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Chapter 3

Biological investigation

Cytotoxicity test

3 Cytotoxicity test of 5 compounds[140-143]

3.1 Brine shrimp lethality bioassay

Brine shrimp lethality bioassay is rapid general bioassay for the bioactive compounds of the natural and synthetic origin. Bioactive compounds are almost always toxic at high dose. Pharmacology is simply toxicology at a lower dose or toxicology is simply the pharmacology at a higher dose.

Brine shrimp lethality bioassay is a bench top bioassay method for evaluating anticancer, antimicrobial, and pharmacological activities of natural products and it is a recent development in the bioassay for the bioactive compounds. By this method, natural products extracts, fractions as well as pure compounds can be tested for their biosactivity. Here, in vivo lethality in a simple zoologic organism (Brine shrimp nauplii) is used as a convenient monitor for screening and in the discovery of new bioactive natural products. This bioassay is indicative cytotoxic and a wide range of pharmacological activity of the compounds.

Brine shrimp lethality bioassay stands superior to other cytotoxic testing procedures because it iis a rapid method utilizing only 24 hours, inexpensive and requires no special equipment. I utilizes a large a large number of organisms for statistical validation and a relatively a small amount of sample. Furthermore, unlike other methods, it does not require animal serum.

Brine shrimp eggs are hatched simulated sea water to get nauplii. Sample solution are prepared by dissolving the test materials in precalculated amount of DMSO. Ten nauplii are taken in a test tubes containing 5 ml of simulated sea water. The samples of different concentrations are added to the pre-marked test tubes with a micropipette. The assay is performed in three replicates. Survivors are counted after 24 hours and 48 hours. These data are processed in a simple program for probit analysis to estimate LC50 values.

3.2.4 Preparation of the control group

Cisplatin served as the positive control. 1 mg of cisplatin was dissolved in DMSO to get an initial concentration of 1 mg/ ml from which serial dilutions were made using DMSO to get 6.25 µg/ml, µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml concentrations and added to the premarked test tubes containing 5 ml of sea water and 10 shrimp nauplii. So, the final concentration of samples in the test tubes were 1.25 µg/ml, 2.5 µg/ml, 5 µg/ml, 10 µg/ml, 20 µg/ml, 40 µg/ml.

The control group containing 10 living brine shrimp nauplii in 5 ml simulated sea water received the positive control solutions.

As for negative control, 1 ml of DMSO was added to each of three marked glass vials containing 4 ml of simulated sea water and 10 shrimp nauplii to use for negative control. If the brine shrimps in these vials show a rapid mortality rate, then the test is considered as the nauplii died due to some reason other then the cytotoxic of the compounds.

3.2.5 Counting of nauplii

After 24 and 48 hours, the vials were observed using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of the lethality of the brine shrimp nauplii was calculated for each concentration

4.1 Cisplatin & its metabolites induced Histo-Pathological changes in liver and kidney of mice

4.1. Introducton

The most common procedure used in histologic research is the preparation of tissue sections or slices that can be studied with the light microscope. Under the light microscope, tissues are examined visually in a beam of transmitted light. Because most tissues and organs are too thick to pass through them, they must be sliced to obtained thin, translucent sections that are attached to glass slides for microscopic examination[144].

4.1.2 Materials & methods

1. Specimens are Cisplatin & its metabolites induced liver and kidney of mice.
2. Biopsy material: Small pieces of tissues are cut from large specimens. The material is processed and stained for microscopic examination.

4.1.3 Collection of mice

Animal House, ICDDR'B, Mohakhhali, Dhaka. (vide memo no. 0461)

Total number of test animal (Mouse: *Mus musculus*)[145]: 50

Average wt of mouse: 40 g

Total number of groups: 3

Group A: Cisplatin

Group B: Cisplatin + D. alata[146, 147]

Group C: Cisplatin + Moringa oleifera[148, 149]

Group D- Metabolite 1

Group E- Metabolite 1+D. alata

Group F- Metabolite 1 +Moringa oleifera

Group G- Metabolite 2

Group H- Metabolite 2+D. alata

Group I- Metabolite 2 +Moringa oleifera

Group J- Metabolite 3

Group K- Metabolite 3+D. alata

Group L- Metabolite 3+M. olifera

Group M- Control.

4.1.4 Animal handling/Feeding[150] - Properly maintained.

4.1.5 Animal Dose & Duration[151]

A. Injectable:

(a) Cisplatin : 16 mg / kg body wt, given every weeks for 2 weeks

(b) Metabolite2 : 16 mg / kg body wt, given every weeks for 2 weeks

(c) Metabolite 4 : 16 mg / kg body wt, given every weeks for 2 weeks

B. Powdered extract250mg/kg body wt

4.1.6 Drug dissolving solvent

a) Dimethyl sulfoxide (DMSO)

b) Propylene glycol (Merck Specialities Private Limited, Mumbai, India)

5 Biochemical investigations in Mice

5.1 Introduction

Several lines of evidence indicate that free radicals are involved in the nephrotoxicity caused by cisplatin, and the damage is suggested to be the consequence of decreased renal antioxidant enzyme activity with enhanced lipid peroxidation. However, administration of antioxidants has been shown to ameliorate cisplatin-induced nephrotoxicity in animals[163].

Aqueous methanolic (50% MeOH) extracts of the tubers (peel and flesh) of nine cultivars of greater yam (Dioscorea alata) were determined to have relatively high antioxidant activities among which two cultivars (Ubing upo, purple, and Ube A 096, white) had activities as K L J K D V W R F R S K H Q R O V H W R F R S K H U R O and butylhydroxyanisole (BHA). The aqueous methanol extract of the purple variety was fractionated on XAD-2 column chromatography serially with water, 25% MeOH, 50% MeOH, 75% MeOH, 100% MeOH and acetone. High activities were found in the 50% and 75% MeOH fractions; these were dried[165].

Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to human against infections and degenerative diseases. Current research is now directed towards natural antioxidants originated from plants due to their therapeutics. Moringa oleifera is used in Indian traditional medicine for a wide range of various ailments. To understand the mechanism of pharmacological actions, antioxidant properties of the Moringa oleifera leaf extracts were tested in two stages of maturity using standard in vitro models.

The successive aqueous extract of Moringa oleifera exhibited strong scavenging effect on 2, 2-diphenyl-2-picryl hydrazyl (DPPH) free radical, superoxide, nitric oxide radical and inhibition of lipid per oxidation. The free radical scavenging effect of Moringa oleifera leaf extract was comparable with that of the reference antioxidants. The data obtained in the present study suggests that the extracts of Moringa oleifera both mature and tender leaves have potent antioxidant activity against free radicals, prevent oxidative damage to major biomolecules and afford significant protection against oxidative damage[166].

5.2 Steps

Reduced glutathione (GSH), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and diacetylmonoxime (DAM) were purchased from Bangladesh Medical Equipment, Hatkhola, Dhaka, Bangladesh. Cisplatin was purchased from Bangladesh Medical Equipment, Hatkhola, Dhaka, Bangladesh.. Other chemicals and reagents used were of analytical grade. Tuber part of D. alata and seed pods of Moringa oleifera were collected from the local vegetable market of Sirajgonj district, Bangladesh.

The type specimen was deposited in the herbarium of Department of Botany, University of Dhaka, Dhaka, Bangladesh. The tuber parts and seed pods of D. alata and Seed pods of Moringa oleifera were cut into small pieces, dried at 45-50°C for 48 h, and powdered. Two hundred grams of the powdered material was extracted with petroleum ether using a Soxhlet apparatus. The defatted material was air-dried, then suspended in 70% methanol and boiled for 8 h.

Serum creatinine and urea were estimated by the method of Brod and Sirota[168] and Marshell et al.[169], respectively. The activity of glutathione peroxidase (GPX) by the method of Hafeman et al.[170], levels of reduced glutathione (GSH) by the method of Moron et al.[171] and malondialdehyde (MDA) by the method of Ohkawa et al.[172] using 1,1,3,3 tetramethoxypropane as standard. The protein content was estimated by the method of Lowry et al.[173] using bovine serum albumin as standard.

Experimental data were expressed as mean ± SD. Student's 't' test was applied for expressing the significance, and P values less than 0.05 were considered as significant. Serum creatinine and urea levels were significantly elevated ($P < 0.001$) in the cisplatin-treated animals compared to the normal group. The increase of serum creatinine and urea levels was 7 fold, 5.7-fold respectively. Treatment of animals with methanolic extract of D. alata and M. olifera significantly reduced the elevated levels of serum creatinine and urea. The extract treatment was able to lower the serum creatinine and urea to almost normal level (Table 1).

The renal GSH concentration decreased by over 40% ($P < 0.001$) in cisplatin-treated animals compared to the normal group. However, the administration of methanolic extract of D. alata and M. olifera at a concentration of 250 mg/kg body wt prior to cisplatin treatment increased the renal GSH concentration to the normal level (Table 2).

The present study was carried out using higher doses of cisplatin (16 mg/kg body wt, i.p). This corresponds to the dose of cisplatin normally used in clinical practice[167]. This dose is also found to produce nephrotoxicity and ototoxicity.

Several protective agents have been evaluated against cisplatin-induced nephrotoxicity in experimental and clinical studies. They include diethyldithiocarbamates[174], glutathione[3], glycine[175], methionine[176], procaine and procainamide[177]. However, none of these compounds has proved to be clinically efficacious as complete protection in patients.

The results of the present study show that reduced GSH level significantly decreased in the cisplatin-treated animals compared to the normal group. These observations support the hypothesis that the mechanism of nephrotoxicity in cisplatin-treated animals is related to depletion of antioxidant defence system. Treatment with *D. alata* and *M. olifera* extract (250 mg/kg body wt, p.o) prior to cisplatin administration prevents the depletion of antioxidants.

The tested doses of methanolic extract of *D. alata* and *M. olifera* (250 mg/kg body wt, p.o) show no signs of toxicity in mice. Survival rate of animals treated with cisplatin and with *D. alata* and *M. olifera* extract (250 mg/kg body wt, p.o) supports the nephroprotective effect of this mushroom.

Free radicals are known to play an important role in cisplatin-induced nephrotoxicity. The free radicals and reactive oxygen species induce oxidative stress in kidneys[180,181]. Due to cisplatin administration, platinumsulphydryl group complexes formed are taken up by renal cells and stabilized by intracellular GSH for several hours. In case of intracellular GSH depletion, the complexes undergo rapid transformation to reactive metabolites[182]. Thus GSH depletion results in increased toxicity of cisplatin. GSH depletion also results in lipid peroxidation and this seems to be the prime factor that permits lipid peroxidation and impaired antioxidant enzyme activities.

These observations support the conclusion that the mechanism of nephrotoxicity in cisplatin-treated rats is related to depletion of antioxidant systems. Thus, nephroprotection by the mushroom extract might be directly related to its antioxidant activity.

Preliminary phytochemical analysis indicates that the major constituent of the extract is a polysaccharide. This conclusion is based on the positive reaction of the extract to anthrone test[183] and also to phenol-sulphuric acid reagent[184].

