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The Renoprotective Effect of Methanolic Extract of Portulaca Oleracea on Cisplatin-Induced Nephrotoxicity in Wistar Rats

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Abstract
This study was carried out to investigate the renoprotective effect of Portulaca oleracea on cisplatin-induced nephrotoxic wistar rats. Twenty four (24) female wistar rats were randomly divided into six (6) groups - Group A were given no treatment and served as the control group; Group B was given only a single dose of cisplatin (2ml/kg) and served as the cisplatin control group. Groups C and D were orally given 400mg/kg and 800mg/kg body weight of methanolic extract of portulaca oleracea (MEPO) respectively 6 hours after cisplatin injection (2ml/kg) . Groups E and F were orally given 400mg/kg and 800mg/kg body weight of MEPO respectively 6hours before cisplatin injection (2ml/kg) for 7 days. The effect of the treatment on relative kidney weight, serum creatinine level, serum uric acid and histoarchitecture of the rat kidney were accessed. Results showed significantly decreased serum creatinine levels ($p<0.05$) in rats treated with 400mg/kg b.wt. and 800mg/kg b.wt. MEPO as compared with the cisplatin control group. Serum uric acid was significantly decreased in groups C, D, E, and F when compared with control A. The relative average weight of the kidney increased significantly in all treated groups except group treated with 800mg/kg b.wt. MEPO 6hours before cisplatin. Kidney histological slides showed both recovery from and prevention of effects of induced toxicity at all treatment doses. Results suggest that *Portulaca oleracea* extract may be used to cure or prevent cisplatin-induced renal toxicity without any adverse effect; hence it can serve as a novel combination agent with cisplatin to limit renal injury.
Keywords: Cisplatin, portulaca oleracea, renoprotection, nephrotoxicity.

INTRODUCTION

Plant-based drugs have been used against various diseases for a long period of time. The primitive man used herbs as therapeutic agents and medicament, which they were able to procure easily[3].
Portulaca oleracea L. belongs to the family of Portulacaceae. It is commonly called Purslane in English language, babbajibji in Hausa language and esan omode or papasan in Yoruba language[5]; however it is called ntioke, ntilimoke, ntiike,or idiridi in Igbo language[21]. It has been reported to be rich in α-linolenic acid and β-carotene and a healthy food for patients with cardiovascular diseases[14]. It is used in the Arabian peninsula as antiseptic, anti-scorbutic, and antispasmodic[7]; In China, it is used as an anti-bacterial and anti-viral agent, and for the treatment of viral hepatitis and in diabetes management[11].
The kidneys are a primary component of the body's defense against toxins and other foreign substances in the environment. Despite the importance of the excretion of metabolic waste products and potential toxins, the threat to life in renal failure typically comes not from the accumulation of metabolic wastes or environmental toxins, but from the loss of the body's ability to balance the daily intake of salts and water by an appropriate rate of excretion[12].
Nephrotoxicity is a poisonous effect of some substances, both toxic chemicals and medication, on the kidneys. Drugs are a common source of acute kidney injury. Compared with 30 years ago, the average patient today is older, has more comorbidity, and is exposed to more diagnostic and therapeutic procedures with the potential to harm kidney function[10]. Drugs shown to cause nephrotoxicity exert their toxic effects by one or more common pathogenic mechanisms. Drug-induced nephrotoxicity tends to be more common among certain patients and in specific clinical situations[13]. The essential values of some plants have long been published; however a number of them remain unexplored yet, therefore there is a necessity to explore their uses and to conduct pharmacognostic and pharmacological studies to ascertain their therapeutic properties[3].

MATERIAL AND METHOD

LOCATION AND DURATION: This study was conducted in the Department of Anatomy, College of Health Sciences, Nnamdi Azikiwe University Nnewi campus and lasted for about three months.
PLANT COLLECTION: fresh aerial parts of Portulaca Oleracea plant was harvested from the surroundings of Nnamdi Azikiwe University Awka and authenticated by Department of Pharmacognosy and Traditional Medicine, College of Pharmacy, Nnamdi Azikiwe University, Agulu Campus with reference number PCG477. The plant was washed, cut into smaller parts (for easy drying), shade-dried for two weeks and finely powdered

