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PROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF *OROXYLUM INDICUM* AGAINST CISPLATIN-INDUCED ACUTE RENAL FAILURE

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ABSTRACT

The objective of present study was to investigate the effect of ethanolic extract of roots of *Oroxylum indicum* against cisplatin-induced renal injury in male albino rats. Cisplatin (6mg/kg body wt i.p.) induced nephrotoxicity evidenced by significant increase in Blood Urea nitrogen, Serum Creatinine, Urinary Total Proteins, Lipid Per oxidation levels and decreased Creatinine Clearence level. On oral administration of ethanolic extract (200mg/kg; 400mg/kg body wt) for 3days starting one hr before cisplatin administration significantly reduced elevated levels of Blood Urea nitrogen, Serum Creatinine, Lipid Per oxidation, reduced the protein excretion and increased Creatinine Clearence level. Histological studies also substantiated the above results. The effect of ethanolic extract of roots of *O. indicum* was dose dependent.

Key words: *Oroxylum indicum*, Cisplatin, Lipid peroxidation, Nephroprotector activity.

INTRODUCTION

Nephrotoxicity can be defined as renal disease or dysfunction that arises as a direct or indirect result of exposure to medicines and industrial or environmental chemicals. Over the past several years, the number of persons suffering from renal problems is increasing and estimated that 26 million people are suffering with kidney problems. The reasons for this are exposure to medicines, industrial/environmental chemicals, age, pre-renal disease etc. Drugs such as cisplatin, gentamicin, paracetamol, adriamycin induces nephrotoxicity. Cisplatin (Cis-diammine dichloro platinum-II) an antineoplastic agent has a great spectrum of clinical activity to treat a variety of cancers such as ovarian, testicular, bladder, head and uterine cervix carcinoma (Sleiffer *et al*., 1985; Thigpen *et al*., 1994; Deconti *et al*., 1973) but clinical use of cisplatin was limited because of its nephrotoxicity (Hanigan 2003; Ramesh and Reeves 2004; Jing *et al*., 2007). Although the mechanism of cisplatin-induced renal toxicity is still unclear, several reports suggested that oxygen free radical play a major role (Baldew *et al*., 1989; Bompart *et al*., 1989; Sugihara, 1987; Rao and Rao 1998). Till today there is no specific drug in allopathy which was proved to be clinically effective as a complete protective agent against cisplatin-induced nephrotoxicity Hence, there is a continuous search for agents which provide nephroprotection against renal impairment. Earlier reports evident that number of plant like *Pongamia pinnata* (Annie Shirwaiker *et al*. 2003), *Nigella sativa* (All and Gerald blunder, 2003) *Cassia auriculata* (Annie Shirwaiker *et al*. 2003), *Zingiber officinale* (Ajith *et al*., 2006) exhibited good nephroprotection against cisplatin-induced nephrotoxicity.

Oroxylum indicum Vent.(*O.indicum*) Monotypic genus, is important medicinally, distributed in India, Srilankha, Philippines and Indonesia. In India it is distributed in Eastern and Western ghats and North East India. The tribal people of Chittor district of Andhra Pradesh use roots of *Oroxylum indicum* to treat the urinary disorders (Madhavachetti *et al*., 2007) but no scientific studies have yet been undertaken to verify the claims.Hence, the present study is designed to evaluate the nephroprotector potential of roots of *O.indicum* against cisplatin-induced nephrotoxicity.

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MATERIALS AND METHODS

Plant material

O.indicum roots were collected from Talakona, Chittor district and authenticated by Dr. Madhava chetti , Department of botany, S.V University, Tirupathi and voucher specimen was deposited in S.V.U Botany department.

Preparation of Ethanolic Extract

The roots were (2kgs) allowed to dry under shade. The dried roots were powdered in a wiley mill and extracted with rectified spirit (2L x 3). The extract was concentrated under reduced pressure to get solid mass (16 gms). This solid mass was used for pharmacological studies.

Animals

Healthy Wister adult male albino rats between 2 and 3 months of age and weighing about 150-200 gm were used in the present study. Housed in poly propylene cages and fed with standard rat pellet diet, water *ad libitum*. Animals were acclimatized to our lab environment for about a week. Animals were handled according to the rules and regulations of Institutional Animal Ethics Committee (IAEC).

