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Evaluation of the Side Effects of Cisplatin Drug in a Nephrotoxicity Model of Wistar Rats

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Abstract
This study critically assessed the observable side effects of cisplatin (anti-tumor) drug in a Portulaca oleracea treated nephrotoxicized wistar rats. Twenty four albino female rats were grouped into four-groups A, B, C, & D. Group A served as the control group; group C and D were all nephrotoxicized using 2ml/kg cisplatin and treated with 400mg/kg and 800mg/kg doses of Portulaca oleracea methanolic extract respectively. Group B were nephrotoxicized but remained untreated. Significant Increase in serum creatinine ($P<0.05$) in group B rats showed presence of nephrotoxicity and results further showed recovery (significant decrease at $P<0.05$) in serum creatinine level of groups C &D rats due to the administration of portulaca oleracea. Physically observable features were examined in the rats at the 4th & 7th day of drug administration which showed the presence of side effects (ototoxicity, loss of appetite, black stool, dizziness, fever, etc) which were seen to be increasing with prolonged administration. This research posits seemingly untreatable side effect of cisplatin chemotherapy which increases with increased chemotherapy duration and decreased side effects in the combination of portulaca oleracea in cisplatin chemotherapy.
Keywords: chemotherapy, cisplatin and nephrotoxicity.

INTRODUCTION

Cisplatin (CP) is a chemotherapy drug. It was the first member of a class of platinum-containing anti-cancer drug which reacts in vivo, binding to and causing crosslink of DNA which ultimately triggers apoptosis[22]. It is administered intravenously as short term infusion in normal saline for treatment of various types of cancers including sarcomas, some carcinomas (eg. small cell lung cancer and ovarian cancers), lymphomas and germ cell tumors[16]. It is particularly effective against testicular cancer[17]. Chemotherapy for lymphoproliferative disease, leading to tumor lysis syndrome with uric acid and calcium phosphate crystal deposition, has also been associated with renal failure[7]. Cisplatin is accompanied by decrease in glomerular filtration rate (GFR), increase in blood urea nitrogen and serum levels of creatinine and tubular injury[12]. Nephrotoxicity is a dose limiting side effect of Cisplatin that can limit its use[18].

Common neurological side effects of cisplatin include visual perception and hearing disorder which can occur soon after treatment begins [19,20]. Cisplatin is one of the most emetogenic chemotherapy agent showing nausea and vomiting as its side effects [22], ototoxicity, electrolyte disturbance, myelotoxicity, and hemolytic anemia[21]. Portulaca oleracea (purslane) extract used in the treatment of nephrotoxicity is a widespread weed ranked 8th most common plant in the world [14], has a long history of use for human food, animal feed and medicine. It is used in liver and kidney disorder, as an emollient, astringent and diuretic [10], depurative, anti-bacterial, anti-scorbutic, and febrifuge[25]. It has been described as a 'power food' of the future because of its high nutritive and anti-oxidative properties[15].

MATERIAL AND METHOD

Fresh aerial parts of PO was harvested, dried, finely grated and extracted using methanol in soxhlet apparatus. Twenty four female wistar rats were obtained, acclamatized for two weeks under standard housing condition, fed ad-libitum with water and standard rat chow. The rats were divided into four groups A-D. group A served as the control group; group B received only a single dose of cisplatin (2ml/kg), Group C and D were given cisplatin (2ml/kg) and were treated with 400mg/kg and 800mg/kg methanolic extract of portulaca oleracea respectively. Portulaca oleracea treatment was given two hours after cisplatin drug administration. Cisplatin drug was administered intraperitonially while the PO extract was administered orally. Physically observable changes and features were accessed during the 4th and 7th day of cisplatin administration. These side effects were divided into four groups based on convenience. The water consumption of the rats was also monitored before and after drug administration. The cisplatin side effects on the rats were observed before and after drug administration. The animals were anaesthetized after the 7th day of drug administration and 5ml of blood sample each were collected by cardiac puncture into centrifuge tubes. The serum was separated by centrifugation for twenty minutes at 4000rpm and the serum creatinine level assessed using the Jaff's method as modified by Ochei and kolhatka[13].

The results were analyzed using SPSS (version 16.0) and the differences between mean and the main effects of the groups were determined by one way anova and the results expressed as mean value ± SD. P< 0.05 were seen to statistically significant.