with a mechanical grinder yielding 550g of powder.
PREPARATION OF EXTRACT OF PLANT: a methanolic extract of the powdered portulaca oleracea (430g) was prepared using soxhlet apparatus and the extract (1L) concentrated using a rotary evaporator at reduced pressure of 40°C yielding 69.79g dry extract. The extract was made up to solution at varying doses per ml on each day of administration and given according to body weight and groups.
DRUGS/CHEMICAL: Cisplatin drug was obtained from Christ the king pharmarcy, Nnewi and was certified by Faculty of pharmacy, Nnamdi Azikiwe University, Agulu Campus
ETHICAL APPROVAL: Ethical approval was gotten from the ethical committee of the faculty of Basic Medical Sciences College of Health Sciences, Nnamdi Azikiwe University Nnewi Campus for animal based research.
ANIMALS: Twenty four (24) female wistar rats were obtained from the animal house of College of Health Sciences, Nnamdi Azikiwe University, Okofia Nnewi Campus and acclimatized for two (2) weeks (to exclude any intercurrent infection) under standard housing condition (ventilated room with 12/12 hour light/dark cycle at 24 ± 2^0C). The rats were fed ad libitum with water and standard rat chow. The experiment was conducted in accordance with the guiding principles in the use of animals in toxicology, adopted by the society of toxicology in July 1989.
EXPERIMENTAL DESIGN: The rats were randomly divided into six (6) groups (A-F) irrespective of their varying weight. Group A were given no treatment and served as control group for the experiment. Group B was given only a single dose of cisplatin (2ml/kg); it serves as cisplatin control group. Group C was given 400mg/kg of body weight of Methanolic extract of portulaca oleracea (MEPO) 6hours after a single dose cisplatin injection (2ml/kg). Group D was administered 800mg/kg of body weight of MEPO 6 hours after cisplatin injection (2ml/kg). Group E was administered 400mg/kg body weight of MEPO 6 hours before cisplatin injection. Group F was administered 800mg/kg body weight of MEPO 6 hours before cisplatin injection. MEPO extract administration was by oral route via an oral cannula. Cisplatin drug was administered intraperitonially. The daily dose for all administration was given for 7 days. The animals were fasted overnight after the 7^{th} day of drug administration and on the 8^{th} day, 5ml blood sample was collected by cardiac puncture into centrifuge tubes. The serum was separated by allowing blood sample for 15 minutes at 25°C then centrifuged at 4000rpm for 20 minutes, then kept in plastic vials at -20°C until analysis. After blood collection , the animal were euthanized under diethyl ether anaesthesia and their kidney excised, rinsed in cold saline and fixed in 10% formal saline prior to histological processing.
BIOCHEMICAL ASSAY: Serum creatinine levels were measured using the JAFF'S method as modified by Ochei and Kolhatka, 2000[19]. Serum Uric acid of the rats was also observed.
TISSUE PROCESSING: For easy study of sections under microscope the tissues were trimmed down to a size of about 3mm x 3mm thick and fixed in 10% formaldehyde. After fixation, dehydration of the fixed tissues was done in ascending grades of alcohol 50%, 70% and 95% absolute and cleared in xylene. Staining was done with heamatoxylene and eosin and mounted using DPX. After which, the sections were viewed under the light microscope. Photomicrographs of these sections were obtained using the digital photomicroscope .
STATISTICAL ANALYSIS: Data generated were analyzed using the SPSS software (v. 16.0). The results were expressed as mean value ± SD. Differences between mean and the main effects of treatment group were determined by the one way analysis of variance (ANOVA). $P<0.05$ was considered significant.

RESULTS

Table 1.0 relative kidney weight of rats after drug administration (mean±S.D)
Table 1.0 shows the relative kidney weight after drug administration. Group B showed significant decrease in kidney weight when compared with groups A, C, D, and E. ($p<0.05$). Group F rats had a significantly lower kidney weight than group A, C and D ($p<0.05$).