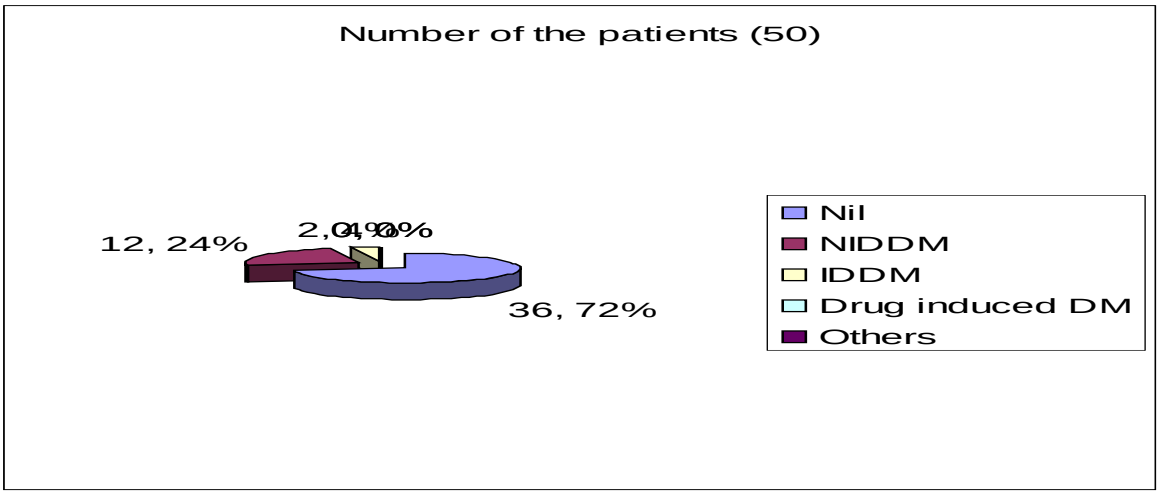
Because it has become naturalized throughout tropical South America, Africa, Australia, the US southeast, *D. alata* has many different common names from these regions. In English alone, aside from purple yam, other common names include greater yam, Guyana arrowroot, ten-months yam, water yam, white yam, winged yam, or simply yam. In other cultures and languages it is known variously as *satalu* or violet yam in India and one study revealed that *M. fera* seed powder and tuber part of *D. alata* at the dose rate of 600 mg/kg is as efficacious as silymarin in exerting nephroprotective and antioxidant effects[154].

The specimen was identified with the help of the available literature and the identification was confirmed by the Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh (vide memo no. 0354). The experimental results reveal that the methanolic extract of *M. fera* seed powder and tuber part of *D. alata* could help prevent nephrotoxicity manifested consequent to cisplatin chemotherapy. The effect is mainly due to the capacity of the extract to restore renal antioxidant defence system.

Chapter 5

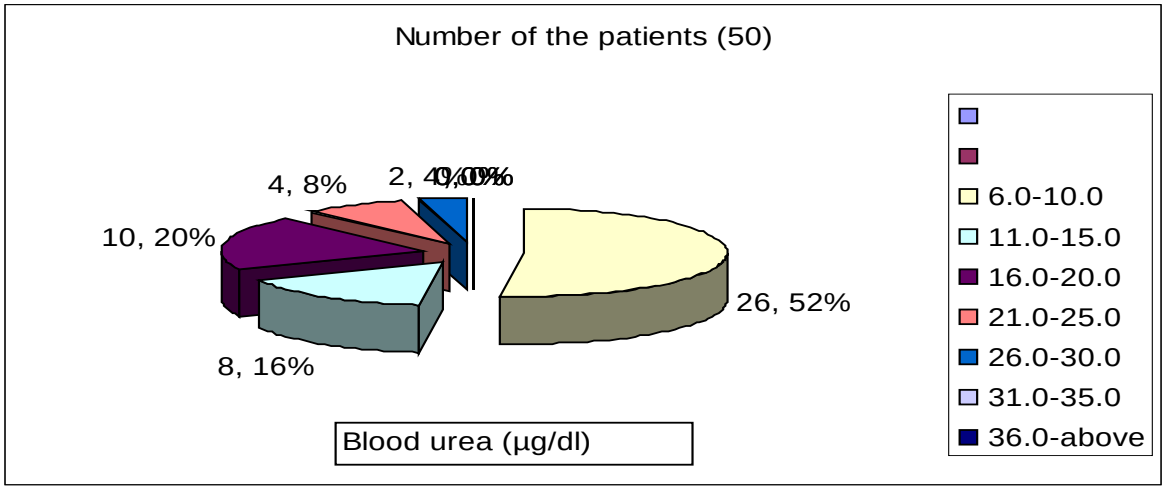
Results

Figure 14 : Distribution of cancer patient according to the state of glyceamic condition.



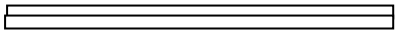
In figure 10 a retrospective study of 50 admitted cancer patients in KYAMC Cancer Center, Sirajgonj demonstrated that 4% (n=2) of the were suffered from IDDM, 24% (n=12) were NIDDM and 72% were free of DM.

Figure 15 : Distribution of cancer patient according to the state of uremia.



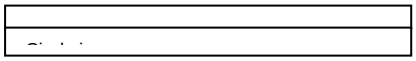
4% (n=2), 8% (n=4) and 20% (n=10) of total cancer patients were suffered from severe to moderate to mild hyperureamia respectively indicating strong involvement of renal system of cisplatin toxicity (figure 12)

Figure 19 : RT and are of cisplain and its metabolites in blood after 3 hour.



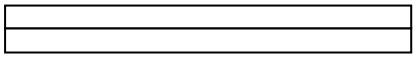
In figure 16, graphic presentation showed that the detection of cisplatin and its suspected metabolites by measuring the peak time and area by using HPLC after 3 hours of cisplatin administration in a cancer patient. Here peak time, area and concentration of cisplatin (C), metabolite 1 (CM2) and metabolite 2 (CM3) were 2.792 min., 48.7, 440.6 µg/ml, 3.25 min., 432.6, 135.46 µg/ml, 4.17 min., 203.5, 83.08 µg/ml respectively. Flow rate= 0.5 ml/min., Temp. = 40° C, solvent system 20% acetonitril: 80%MeOH, injected amount was 10 µl.

Figure 21 : RT and area of cisplain and its metabolites in blood after 12 hour.