Acute toxicity studies

Animals were divided into 6 groups each group containing 6 animals. First group stands for normal and the remaining five groups received different doses (100, 300, 600, 1000 and 3000 mg/kg body wt) of alcoholic extract of roots of *O.indicum* to study the lethal dose.

Screening of nephroprotector activity of alcoholic extract of plant *O.indicum*

Cisplatin was prepared in distilled water to give 1mg/ml solution. The alcoholic extract of the plant was suspended in 2% gum acacia used for studies. Animals were divided in to 6 groups (n=6). Group-I animals treated with vehicle (2% gum acacia) was kept as normal. Animals which belongs to Group-II injected with a single dose of Cisplatin (6mg/kg body wt, i.p.) was kept as control. Group-III received alcoholic extract of roots of *O.indicum* (400mg/kg body wt) only. Group-IV and V were treated with alcoholic extract of *O.indicum* of 200 and 400 mg/kg body wt, p.o. plus Cisplatin. The extract was administered by oral gavage 1hr before and 24 and 48 hrs after Cisplatin injection to Group-IV (extract 200mg/kg plus Cisplatin 6mg/kg body wt) and Group-V (extract 400mg/kg plus Cisplatin 6mg/kg body wt) animals.

After 48 hrs of cisplatin administration urine samples were collected and urinary functional parameters were determined. 72 hrs after Cisplatin injection, animals were sacrificed, blood was collected directly from the heart of each animal and BUN, SC, Clcr, was determined. Kidney tissue homogenates were used for the assay of reduced LPO.

Assessment of renal function

Estimation of serum markers

Blood Urea Nitrogen**:** BUN is determined by DAM method (Godkar, 1994). Absorbance was read from spectrophotometer (Systronics).

Serum Creatinine: Creatinine levels in serum was estimated by the Jaffe's Alkaline Picrate method (Godkar, 1994) using a creatinine kit. Absorbance was read from spectrophotometer (Systronics).

Estimation of Urinary functional parameters

Urinary Total Protein (U_{TP}): Urinary total proteins was estimated by Turbidity method (Godkar, 1994). Turbidities of urine test and standards were measured against blank at 640nm.

Creatinine clearance (Cl_{cr})**:** Creatinine clearance was estimated by alkaline picrate method (Godkar, 1994). Absorbance of test and standard were measured against blank at 520nm.

$$\text{Creatinine clearance} = \frac{\text{Urinary creatinine} \times \text{Urinary volume/hr}}{\text{Serum Creatinine}}$$

Lipid peroxidation(LPO)

The concentration of kidney tissue LPO was determined by Thiobarbituric acid test (Ohkawa *et al*., 1979) and the absorbance was measured at532 nm.

Histological Studies

Two animals from each group were sacrificed on day fifteen or sixteen and kidneys were isolated. The kidney sections were stained with hematoxylin and eosin and observed under light microscope.

Statistical analysis

The results are expressed as mean±SEM and the data was analysed by one way analysis of variance followed by post hoc Student-Keuls test using SPSS computer software for *invivo* studies. Statistical significance was set at $P \leq 0.05$.

RESULTS

Acute toxicity of *O.indicum*

The alcoholic extract of root of *O.indicum* when orally administered in the dose range of 100-2000 mg/kg body wt. did not produce any significant changes in the autonomic or behavioral responses, including death during the observation period.

Animals which received the alcoholic extract roots of *O.indicum* alone (group III) for three days exhibited no change in serum markers level and urinary functional parameters. Hence, the alcoholic extract of root of *O.indicum* did not show any deteriorative effects on kidney. To assess the neproprotector activity of extract the data obtained from alcoholic extract treated groups (IV, V) was compared with group II (animals which received only cisplatin injection).

Effect of alcoholic extract on serum markers level

Table 1 lists the effect of alcoholic extract of roots of *O.indicum* on cisplatin-induced nephrotoxicity. Intraperitonal administration of cisplatin at 6 mg / kg *i.p.* caused significant elevation of BUN in group II animals, when compared to normal control animals (group I). On administration of alcoholic extract in group IV and V animals a significant reduction in the levels of BUN was observed when compared to group II animals. Administration of alcoholic extract of roots of *O.indicum* before and after cisplatin challenge significantly lowered

the elevated level of SC. However, the levels of BUN, SC in 400mg/kg body wt *O.indicum* treated group were lower than 200mg/kg body wt *O.indicum* treated group.