GROUPS	RELATIVE KIDNEY WEIGHT (grams)
CONTROL	0.7950±0.02082
CISPLATIN ONLY	0.6150±0.07141
400MG MEPO AFTER CISPLATIN	0.7900±0.07483
800MG MEPO AFTER CISPLATIN	0.8775±0.06185
400MG MEPO BEFORE CISPLATIN	0.7300±0.07165
800MG MEPO BEFORE CISPLATIN	0.6475±0.04272

Table 2.0 kidney analyte concentrations of treated groups (mean±S.D)

GROUPS	SERUM CREATININE (mg/dl)	SERUM URIC ACID (mg/dl)
CONTROL	0.2067±0.00577	2.4700±0.01732
CISPLATIN ONLY	0.6100±0.00000	2.0300±0.01732
400MG MEPO AFTER CISPLATIN	0.3850±0.03536	1.7000±0.01732
800MG MEPO AFTER CISPLATIN	0.2400±0.00000	1.9633±0.02517
400MG MEPO BEFORE CISPLATIN	0.2867±0.00577	1.8600±0.01732
800MG MEPO BEFORE CISPLATIN	0.0667±0.01155	2.1033±0.36116

Table 2.0 shows the serum creatinine and uric acid concentrations of various groups. Group B showed a significantly higher serum creatinine when compared to all the other groups ($p<0.05$). The control group showed a significantly lower than all treated groups except group F which was significantly lower than all the groups including the control group ($p<0.05$). the serum uric acid showed a significant decrease in group B when compared to group A. group C, D, E, and F showed a significant decrease in serum uric acid when compared with group A while group C showed a significant decrease with group B.

Table 3.0 histological features observed in the kidney tissues from the various groups

GROUPS	TUBULAR NECROSIS AND DISRUPTION	PELVIS NECROSIS AND HAEMORRHAGE	INFLAMMATORY CELLULAR INFILTRATION	HYPOPERFUSED GLOMERULI
CONTROL	-	-	-	-
CISPLATIN ONLY	+++	+++	++	-
400MG MEPO AFTER CISPLATIN	+	-	+	-
800MG MEPO AFTER CISPLATIN	+	-	-	-
400MG MEPO BEFORE CISPLATIN	+	-	-	-
800MG MEPO BEFORE CISPLATIN	-	-	-	-

KEY

-	Absence
+	Mild
++	Moderate
+++	Severe

TABLE 3.0 shows the grading of the observed histological features of the kidney tissues of various groups. Group A showed normal kidney histological architecture. Group B showed severe pelvis and tubular necrosis with pelvis and tubular disruptions and haemmorhage. It further showed a moderate inflammatory cellular infiltration. Group C showed a mild tubular necrosis with pelvis inflammation; however this group showed no pelvis necrosis, haemmorhage or hypoperfused glomeruli. Groups D and E showed a normal kidney histological architecture except with a mid tubular necrosis and disruption while group F showed a complete normal tissue histological architecture without any tissue pathology.

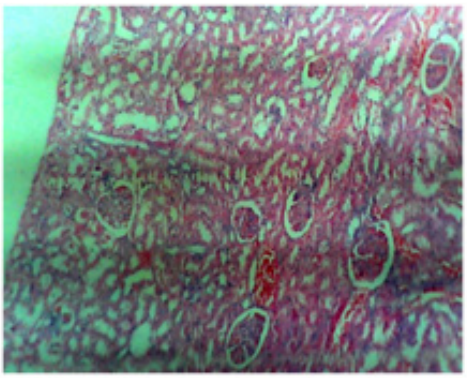

PLATE I - GROUP A (CONTROL): The kidney tissue showing a normal cortex (H&E method – X400)The capillaries forming the glomerulus, urinary space and trhe capsule can be clearly seen. The proximal convoluted tubules (dark- stained) and the distal convoluted tubule (light stained) can also be seen to be normal.

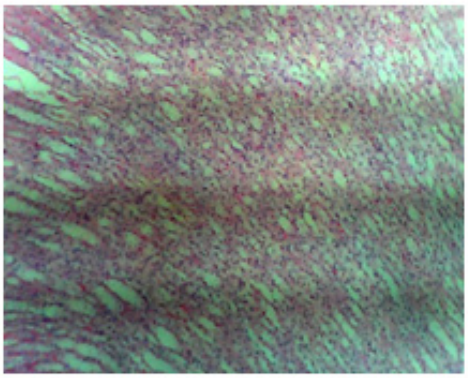

PLATE II – GROUP A (CONTROL): kidney showing normal renal pelvis (H & E method – X100)