RESULTS

Table 1.0 showing the serum creatinine level of wistar rats after drug administration

GROUPS	SERUM CREATININE (mg/dl)
GROUP A	0.21± 0.01*
GROUPB	0.61±0.00**
GROUP C	0.39±0.04***
GROUP D	0.24±0.00***

*the serum creatinine level of the normal rats (untreated rats) were significantly lower (P<0.05) than all other groups. **There is a significant increase in serum creatinine (P<0.05) of Group B rats due to administration of cisplatin when compared to the other groups. ***There was a significant decrease (P<0.05) in the serum creatinine of groups C&D when compared to Groups A

Table 2.0 showing the average water consumption of animals before and during drug administration

GROUPS	WATER CONSUMPTION (ML/DAY) BEFORE ADMINISTRATION	WATER CONSUMPTION (ML/DAY) AFTER ADMINISTRATION
A	30.00±2.00	29.00±1.41
B	28.67±1.53	32.00±2.83
C	29.00±1.00	22.00±2.83
D	33.00±2.65	38.00±0.00

The increase or decrease in the water consumption of the animals before or after the drug administration were not statistically significant (P<0.05) except group D that showed a statistically significant increase after drug administration when compared to the other groups.

Table 3.0 showing the average body weight of animals before and after drug administration.

GROUPS	BODY WEIGHT (GRAMS) BEFORE ADMINISTRATION	BODY WEIGHT (GRAMS) AFTER ADMINISTRATION
A	150.60±11.07	167.23±13.12
B	148.15±13.43	143.88±10.62
C	152.03±18.91	174.98±4.20
D	166.70±12.00	183.93±5.74

There was a significant decrease in the average weight of rats in group B (P<0.05) when compared to rats in Group A. however, significant increase in the average weight was seen in rats of groups A, C and D when compared with group B (P<0.05).

Summary of group 1 side effects observed at day 4 and 7 during drug administration (tables 4.0&5.0)

Group A showed none of the group I side effects. Both fast breathing and blurred vision were not observed in any of the groups at all at both 4th and 7th day of drug administration. Wheezing manifested only in groups C & D at day 7 of side effect assessment. There were signs of moderate troubled walking in group D rats on the 4th day (which disappeared at day 7) and in group B in the 7th day. All the treated groups except A were seen to be moderately tired at day 4 except group C with group D being severely tired at day 7 of drug administration.

Table 4.0 Observable physical features at day 4 during drug administration – Group 1 side effects

GROUPS	DIZZINESS/TIREDNESS	TROUBLED WALKING	WHEEZING	BLURRED VISION	FEVER/SHIVERING	FAST BREATHING
A	-	-	-	-	-	-
B	+	-	-	-	+	-
C	-	-	-	-	-	-
D	+	+	-	-	-	-

KEY

+	MODERATE
++	SEVERE
-	ABSENT

Table 5.0 Observable physical features at day 7 during drug administration – Group 1 Side effects

GROUPS	DIZZINESS/TIREDNESS	TROUBLED WALKING	WHEEZING	BLURRED VISION	FEVER/SHIVERING	FAST BREATHING
A	-	-	-	-	-	-
B	+	+	-	-	-	-
C	+	-	+	-	-	-
D	++	-	+	-	-	-

KEY

+	MODERATE
++	SEVERE
-	ABSENT

Summary of group 2 side effects observed at day 4 and 7 during drug administration (tables 6.0&7.0)

Group 2 side effects were not seen at all in groups except for group D rats that showed moderate doughiness at both the 4th and 7th day.

Table 6.0 Observable physical features at day 4 during drug administration – Group 2 side effects

GROUPS	PIN POINT REDNESS	MOUTH/LIP SORE	DOUGHINESS/SPRINGBACK	PALE TOES AND EARS	FACE AND MUSCLE SWELLING
A	-	-	-	-	-
B	-	-	-	-	-
C	-	-	-	-	-
D	-	-	+	-	-

KEY

+	MODERATE
++	SEVERE
-	ABSENT

Table 7.0 Observable physical features at day 7 during drug administration – Group 2 side effects

GROUPS	PIN POINT REDNESS	MOUTH/LIP SORE	DOUGHINESS/SPRINGBACK	PALE TOES AND EARS	FACE AND MUSCLE SWELLING
A	-	-	-	-	-
B	-	-	-	-	-
C	-	-	-	-	-
D	-	-	+	-	-

KEY

+	MODERATE
++	SEVERE
-	ABSENT

Summary of group 3 side effects observed at day 4 and 7 during drug administration (tables 8.0 & 9.0)