Effect of alcoholic extract on Urinary parameters

The deterioration of renal functions induced by cisplatin and the effect of oral administration of the alcoholic extract of roots of *O.indicum* are given in Table 2. Animals administered with cisplatin excreted high amount of U_{TP} when compared with normal group I animals. Whereas, Treatment with ethanolic extract in group IV (200mg/kg bd wt) and group V animals (400mg/kg bd wt) reversed the effect caused by cisplatin in dose dependent manner.

The animals received cisplatin alone exhibited decreased levels of Cl_{cr} when compared with normal animals. On oral administration of extract showed significant increase in Cl_{cr} in group IV and V animals.

Effect on LPO

Kidneys were isolated from the animals to estimate the levels of MDA which was expressed nmol/mg protein. Animals which were treated with cisplatin alone exhibited elevated levels of MDA, when compared to normal control animals. Animals which treated with extract exhibited dose dependent reduction of MDA levels when compared to group II animals.

Table 1. Effect of ethanolic extract of roots of *Oroxylum.indicum* against Cisplatin-induced nephrotoxicity

Group	Treatment mg/kg	BUN (mg/kg)	SC (mg/dl)	LPO (nmol/gm tissue)
I	Normal(1%tween)	32.12±1.16	0.70±0.10	10.5±0.06
II	Cisplatin(6mg/kg) body wt	68.34±2.13*	1.62±0.17*	21.3±0.54*
IV	Extract (200mg/kg) + Cisplatin(6mg/kg)	46.44±1.71	1.24±0.15	14.2±0.22
V	Extract (400mg/kg)+ Cisplatin(6mg/kg)	33.80±1.30	0.81±0.12	9.0±0.11

Each value represents the mean ±S.E.M from 6 animals in each group.
*p<0.001 when compared with normal control group.
a:p<0.05 when compared with control group.
b:p<0.01 when compared to control group.
c:p<0.05 when compared to normal control animals.

Table 2. Effect of ethanolic extract of roots of *Oroxylum.indicum* against Cisplatin-induced nephrotoxicity

Group	Treatment (mg/kg)	Clcr (ml/hr/100 Bd.wt)	U_{TP} (mg/24hrs)
I	Normal(1%tween)	18.14±1.5	8.25±0.50
II	Cisplatin(6mg/kg) body wt	6.48±0.57*	18.42±1.70*
IV	Extract (200mg/kg)+ Cisplatin(6mg/kg)	12.00±1.2	12.02±1.12
V	Extract (400mg/kg)+ Cisplatin(6mg/kg)	17.50±1.42	9.32±0.72

Each value represents the mean ±S.E.M from 6 animals in each group.
*p<0.05 when compared to normal control group.
a:p<0.05 when compared to control group.
b:p<0.05 when compared to control group.
c:p<0.01 when compared to normal control animals.

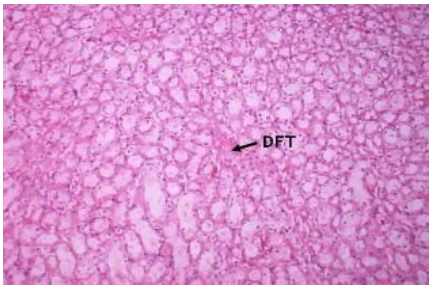

Plate 1:Section of normal kidney showing normal organization. DFT= Dense fatty tissue

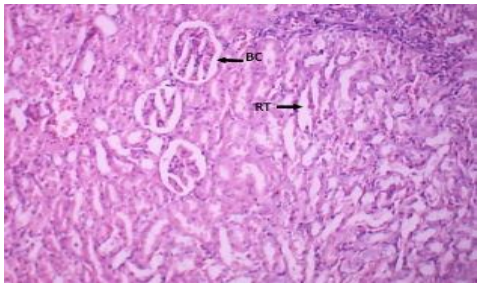

Plate 2: Section of rat kidney treated with Ethanolic extract of *Oroxylum indicum*. BC=Bowmanns capsule ; RT= Renal tubule