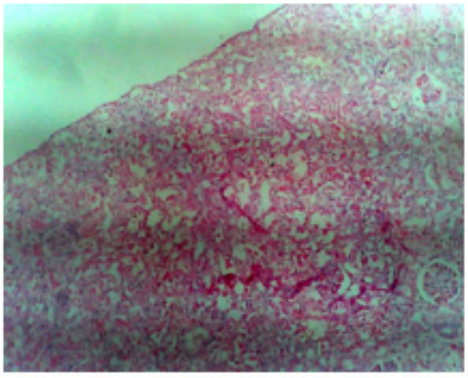

PLATE III- GROUP B (CISPLATIN ONLY): kidney showing extensive pelvis haemmorhage and necrosis (H & E method – X100). Sites of extensive haemmorhage (dark red-stained) are seen within the pelvis along with cells showing necrosis. Ruptured arteries can also be seen. The tissue is under the effect of cisplatin drug administered.

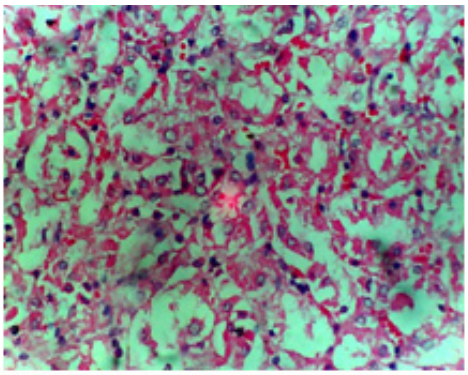

PLATE IV - GROUP B (CISPLATIN ONLY): kidney showing tubular necrosis and mild disruption (H & E method – X400). Areas of mild disruption are seen in the renal tubules with necrosis of the cells. The tubules also show the dark stained macula densa scattered within it. The Tissue is under the effect of cisplatin drug administered.

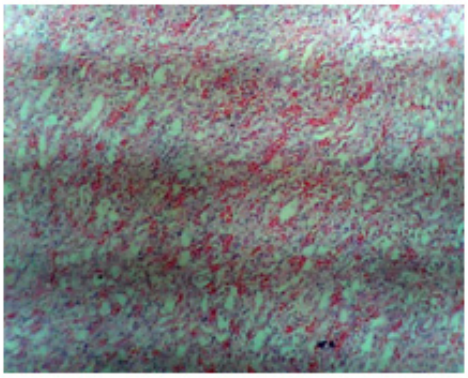

PLATE V - GROUP B (CISPLATIN ONLY): kidney showing severe haemmorhage (H & E method – X100). Sites of extensive haemmorhage (dark red-stained) are seen within the pelvis along with cells showing necrosis. Ruptured arteries can also be seen. The tissue is under the effect of cisplatin drug administered.

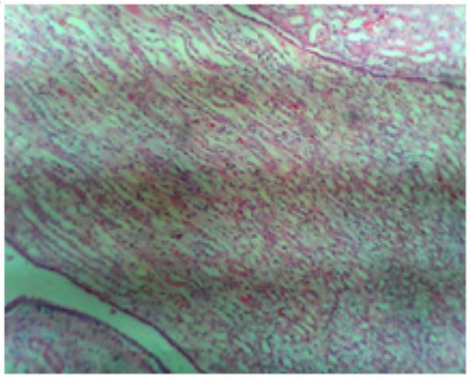

PLATE VI - GROUP C (400MG MEPO AFTER CISPLATIN): kidney showing essentially normal pelvis (H & E method – X100). The plate shows an essentially normal pelvis with sites of division into calyx of the sinus also seen. No pathology can be detected in the tissue.

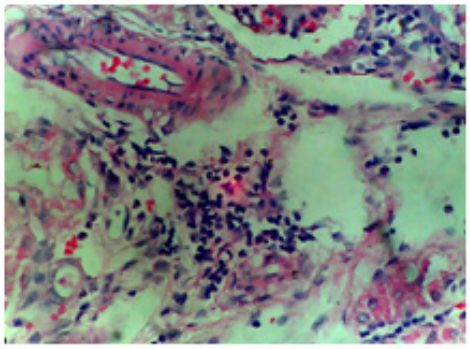

PLATE VII - GROUP C (400MG MEPO AFTER CISPLATIN) : kidney showing focal area of mild pelvis inflammation (H & E method – X1000). A higher magnification showed a normal kidney histological architecture with focal ares of mild inflammation in the pelvis. The aggregates of the dark-stained macula densa can also be seen within the pelvis. The tissue is showing characteristics of tissue undergoing healing process.