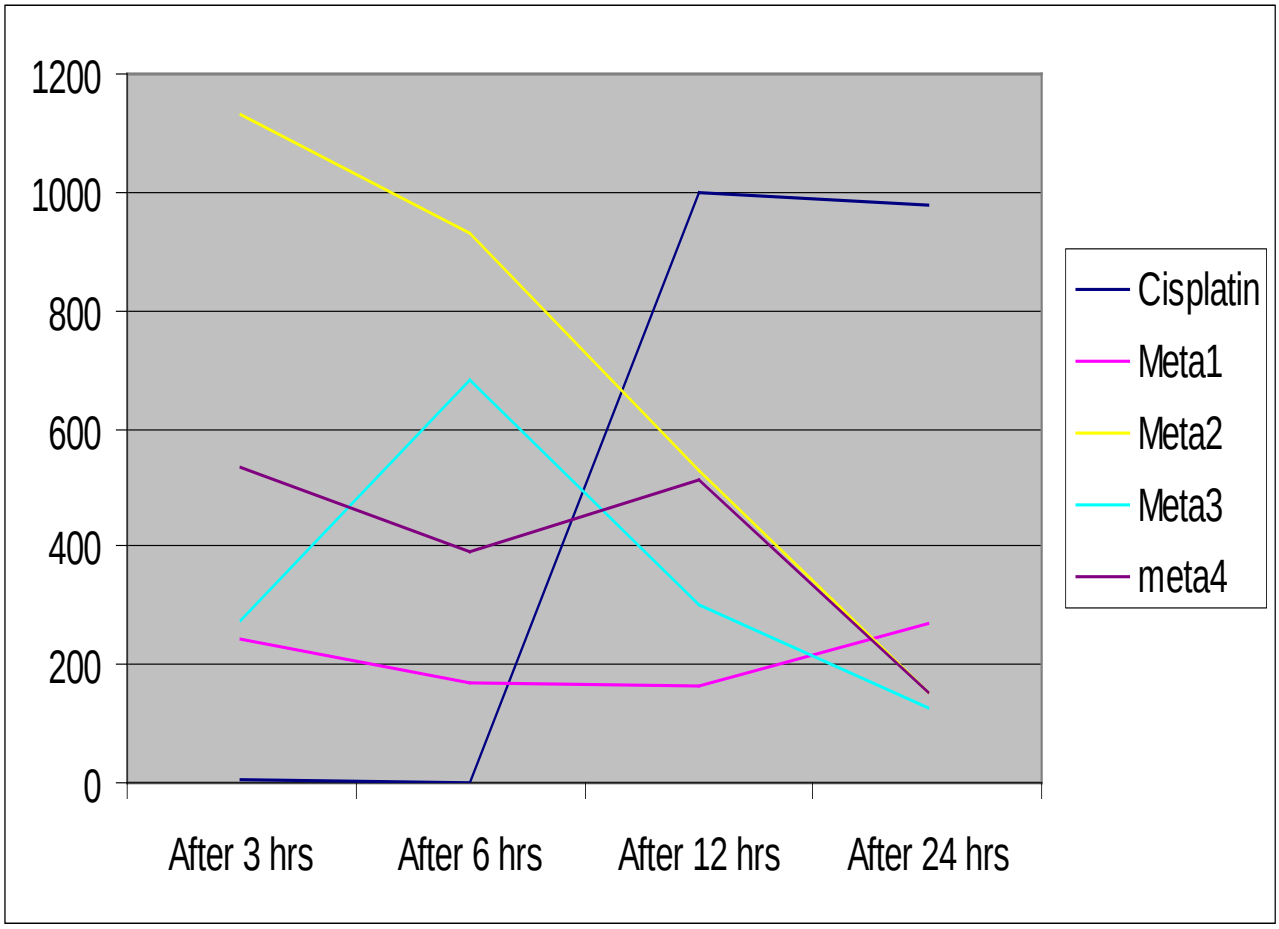
In figure 18, graphic presentation showed that the detection of cisplatin and its suspected metabolites in plasma by measuring the peak time and area by using HPLC after 3 hours of cisplatin administration in a cancer patient. Here peak time, area and concentration of metabolite 1 (CM2), metabolite 2 (CM3) and metabolite 3 (CM4) were 3.16 min., 46484.3,723.40µg/ml, 3.788 min., 1179.6,165.19µg/ml, 4.514 min., 591.2, 83.08µg/ml respectively. Flow rate= 0.5 ml/min., Temp. = 40° C, solvent system=20% acetonitril: 80%MeOH, injected amount was 10 µl.

Figure 23 : RT and area of cisplain and its metabolites in urine after 3 hours.