No signs of bloody urine were seen in all groups; at day 7, moderate damper bedding were seen in groups B and D. moderate black and bloody feces were observed in group B and C rats on the 4th day of drug administration and groups B, C, and D on the 7th day. No trace of vaginal discharge were seen on the 7th day

Table 8.0 Observable physical features at day 4 during drug administration – Group 3 side effects

GROUPS	BLOODY URINE	DAMPER BEDDING	BLACK AND BLOODY FECES	VAGINAL DISCHARGE
A	-	-	-	-
B	-	-	+	-
C	-	-	+	-
D	-	+	-	-

KEY

+	MODERATE
++	SEVERE
-	ABSENT

Table 9.0 Observable physical features at day 7 during drug administration – Group 3 side effects

GROUPS	BLOODY URINE	DAMPER BEDDING	BLACK AND BLOODY FECES	VAGINAL DISCHARGE
A	-	-	-	-
B	-	+	+	+
C	-	-	+	-
D	-	+	+	-

KEY

+	MODERATE
++	SEVERE
-	ABSENT

Summary of group 4 side effects observed at day 4 and 7 during drug administration (tables 10.0&11.0)

All the treated groups showed moderate ototoxicity at both 4th & 7th day of drug administration. Group D showed moderate hunched over position on the 4th day of administration only. Group B showed severe loss of appetite at both 4th and 7th day and group C & D showed moderate appetite loss on day 4 of drug administration which were reversed on day 7. Death occurred in group B rats after the 7th day of drug administration.

Table 10.0 Observable physical features at day 4 during drug administration – Group 4 symptoms

GROUPS	LOSS OF APPETITE	OTOTOXICITY	HUNCHED OVER	DEATH
A	-	-	-	-
B	++	+	-	-
C	+	+	-	-
D	+	+	+	-

KEY

+	MODERATE
++	SEVERE
-	ABSENT

Table 11.0 Observable physical features at day 7 during drug administration – Group 4 symptoms

GROUPS	LOSS OF APPETITE	OTOTOXICITY	HUNCHED OVER	DEATH
A	-	-	-	-
B	++	+	-	+
C	-	+	-	-
D	-	+	-	-

KEY

+	MODERATE
++	SEVERE
-	ABSENT

DISCUSSION

Drugs cause approximately 20 percent of community- and hospital acquired episodes of acute renal failure[1,2,3]. Among older adults, the incidence of drug-induced nephrotoxicity may be as high as 66 percent[4]. Compared with 30 years ago, patients today are older, have a higher incidence of diabetes and cardiovascular disease, take multiple medications, and are exposed to more diagnostic and therapeutic procedures with the potential to harm kidney function[5]. A lthough renal impairment is often reversible if the offending drug is discontinued . the condition can be costly and may require multiple interventions, including hospitalization[6]. Cisplatin is the most commonly used antitumor drug in the clinic and has been implicated to cause nephrotoxicity as its major side effect[12],hence its use in this research. Major cisplatin-induced side effects including nephrotoxicity and hepatotoxicity are popularly known and widely researched on which sometimes seem to be chronic in nature. However, this research focused on the physically observable side effects and its possible attenuation with a combined Portulaca oleracea extract treatment. About 21 physically observable side effects were evaluated in this research.

Cisplatin drug administered was able to cause a significant nephrotoxicity as evidenced by increased serum creatinine ($P<0.05$) in group B as compared to group A (table 1.0). this effect seems to be attenuated by portulaca oleracea extract in groups C and D which showed a significantly decreased serum creatinine ($P<0.05$) when compared to group B. this agrees with the research by Gholamreza Karimi et al 2010[11] on the nephro-protective effect of aqueous and ethanolic extracts of portulaca oleracea on cisplatin-induced renal toxicity. Nephrotoxicity of cisplatin is usually associated with their accumulation in renal cortex, dependent upon their affinity to kidney and on kinetics of drug trapping process [9]. Cisplatin induced oxidative stress can activate some protein kinases which sensitizes the injured cell to apoptosis [11].

Cisplatin showed no significant effect on the water consumption of the rats as seen in the group B (the cisplatin control group) when compared to the normal control group (group A). The combination treatment with cisplatin and portulaca oleracea extract in groups C and D caused a significant decrease and increase ($P< 0.05$) in average water consumption respectively when compared to the control group and cisplatin group (table 2.0). the opposite significant effects seen in the treated groups is suspected to be due to the dose-effect relationship of portulaca extract given to the groups as group B showed no significant effect ($P<0.05$).