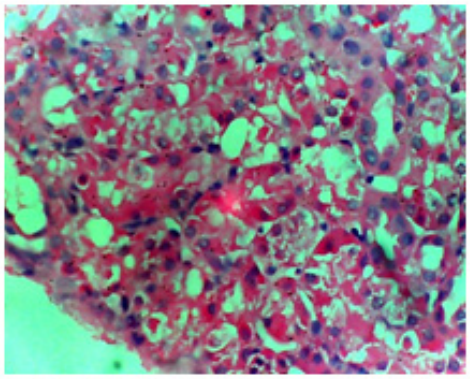

PLATE VIII - GROUP C (400MG MEPO AFTER CISPLATIN): kidney showing focal area of mild tubular necrosis (H & E method – X1000). A higher magnification showed normal kidney histology but with few focal areas of mild tubular necrosis seen in the renal cortex of the tissues accessed in group C. the dark- stained macula densa can be seen surrounding the pelvis.

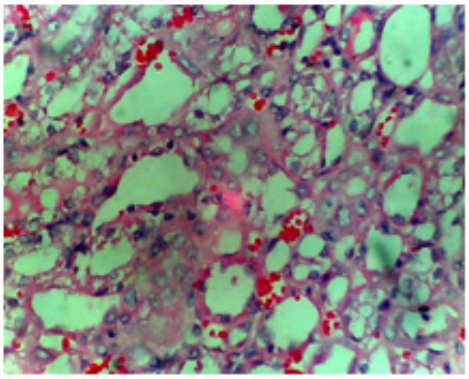

PLATE IX - GROUP D (800MG MEPO AFTER CISPLATIN): kidney showing area of normal convoluted tubule (H & E method – X1000). A higher magnification of the renal cortex shows area of normal convoluted tubule. The dark-stained proximal convoluted tubule and light-stained distal convoluted tubule can be seen. The interlobar arteries can be seen undistorted in their positions.

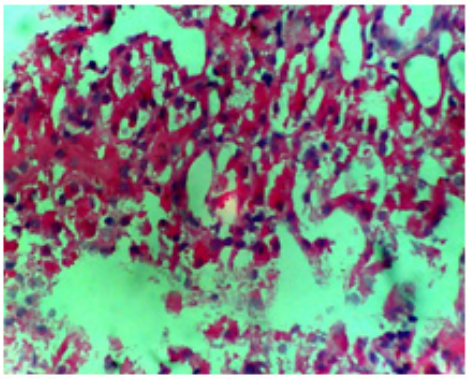

PLATE X - GROUP D (800MG MEPO AFTER CISPLATIN): kidney showing mild tubular disruption& necrosis (H & E method – X1000). A higher magnification of group D tissues shows the area of normal kidney histology with areas of mild tubular disruption and necrosis. Plate X shows a recovery characteristic features.

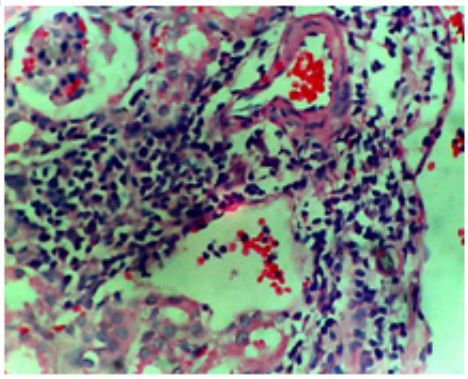

PLATE XI - GROUP E (400MG MEPO BEFORE CISPLATIN): kidney showing focal area of mild cortical inflammation withmild hypoperfused glomeruli (H & E method – X1000). A higher magnification of the glomeruli showed focal area of mild cortical inflammation and hypoperfusion. The arteries are also arranged within the glomerulus with aggregates of darkly stained macula densa surrounding the glomerulus.