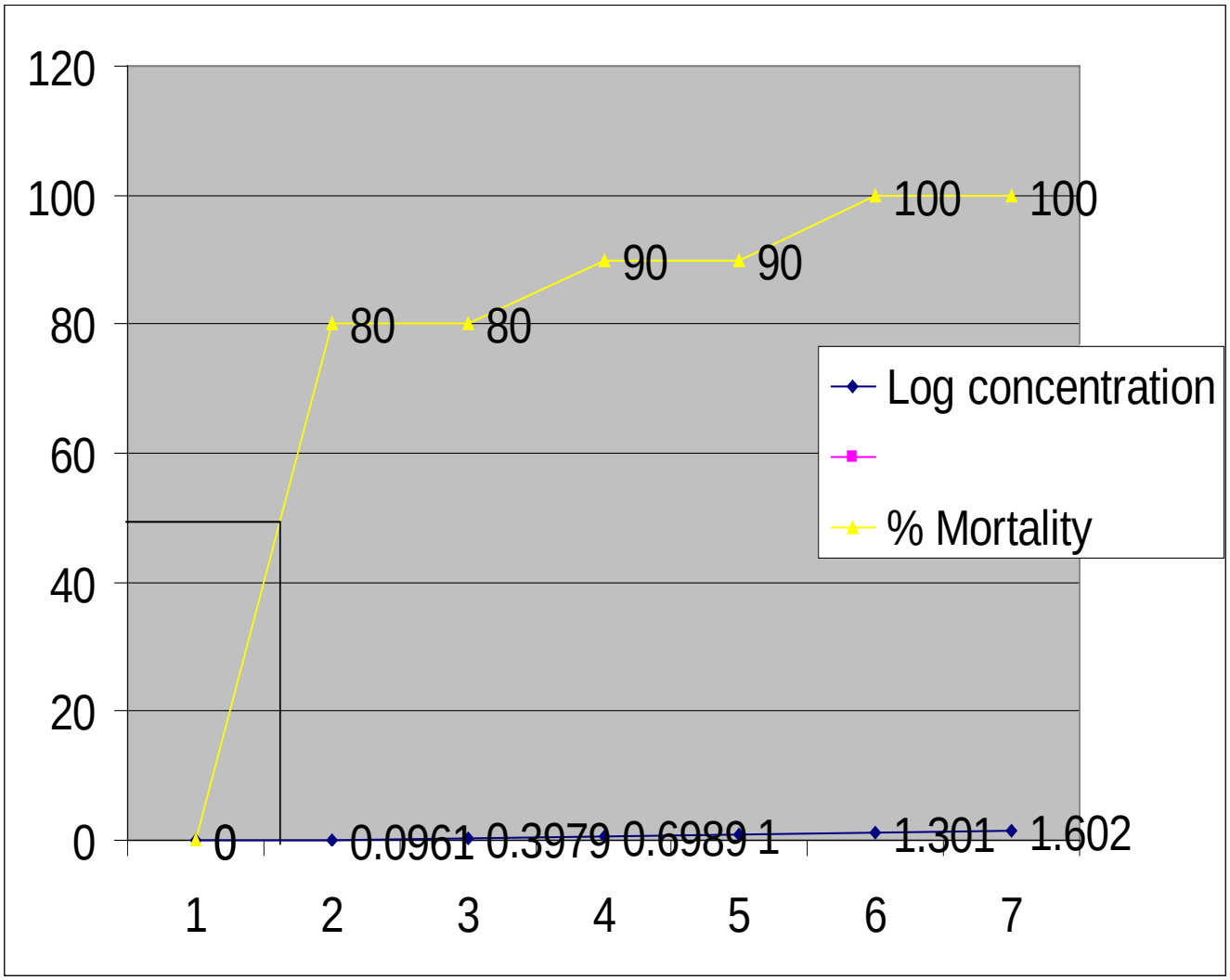
In figure 20, graphic presentation showed that the detection of cisplatin and its suspected metabolites by measuring the peak time and area by using HPLC after 3 hours of cisplatin administration in a cancer patient. Here peak time, area and concentration of cisplatin ©, metabolite 2 (CM3) and metabolite 3 (CM4) were 3.16 min., 46684.3, 723.4 µg/ml, 3.788 min., 1179.6, 167.34 µg/ml, 4.514 min., 591.2, 73.89 µg/ml respectively. Flow rate= 0.5 ml/min., Temp. = 40° C, solvent system=20% acetonitril: 80%MeOH, injected amount was 10 µl.

Graph 2 : Mean urinay concentration of cisplatin& its metabolites in urine sample of 50 patients.



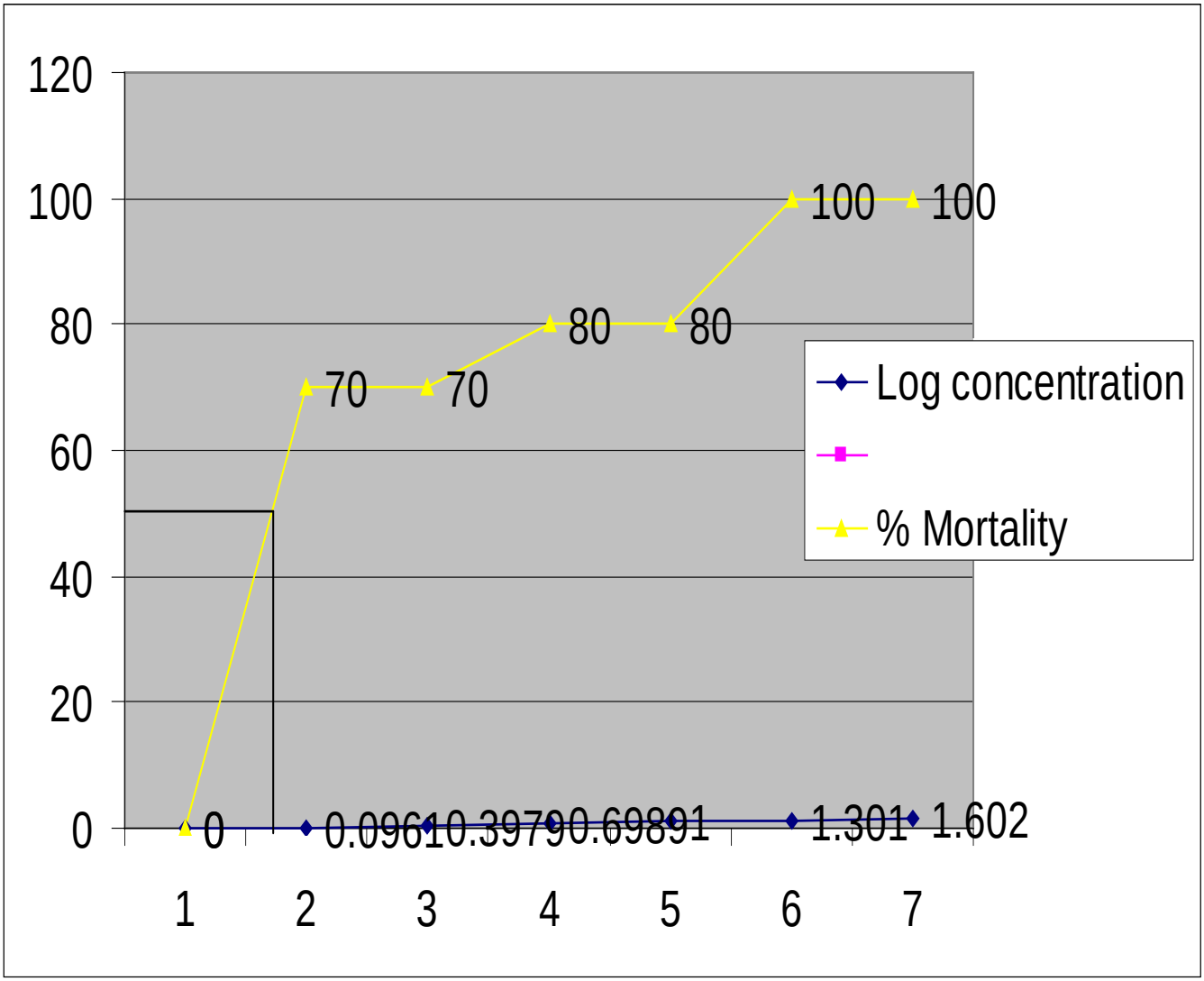
In graph 2, graph showed that the mean±SD urine concentration of cisplatin and its suspected metabolites M1 to M4 named CM2, CM3, CM4 and CM5 at different time intervals (at 3,6,12 and 24 hour) of 50 cancer patients.