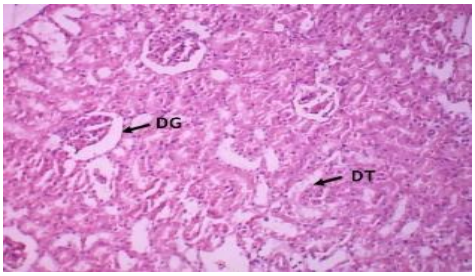

Plate 3; Section of rat kidney treated with cisplatin(6mg/kg).
DG= Degenerative Glomeruli;
DT= Degenerative Tubule

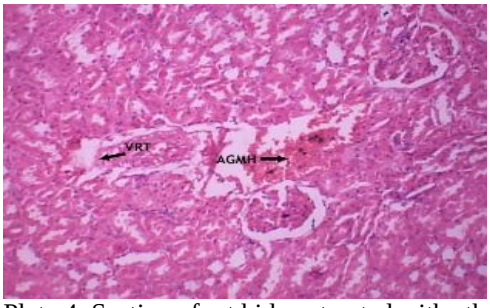

Plate 4; Section of rat kidney treated with ethanol extract of *Oroxylum indicum* (200mg/kg+cisplatin)

showing moderate degenerative changes.
VRT= Vacuolization of Renal tubules;
AGMH= Atropic Glomeruli with mild haemorrazic changes

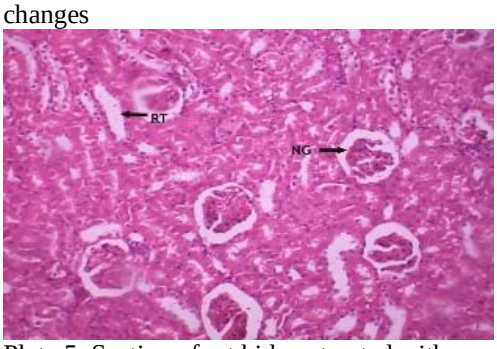

Plate 5: Section of rat kidney treated with ethanolic extract of *Oroxylum indicum* (400mg/kg) showing regenerative changes.
RT= Normal rénal Tubule ; BC= Normal Bow man's capsule
EC= Normal Epithelial cells (indicating regenerative changes

Histological studies

Histological studies showed a degenerative glomeruli and degenerative tubule in kidneys of animals treated with Cisplatin (6mg/kg) alone. Group-IV(200mg/kg+ Cisplatin) showed moderate degenerative changes like vacuolization of renal tubules and atropic glomeruli with mild hemorrhagic changes, Group-V(400mg/kg) showed regenerative changes such as normal renal tubule, normal bowman's capsule and normal epithelial cells.

Discussion

Cisplatin is one of the most effective anticancer drugs administered to treat a variety of cancers such as ovarian cancer, testicular, bladder, head and neck and uterine cervix carcinomas (Sleijfer *et al.*, 1985). High doses of cisplatin is more effective than low doses in ovarian and colorectal cancer (Di Re *et al.*, 1990), however high dosage treatment induces nephro-and neuro toxicity. Nephrotoxicity is a dose limiting factor in clinical uses of cisplatin. The mechanism of cisplatin nephrotoxicity is not fully understood several mechanisms were proposed such as cisplatin induces apoptosis in sensitive non-renal cells (Buttke *et al.*, 1994; *Hara et al.*, 2001), cisplatin generates active oxygen species such as superoxide anion and hydroxyl radical by interaction with DNA. This generation of active oxygen species may play a role in the cytotoxicity of cisplatin (Masuda *et al.*, 1994) however the generation of free oxygen radicals in tubular cells has been proposed as an important pathogenic process (Ishikawa *et al.*, 1990).

Number of people suffering from kidney disorder's increasing day to day but till today there is no effective drug is available to treat renal toxicity. Number of reports evidenced that anti oxidants such as Vitamin C, Vitamin D, Selenium and plants contain anti oxidant principles i.e., *Pongamia pinnata* (Annie Shirwaikar *et al.*, 2003*), Echinacea pallida* (Mustea *et al.*, 1998). *Nigella sativa (*All and Gerald blunder, 2003) showed protection against cisplatin induced nephrotoxicity. *O.indicum* also one such plant which contain antioxidant principles such as flavonoids and phenolic compounds (Li-juan chen *et al*., 2003). Hence present study was focused on the effect of ethanolic extract of roots of *O.indicum* on the renal damage induced by Cisplatin.

