.,* 1995; 48(3): 761-770.

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