Graph 5 : Cytotoxicity test of CM3



Cytotoxicity test of Metabolite 2 (CM3) was done by using brine shrimp lethality bioassay by plotting the different log concentration of cisplatin (0.0961, 0.3979, 0.6989, 1, 1.3010) against % mortality (0 to 100%) and LC_{50} of cisplatin was calculated 1.108 (table 5)

Graph 7: Cytotoxicity test of CM5



Cytotoxicity test of Metabolite 4 (CM5) was done by using brine shrimp lethality bioassay by plotting the different log concentration of cisplatin (0.0961, 0.3979, 0.6989, 1, 1.3010, 1.6020) against % mortality (0 to 100%) and LC_{50} of cisplatin was calculated 11.25 µg/ml(table7)

Table 3: Results of brine shrimp lethality bioassay

Concentration© (µg/ml)	Log C	% Mortality				LC50 (µg/ml)
		Compound 1*(C)				
		Group A	Group B	Group C	Average	
1.25	0.0969	30	50	40	40	1.8
2.5	0.3979	50	70	60	60	
5	0.6989	60	80	70	70	
10	1	70	80	60	70	
20	1.3010	80	100	90	90	
40	1.6020	100	100	100	100	

LC_{50} of administered cisplatin was 1.8 µg/ml

*C=Cisplatin

Table 4: Results of brine shrimp lethality bioassay

Concentration© (µg/ml)	Log C	% Mortality				LC50 (µg/ml)
		Compound 2 (*CM2)				
		Group A	Group B	Group C	Average	
1.25	0.0969	70	60	50	60	1.2
2.5	0.3979	70	60	50	60	
5	0.6989	80	60	70	70	
10	1	80	70	60	70	
20	1.3010	90	70	80	80	
40	1.6020	100	100	90	97	

LC_{50} of metabolite 1, CM2 was 1.2 µg/ml

*CM= Cisplatin metabolite

Table 7: Results of brine shrimp lethality bioassay

Concentration (C) (µg/ml)	Log C	% Mortality				LC50 (µg/ml)
		Compound 5 (*CM5)				
		Group A	Group B	Group C	Average	
1.25	0.0969	50	60	40	50	1.25
2.5	0.3979	50	70	60	60	
5	0.6989	60	90	90	80	
10	1	70	90	80	80	
20	1.3010	100	100	100	100	
40	1.6020	100	100	100	100	

LC_{50} of metabolite 4, CM5 was 1.25 µg/ml

*CM= Cisplatin metabolites

Table 8 : Results of brine shrimp lethality bioassay

Concentration (C) (µg/ml)	Log C	% Mortality				LC50 (µg/ml)
		Cisplatin(standard solution)				
		Group A	Group B	Group C	Average	
1.25	0.0969	30	40	50	40	1.75
2.5	0.3979	50	60	70	60	
5	0.6989	70	80	60	70	
10	1	70	80	60	70	
20	1.3010	100	100	100	90	
40	1.6020	100	100	100	100	

LC_{50} of cisplatinstandard solution was 1.25 µg/ml

Treatment of animals with methanolic extract of D. alata and M. olifera significantly reduced the elevated levels of serum creatinine and urea. The extract treatment was able to lower the serum creatinine and urea to almost normal level (Table 1)