The effect of cisplatin on the average body weight was also observed; the cisplatin control group showed a significant decrease ($P<0.05$) in average body weight when compared to the other groups (table 3.0)

The physically observable side effects observed in this research which were grouped into four were not present in the control group showing their incidence in other groups to be as a result of cisplatin. Cisplatin side effects have been noted to be moderate or more severe; may get better or worse through the course of treatment or more side effect may develop as the course goes on[24].

In group 1 side effects (tables 4.0&5.0), fast breathing and blurred vision was not observed at both days of assessment during drug administration. Fever, present only in cisplatin on the 4^{th} day was inconstant as it were absent on the next day of assessment. Wheezing as a side effect was observed to show a slow onset in the portulaca oleracea treated group (group C &D) by its appearance in the 7^{th} day of drug administration. This slow onset have received suppression by the anti-oxidative property of portulaca oleracea[15]. Cisplatin has the ability to lower WBC and platelet count hence increasing the risk of infections like fever, chills, pain or bleeding [8]. Troubled walking noticed in group D rats were seen to disappear in the 7^{th} day of assessment (table 5.0); this may be as a result of the higher dose of portulaca oleracea extract administered or damage to certain nerves in the body causing peripheral neuropathy resulting to troubled walking[8].

Groups B & D showed moderate dizziness at the first assessment (table 4.0) and only D showed severe tiredness during the 7^{th} day of drug administration. Dizziness, spotted redness, fast breathing and swelling of the face are seen as allergic reactions and starts immediately after treatment [23]. However, based on this research finding, this is true with dizziness only (Tables 4.0 & 5.0); the allergic side effects have been described as a rare side effect [8].

Group 2 side effects were not seen at all in all groups except for group D rats that showed moderate doughtiness at both 4^{th} and 7^{th} (table 6.0&7.0), suggesting an association between dizziness (group 1 side effect) and doughtiness which were all shown by group D.

In group 3 side effects, there were no signs of bloody urine in all groups but damper beddings were seen in groups B (on the 7^{th} day) and D (on the 4^{th} and 7^{th} day) after drug administration (tables 8.0 & 9.0). This finding shows a correlation between damper bedding and average water consumption (table 2.0) as there was a significant increase ($P<0.05$) in average water consumption of Group D rats who compared with other groups to compensate for the increased urine output/damper beddings (tables (8.0&9.0). All the groups except for A shared presence of black and bloody feces either on the 4^{th} or 7^{th} day. Group B rats showed traces of vaginal discharge on the 7^{th} day of drug administration (table 9.0)

In the grouping side effects, ototoxicity, which is a major side effect of cisplatin and were noticed at a moderate level in groups B, C & D, each showing early onset (on the 4^{th} day of drug administration) continuing till the 7^{th} day (tables 10.0 & 11.0).hearing disorder and visual perception are all neurological side effects[19,20] and can

occur soon after treatment begins. The neurological side effects has been said to be caused by non-competitive inhibition of an archetypal membrane –bound mechanosensitive sodium-hydrogen ion transporter known as NHE-1 by cisplatin. This transporter is mostly found on the cells of the PNS near ocular and aural stimuli receiving centers. This inhibition is said to be dose-dependent and reversible[19]. Complete hearing loss may be caused by ciplatin's ability to bind melanin in the stria vascularis[22]. Group D showed a hunched over position on the 4th day (table 10.0; this supports and shows a relationship between the earlier side effects(dizziness and doughtiness)and hunched over position seen in this same group – (tables 4,5,6&7). All groups except A showed loss of appetite during drug administration but while groups C & D showed a moderate loss, group B was severe hence showing the chronic effect of cisplatin drug. This agrees with the average body weight which was significantly decreased ($P<0.05$) when compared to other groups (table 3.0). This supports the finding on cisplatin induced loss of appetite which is said to occur few days after cisplatin treatment. Death was seen to occur in group B which received only cisplatin drug; two out of the six rats used for the group died after the drug administration showing a severe and terminal side effect of cisplatin when not in combination with its side-effect reducing drug like portulaca oleracea.

CONCLUSION

This research work posits that portulaca oleracea extract or any other phytochemically equivalent drug can be a good combination with cisplatin in chemotherapy to reduce its side-effect.

However, cisplatin side effect has been discovered not to have a definite pattern or association; it can be moderate or severe and can increase or decrease during the course of treatment. Ototoxicity (loss of hearing) has been noted to be a major side effect of cisplatin which among others may or may not be reversed even after cisplatin chemotherapy. Further research should be carried out on the systemic side effects of cisplatin and a wholistic combination therapy possibility for more reduced side effects in cisplatin chemotherapy.

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