Protective effect of ethanolic extract of roots *O.indicum* was tested at two doses i.e, 200mg/kg and 400mg/kg against Cisplatin-induced nephrotoxity.

Animals which received plant extract (400mg/kg) alone for 48 hrs there was no change in serum markers levels, urinary functional parameters and LPO levels compared to normal control animals. These results showed that ethanolic extract of *O.indicum* did not show any deteriorate effect on kidney.

Urea is the major nitrogen containing metabolic product of protein catabolism in human. It is accounts for more than 75% of the non protein nitrogen eventually excreted. More than 90% of urea is excreted through the kidneys. Creatinine formed as the end product of creatine metabolism is a waste product. It is filtered at the glomeruli and secreted by the tubules and its excretion in urine per 24hrs is 1.5-3.0gm. During renal damage, the kidneys unable to excret the urea and creatinine hence these serum markers levels are increased in blood. Cisplatin-induced nephrotoxicity in rats was established by elevated blood urea nitrogen (BUN), serum creatinine (SC) levels and marked drop in creatinine clearance levels. Present study demonstrates cisplatin-induced renal injury, which was evidenced by the elevated levels of BUN, SC, reduced the Clcr and excreted high amount of urinary protein.

The alcoholic extract of Animals which received extract at doses 200mg/kg and 400mg/kg body wt showed significant decrease in BUN, SC and increase in Clcr levels and bring about marked recovery in kidneys as evidenced microscopically.

The protein most commonly found in the urine are those derived from the blood plasma and consist of a mixture of plasma albumin and a globulin. These are not normally filtered through the glomeruli, but in kidney diseases, due to the alteration in glomerular permeability, these proteins appear in urine. Animals received cisplatin alone exhibited high amount of protein excretion in urine, which indicate that cisplatin-induced renal damage. Extract significantly reduced the urinary protein excretion in dose dependent manner, which is indicative of extract is inducing regenerative changes in kidney.

Number of studies suggested that cisplatin induces nephrotoxicity through LPO (Devi Priya *et al.,* 1999*;* Rao *et al.,* 1998). Lipid peroxidation is a degenerative path way of membrane components mediated through free radicals produced in the cells. In present study on administration of cisplatin a significant increase in the malondialdehyde (MDA) content was observed when compared to the normal group. Upon treatment with ethanolic extract of roots of *Oroxylum indicum* (200mg/kg and 400mg/kg) there was significant decrease in MDA content is indicating decrease in lipid peoxidation and the effect was dose dependent manner.

The ethanolic extract of roots of *O.indicum* showed more protective effect against Cisplatin-induced nephrotoxicity. Literature survey revealed that *O.indicum* is endowed with various chemical components such as flavanoids (Roy *et al.,* 2007), tannins, alkaloids, oils etc which is possibly contributes to its diverse use in folklore medicine.Flavonoids are well known potent antioxidants and free radical scavengers. Hence, the probable mechanism of nephroprotection of Presence of flavanoids in *O.indicum* may partly contribute the nephroprotector activity of *O.indicum* may be through its antioxidant and free radical scavenging property. In conclusion the present study provides the corroborative scientific evidence for the folklore uses of *O.indicum* in urinary troubles.

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REFERENCES

Ajith TA, Nivitha, V, Usha S. Zingiber officinale alone and in combition with alpha tocopherol protect the kidney against cisplatin-induced acute renal failure. *Food and chemical Technology*, 45, 2007, 921-927.

All BH, Gerald and Blunder. Pharmacological and toxicological properties of Nigella sativa. *Phytotherapy Research*, 17 (4), 2003, 299-305.

Annie Shirwaikar, S. Malini, S. Chandrika Kumari. (); Protection against cisplatin and gentamicin induced nephrotoxicity in rats. *Inter. Journal of Expt. Biology*, 41, 2003, 58-62.

Baldew GS, Vanden Hamer CJA, Los G, Vermuelen NPE, de Goeij JJM, and Mcvie JG. Selenium induced protection against cis-diaminodichloroplatinum nephrotoxicity in mice and rats. *Cancer Res.*, 49, 1989, 3020-23.