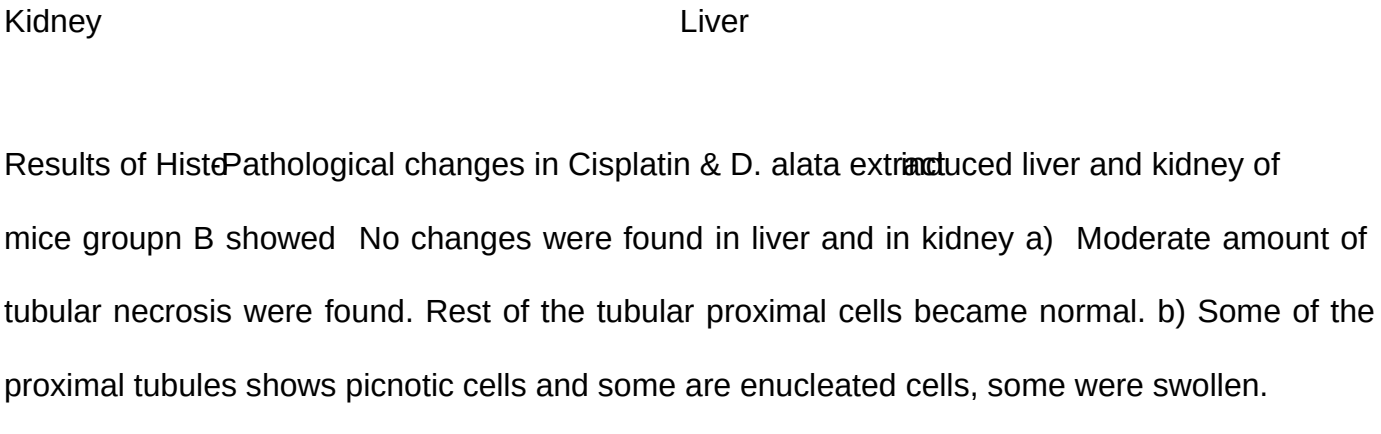
Table 10 : Level of GSH in the kidney of mice treated with normal, cisplatin (16 mg/kg body wt., i.p), cisplatin + methanolic extract of DAME, cisplatin+ MOME, Cisplatin metabolite 3, Cisplatin metabolite 3+ DAME, Cisplatin metabolite 3+MOME , (250 mg/kg body wt, p.o) treated mice

Group	Treatment (mg/kg)	GHS (nmol/mg protein)
Normal	-	12.4±0.88
Cisplatin	16	7.3±1.32a
Cisplatin + DAME	250	10.6±0.28b
Cisplatin + MOME	250	12.3±2.25b
Cisplatinmetabolite3	16	6.9±1.78b
Cisplatin metabolite3+DAME	250	13.02± 0.13a
Cisplatin metabolite3+MOME	250	13.09± 0.55a

Values are mean ± SD;n = 5. All values are significant a P < 0.001 with respect to normal group; b P < 0.001 with respect to cisplatin and cisplatin metabolite 3 group.

In Table 11, The renal GSH concentration decreased by over 40% ($P < 0.001$) in cisplatin-treated animals compared to the normal group. However, the administration of methanolic extract of D. alata and M. olifera at a concentration of 250 mg/kg body wt prior to cisplatin treatment increased the renal GSH concentration to the normal level (Table 2)

Photograph 2 : Histopathology of liver and kidney of mouse after Cisplatin + extract of D. alataa administration



Kidney Liver

Results of Histo-Pathological changes in Cisplatin & D. alata extract induced liver and kidney of mice groupn B showed No changes were found in liver and in kidney a) Moderate amount of tubular necrosis were found. Rest of the tubular proximal cells became normal. b) Some of the proximal tubules shows picnotic cells and some are enucleated cells, some were swollen.

Photograph 4 : Histopathology of liver and kidney of mouse after Metabolite 1 administration

Liver Kidney

Results of Histo Pathological changes in metabolite 2 induced liver and kidney of mice group D showed a little bit hepatocellular swelling and in kidney Massive necrosis of the Proximal con. Tubules with distorted cellular architecture along with other distal tubular abnormalities

Photograph 6 : Histopathology of liver and kidney of mouse after Metabolite 1 + extract of Moringa oleifera administration

Liver Kidney

Results of Histo-Pathological changes in Metabolite 2 + extract of D. alata induced liver and kidney of mice group E showed A little bit hepatocellular swelling and in kidney a) Mild to moderate increase in the restoration of normal physiological renal proximal tubules b) Good numbers of prox. Con. Tubular necrosis remain unchanged.

Photograph 8 : Histopathology of liver and kidney of mouse after Metabolite 2+ extract of D. alata administration

Liver Kidney

Results of Histo-Pathological changes in Metabolite 4 + D. alata induced liver and kidney of mice group F showed No changes were found in liver. Normal hepatocytes . a) Mild increase in the restoration of normal physiological renal proximal tubules b) Less numbers of prox. Con. Tubular necrosis remain unchanged.

Chapter 6

Discussion

Discussion

Cisplatin, the most widely used chemotherapeutic agents for the treatment of several human malignancies. The efficacy of cisplatin is dose dependent, but the significant risk of nephrotoxicity frequently hinders the use of higher doses to maximize its antineoplastic effects. Several advances in our understanding of the biochemical and molecular mechanisms underlying cisplatin nephrotoxicity have recently emerged and are reviewed in this article.

Cisplatin treated total 50 cancer patients those who were admitted in the inpatient department of Oncology, KYAMC, Sirajgonj were studied in this research.

This study revealed that about 56% (n=28) of the total population were placed in age 60 to 68 group while among the elder population where they found less suffered in malignancy denoted to only 8% (n=4) is shown in the graph 8. The incidence of malignant condition with the increases of the age of the patients. Though some study revealed that cisplatin induced severe transient anemia were found[159,160], but in our study we found only 2% of the patients (n=1) suffered from severe anemia, 48% (n=24) were found anemic (figure 1). Serum bilirubin level of 80% (n=40) of the patients were found within normal limit where only 4% (n=2) of the patients were found severe jaundice which is suggested the less involvement of hepatic toxicities (figure 2). This study support the previous work done by Pollera C.F and his colleagues in 1987, where they said that a small amounts of platinum are present in the bile and large intestine after administration of Cisplatin, the fecal excretion of platinum appears to be insignificant. Transient elevations of liver enzymes, especially SGOT, as well as bilirubin, have been reported to be associated with Cisplatin administration at the recommended doses[161]. Only 8% (n=4) were found edematous condition among the patients (figure 3).

Lymph node enlargement among the 50 patients were found 24% (n=12).