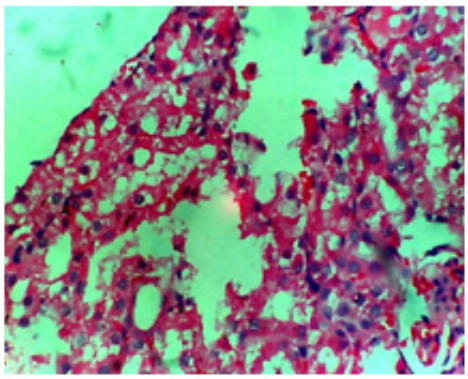

PLATE XII - GROUP E (400MG MEPO BEFORE CISPLATIN): kidney showing mild tubular disruption & necrosis (H & E method – X400). A higher magnification shows area of normal kidney architecture with areas of mild tubular disruption and necrosis. The tissue shown is suspected to be under healing process.

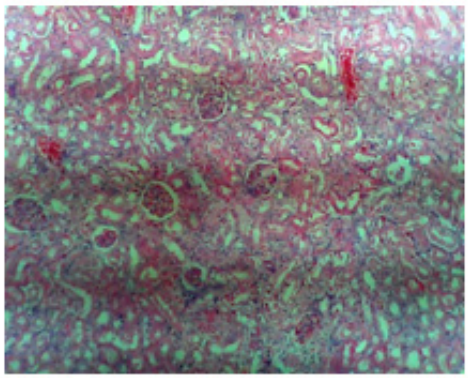

PLATE XIII - GROUP F (800MG MEPO BEFORE CISPLATIN): kidney showing essentially normal cortex (H & E method – X400). The kidney tissue shows a normal kidney histological architecture with no tissue pathology. The glomerulus , capsule and urinary space can be seen. The proximal and distal convoluted tubules can also be well seen. The interlobar arteries of the kidney are seen investing into the glomerulus. The tissue shows complete recovery from effect of cisplatin drug..

DISCUSSION

This study investigated the possible renoprotective effect of orally administered methanolic extract of portulaca oleracea on cisplatin induced nephrotoxicity in wistar rats. The study was designed to ascertain the nature of this effect, whether curative or preventive, the dose-relationship effect and the effect of portulaca oleracea on relative kidney weight.

Cisplatin is the most common antitumor drug in clinic hence its use in this research work with nephrotoxicity as its most common side effect. Nephrotoxicity of the drug is usually associated with their accumulation in renal cortex, dependent upon their affinity to kidney and on kinetics of drug trapping process[2]. it is a platinum compound which is accompanied by decrease in glomerular filteration rate, increase in blood urea nitrogen, serum levels of creatinine and tubular injury. Cisplatin is known to accumulate in mitochondria of renal epithelial cells and induces ROS primarily by decreasing the activity of antioxidant enzymes and by depleting intracellular concentrations of GSH and also causes peroxidation of membrane lipids[18,24,26]. The cytotoxic action of the drug is often thought to be associated with its ability to bind DNA adducts[32]. Cisplatin induced oxidative stress can activate some protein kinases which sensitize the injured cell to apoptosis[9]. At the dose of 2ml/kg, cisplatin was able to cause a significant nephrotoxicity as evidenced by the increased serum creatinine ($p<0.05$) in the cisplatin control group (table 2.0). This group showed a mean value of 0.6100mg/dl of creatinine as compared with 0.2067mg/dl of the normal control group. There was also a significant decrease of serum uric acid in cisplatin group as compared with the control. The decrease in uric acid as a result of cisplatin administration contradicts the finding of Paoulomi et al, 2012[25] that saw no significant effect of cisplatin administration with uric acid.

The daily intraperitonial administration of 2ml/kg cisplatin showed significant decrease ($p>0.05$) in kidney weight as compared with the control group (tables 1.0)

The histological features of the kidney tissues of rats treated with cisplatin showed extensive pelvis haemmorhage, tubular necrosis and mild disruption when compared with the control group (table 3.0; plates I-V).