Bompart G. Cisplatin-induced changes in cytochrome P-450, lipid peroxidation and drug-metabolizing enzyme activities in rat kidney cortex. *Toxicol Lett.*, 48(2), 1989, 193-9.

Buttke TM and Sandstrom PA. Oxidative stress as a mediator of apoptosis. *Immnol. Today*, 15, 1994, 7-10.

Deconti RC, Toftness BR, Lange RC, Creasey WA. Clinical and pharmacological studies with Cis-diammine dichloro platinum (II). *Can Res.*, 33(6), 1973, 1310-1315.

Devi Priya S. and Shyamala Devi CS. Protective effect of quercetin in cisplatin-induced cell injury in the rat kidney. *Ind. J. Pharmacol.***,** 31, 1999, 422-426.

Di Re F, Bohn S, Oriana S, Spatti GB, Zunino F. Efficacy and safety of high dose cisplatin and cyclophosphamide with glutathione protection in the treatment of bulky advanced epithelial ovarian cancer. *Cancer Chemotherapy and Pharmacology*, 25, 1990, 355-360.

Godkar PB. Kidney function tests. In: Text book of Medical Laboratory, Bhalani Publishing House, Bombay, 1994.

Hanigan MH, Devarajan P. Cisplatin nephrotoxicity:molecular mechanisams. *Can Ther.*, 1, 2003, 47-61.

Hara M, Yoshoda M, Nishijima H, Yoksuka M, Iigo M, Ohtani-kaneko R, Shimada A, Hasegawa T, Akama Y and Hirata K. Melatonin a pineal secretary product with anti oxidant properties, protects against cisplatin-induced nephrotoxicity in rats. *Journal of Pineal. Res.*, 30, 2001, 129-138.

Jing XB, Cai XB,Hu H, Chen BM, Cai JY Reactive oxygen species and mitochondrial membrane potential are modulated during CDDP-induced apoptosis in EC-109 cells. *Biochem. Cell Bio.*, 85, 2007, 265-271.

Masuda H, Tanaka T. and Takahama U. Cisplatin generates superoxide anion by interaction with DNA in a cell free system . *Biochem. Biophys. Res. Commun.*, 203, 1994, 1175

Mustea I, Postesu D, Tamas M, Rasnita TD. Experimental evaluation of protective activity of *Echinaceae pallida* against cisplatin toxicity. *Phytotherapy Research*, 11 (3), 1998, 263-265.

Ohkawa H, Ohishi N and Yagi K. Assay for lipid peroxide in animal tissues by thio barbituric acid. *Reacto Annal. Biochem*, 95, 1979, 351-358.

Ramesh G, Reeves WB. Inflammatory cytokines in acute renal failure in Kidney. *Int Suppi.*, 91, s56-61.

Rao M and Rao MNA. Protective effects of *cystone* a polyherbal ayurvedic preparation on cisplatin-induced renal toxicity in rats. *Journal Ethanopharmacol.*, 62, 1998, 1-6.

Rao M, Ravindr K, and Rao MNA. Protective effect of selenomethionine against cisplatin-induced nephrotoxicity in C57BL/6J mice bearing B16F. Melanona without reducing Antitumor activity. *J. Pharm. Pharmcol.*, 50 (1), 2001, 549-52.

Roy MK, Nakhara K, Na TV, Trakoontivakorn G, Takenaka M, Isobe S. Bacailen, a flavonoid extracted from a methanolic extract of *Oroxylum indicum* inhibits proliferation of a cancer cell line in vitro via induction of apoptosis. *Pharmazie*, 62(2), 2007, 149-53.

Sleijfer DT, Meijer RNH. Cisplatin; A review of clinical applications and renal toxicity. Pharm Week Sci Edi., 7, 1985, 227-237.

Sugihara K, Nakano S, Koda M, Tanaka K, Fukuishi N, and Gemba M. Stimulatory effect of cisplatin on production of lipid peroxidation in renal tissues. *Jpn. J. Pharmacol.*, 43(3), 1987, 247-52.

Thigpen T, Vance R, Puneky LK, Hansurt T. Chemotherapy in advanced ovarian carcinoma ; current standard of care cisplatinum. *Biochem Jour*., 2529(1), 1994, 127-136.