Moderate increased of serum creatinine (µg/dl), blood urea (µg/dl) and albuminurea levels were found as 28% (n=14), 20% (n=10) and 32% (n=16) of the total patients respectively, strongly suggested that cisplatin or its metabolites produced nephrotoxicities ($p < 0.001$**) (figure 6,7 & 26) that correlated to the previous study[162]. To prevent nephrotoxicities adequate hydration were assured before starting cisplatin treatment, though some nephrotoxicities were developed which was revealed in previous works also[163].

Total 14 (28%) out of 50 patients suffered from DM, among them 24% (n=12) was suffered from NIDDM and 4% (n=2) from IDDM (figure 4-8).

From the evidence of HPLC data sheet, four suspected metabolites along with Cisplatin (CP, CM2, CM3, CM4 and CM5) were identified with their specific RT (retention time) and Area (figure 18-19). Total 500 samples (250 blood and their corresponding 250 urine samples) from different time interval (0, 3, 6, 12, 24 hour) were placed in HPLC for evaluate their retention time (RT) and Area, and finally their mean concentrations in both serum and urine. From HPLC data sheet, the statistical analysis reported that the mean plasma and corresponding urine concentration of cisplatin (as mg/dL) in 50 cancer patients at different time (0, 3, 6, 12, 24 hour) interval were measured and calculated as 478.3165 (±4.5643), 3.598 (±1.632), 281.4552 (±9.377), 241.9782 (±4.0988), 2.8988 (±0.5643), 1.6779 (±0.5453), 996.559 (±8.4365), 978.9265 (±7.6435) respectively ($p < 0.05$) (figure 18-19) where there was a sharp increase and highest concentration were found after 3 hour of infusion and gradually decreased the concentration which was supported by previous work[164], on the other hand mean plasma

Previous several studies have attempted to contribute the potential approaches to prevention of cisplatin-induced nephrotoxicity[30-34]. Some of these strategies are very promising specially herbal medicine that were proved to decrease cisplatin induced toxicities[35-38].

Further studies will be performed on the potential approaches of some medicinal plants as Dioscorea alata and Morienga olifera to prevention cisplatin induced nephrotoxicities on cisplatin treated mice. Structure of suspected metabolites will be elucided by using HPLC-Mass spectrometry.

Separation of cisplatin and its possible metabolites from urine samples of 5 patients was done by using column chromatography, glass chromatography, PTLC using a range of solvent systems with different polarity and finally their Rf values were done (table 3 & photograph 1).

Primary screening for cytotoxicity of these five compounds (C, CM2, CM3, CM4 and CM5) along with cisplatin (as standard solution) were done by using brine shrimp lethality bioassay (figure 20-25 & table 49). Among them, comparative to cisplatin, its metabolites are more toxic, specially CM2 (Meta 2, LC_{50}=1.2µgm/ml) and CM3 (1.08µgm/ml), CM4 (Meta 4, LC_{50}=1.182µgm/ml) which was correlate to the previous study ($p<0.01$*).

In vivo animal histopathology study of five compounds including cisplatin to see the Histo-Pathological changes in liver and kidney of mice. Total number of test animal (Mouse) : 50. Average wt of mouse: 40 gm, total number of groups =13. Group A was treated with Cisplatin, Group B with Cisplatin and extract of D. alata, Group C with Cisplatin and extract Morienga olifera , Group D with Metabolite 2 (meta 2), Group E with Metabolite 2 and extract of D. alata,

Chapter 7

Conclusion

Conclusion

Cisplatin-induced nephrotoxicity was confirmed in our study. 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. 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. These 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.

Although extracts of D. alata and Moringa oliefera had some effects on biochemical parameters; its effect on renal histology in injured mice kidney was very promising and significant. 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.

Recommendation

Cisplatin (cisplatinum(II) diamine dichloride) is an anticancer drug extensively used against a variety of cancers. Cisplatin chemotherapy is found to manifest dose dependent nephrotoxicity Cisplatin is nephrotoxic, but the mechanism by which cisplatin kills renal proximal tubule cells is not well defined. Some author suggested that the depletion of renal antioxidant defence system has been suggested to be the main cause of cisplatin induced nephrotoxicity. The final step in this bioactivation is the conversion of a platinum cysteine S conjugate to a reactive thiol by a PLP-dependent cysteine S F R Q M X lyase. LLC-PK$_1$ cells, a proximal tubule cell line with low cysteine S F R Q M X lyase activity, are used to study cisplatin nephrotoxicity.

In previous experiences that demonstrated that many strategies have attempted to prevent or reduce its nephrotoxicity[85, 86]. Early experience suggested that the administration of cisplatin by prolonged continuous infusion and saline hyperhydration, with or without frusemide or mannitol osmotic diuresis, reduces nephrotoxicity[85]. Although contemporary protocols invariably employ hyperhydration and many use low dose rates of cisplatin, it is clear that nephroprotection is not complete[87]. Hydration reduces the risk of nephrotoxicity with high dose carboplatin[88].

In our study we found that with adequate precaution and proper hydration of the patient before and during cisplatin administration about 26% of the patients serum creatinine were high level and 12% of patients were found moderate to severe increase blood urea level (figure 13 & 14) that indicate nephrotoxicity.

Several medicinal plants extract were used to prevent cisplatin induced nephrotoxicity[113-135]. In this contest, we have found that nephroprotective effect of Dioscorea alata and Moringa olifera plants material. It reduces the nephrotoxicity of mice and reduces the serum creatinine and blood urea in mice significantly.

Different parts Dioscorea alata and Moringa olifera plants were taken by peoples of the various parts of the world specially Bangladesh, India, China and Sri Lanka as vegetable food in their meal. These two plants are popular vegetable in these countries. So it is easy to apply the different parts of these plant in a cancer patients receiving cisplatin.

In our study, separation of four suspected metabolites eluted by using different chromatographic techniques, HPLC and UV spectrophometry. Structure of these compounds will be elucided by mass spectrometry in future

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Annexure 1

Annexure 1

Photograph 10 : Dioscorea alata tuber, the edible part of the plant