Portulaca oleracea (PO) ranked as the eight most common plant in the world[20], and described as a 'power food' of the future[29] has a long history of use as human and animal food and in medicine hence its use in this research. Preliminary quantitative and qualitative phytochemical analysis carried out on portulaca oleracea extract showed its high content of saponin (32%) followed by alkaloid (26%) amongst other bioactive compounds observed[22]

Methanolic extract of portulaca oleracea (MEPO) was administered in varying doses 6hours before or after 2ml/kg cisplatin injection with regards to its lethal dose (LD_{50})[17]. This is to observe its attenuating effect on the damages caused by cisplatin drug. From this study, all doses of PO administered showed that there was significant decrease in serum creatinine level ($p<0.05$) in treated groups as compared to the cisplatin group. there was a significant decrease in serum uric acid in animals given cisplatin only (group B) when compared with the control group ($p<0.05$) but groups C, D, E, and F showed a significant decrease when compared with group A. only group C was able to show a significant decrease in serum uric acid when compared with group B ($p<0.05$). this suggests the role of MEPO in reducing serum uric acid level which was seen to be more in group C ($p<0.05$) (table 2.0). Except in groups treated with 800mg/kg of MEPO 6hours before injection, all other treated groups showed significant increase in the average kidney weight ($p<0.05$) when compared with the group given cisplatin alone. The rapid cell growth ability of PO supports the presence of protein and starch already observed in its phytochemical analysis[22].

The histological findings from the rat kidney specimen showed that at all the treated groups, there was an effective renoprotection by different doses of MEPO when compared with the control and cisplatin group. However, there were mild necrosis, inflammation and disruptions noticed in tubules and pelvis of all the treated groups except in rats given 800mg/kg MEPO before cisplatin which showed essentially normal kidney histology when compared with the control and cisplatin group (plates I-XIII). PO showed a cell regenerative and an anti-haemmorhoidal ability in the treated groups when compared with the kidney of rats given cisplatin injection only although this healing effect was seen to be dose related and time related as the group that took 800mg/kg MEPO before cisplatin injection showed complete recovery.

The antioxidant property of PO has been proved[29] and its renoprotective ability could be due to this property. Flavonoid present in PO has been validated as antioxidant acting by free scavenging activity. Also present in PO in high quantity is saponin and alkaloid proved to be a surfactant and an analgesic respectively[27]. This suggests that the renoprotective effect of the MEPO may be due to single or combined effect of its bioactive substances. Other constituents of PO like omega-3, ascorbic acid, β-carotene and glutathione have antioxidant activity[6,23,28]. So this plant may inhibit lipid peroxidation by scavenging free radicals and increasing intracellular concentration of glutathione. The protection of the kidney tissues offered by PO against renal damage supports its already published anti-inflammatory effect[7,8,30].

Some antioxidant agents that have been used to ameloriate cisplatin-induced nephrotoxicity in rats include deferoxamine, methimazole,Vit. C, Vit. E, diethyl diothiocarbamate, L-histidinol, thyroquinone[1,4,15,16], but none of these have been proved to be clinically efficient to provide complete protection in patients without side effects. These natural antioxidants may offer comparatively safer alternatives to synthetic antioxidants which may cause serious or unacceptable adverse side effects[15].

The result of this research reveals that the MEPO had significant protective effect against cisplatin-induced nephrotoxicity and the effect was found to be in dose dependent manner. The animals treated with higher dose of MEPO (800mg/kg) both 6 hours before and after cisplatin showed higher protection with a higher significant decrease in serum creatinine level ($p<0.05$) and higher curative and preventive effect in the kidney tissues (plates I-XIII). These findings agreed with Waala et al 2011[31] and Gholamreza et al 2010[9].

CONCLUSION

Portulaca oleracea protects against acute cisplatin nephrotoxicity and may be considered as a potentially useful medicine for combination therapy with cisplatin. MEPO showed both curative and preventive potentials for nephrotoxicity in a dose dependent manner as 800mg/kg MEPO given both before and after showed better protection than the lower dose (400mg/kg). However, a total histological protection of the kidney tissue was seen to be in the group treated with 800mg/kg 6 hours before cisplatin injection suggesting that portulaca oleracea has a higher preventive effect than a curative one. Based on this study and other related researches, there is necessity for further research on the mechanism of action of PO as a renoprotective agent and to validate the claims of this study for a possible MEPO supplementation in cisplatin chemotherapy.

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