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Cytoprotective Properties of Makabuhay *(Tinospora crispa)* Bark Decoction in Cisplatin-induced Nephrotoxic Mice

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***Abstract*—** Renal failure in cancer patients is one common problem in chemotherapy. Patients are frequently treated with nephrotoxic antibiotics like cisplatin. After a single dose of cisplatin, thirty-three percent (30%) of patients develops nephrotoxicity that leads to acute kidney injury. This study investigated the cytoprotective properties of makabuhay bark decoction in cisplatin-induced nephrotoxic mice. The makabuhay was extracted through decoction. Nine adult albino mice were used and divided into three groups. Nephrotoxicity was induced through repeated low dose cisplatin intraperitoneally and makabuhay extracts were administered orally. The treatment lasted for five consecutive days and animals were sacrificed under anesthesia on the 6th day after an overnight fast. Blood was collected for blood urea nitrogen and serum creatinine levels evaluation. Results showed that there is a significant difference in the blood urea nitrogen levels of makabuhay treated mice compared to other groups. However, serum creatinine levels among treatments did not show any significant difference. On the other hand, kidney sections of mice were prepared and interpreted. The normal control group showed normal kidney tissues. Kidney sections from the cisplatin group showed severe renal degeneration and necrosis with congestion. The makabuhay treated mice presented moderate renal congestion and degeneration. The data from this study, proved that makabuhay possess cytoprotective properties in cisplatin-induced nephrotoxicity using established mouse model.

Keywords— *nephrotoxicity, cisplatin, cytoprotection, blood urea nitrogen, serum creatinine*

I. INTRODUCTION

Renal failure in cancer patients is one common problem in chemotherapy. Since the kidney is highly susceptible to toxic injury by different medicines, several drugs for chemotherapy exert potent nephrotoxicity. Cancer patients are frequently treated with nephrotoxic antibiotics, such as methotrexate, streptozotocin and cisplatin to name a few (Ries & Klastersky, 1986).

Cisplatin is one of the most effective medicines used in chemotherapy and plays a major role in the treatment of different human solid tumors including those of the head, neck, testis, ovary and breast (Kim et al., 2006). However, the clinical application of cisplatin is complicated by its effects on kidney and poses nephrotoxicity or Acute Kidney Injury (AKI) (Goldstein and Mayor, 1983). Since 1971, animal studies have shown a major cisplatin-induced nephrotoxicity that proved to be dose related and early clinical reports showed similar toxicity in cancer patients (Ries & Klastersky, 1986). After a single dose of cisplatin, thirty-three percent (30%) of patients develops nephrotoxicity. Furthermore, cisplatin-induced AKI accounts for nineteen percent (19%) of all cases of AKI, making it a serious health problem (Sharp, 2016).

Makabuhay is a Filipino term which means “prolife or to give life”. It is a folkloric medicinal plant that is widely distributed in Asia and Africa that has been generally used by locals to treat many diseases (Tan and Bajo, 2014). Traditionally, it had been prepared as an aqueous extract for the treatment of flatulence, indigestion and diarrhea (Hipol, Cariaga & Hipol, 2012).

This study would be beneficial to cancer patients who developed nephrotoxicity due to cisplatin chemotherapy. For the reason that, aside from controlling cancer by taking prescribed antineoplastic medicines like cisplatin, resulting kidney injury should be also taken into consideration. This investigation would contribute to the Philippine Pharmacology in quest for new medicinal properties from local plant sources like makabuhay that is commonly found among most localities.

This study aims to investigate cytoprotective properties of makabuhay bark decoction in cisplatin-induced mice nephrotoxicity. Moreover, this study aims to assess body weight, before and after treatment, haematological indices, specifically, blood urea nitrogen and serum creatinine levels. And lastly, to assess histopathological parameters of cisplatin-induced nephrotoxic mice as indicators of cytoprotective effects of makabuhay extracts.

II. RELATED WORK

In the Philippines, makabuhay is a vine naturally found in rainforest or mixed deciduous forest. This plant is a large, glabrous, deciduous climbing shrub belonging to the family of Menispernaceae. It contains a number of chemical constituents that includes alkaloids, glycosides, steroids and polysaccharides that account for the plant medicinal properties (Singh et al., 2003). Tinospora was subjected to various clinical studies using experimental animals. The plant was found to lower blood glucose level of the test animals (Noor and Ashcroft,1998). Jagetia and Rao (2006) investigated the plant's use in the treatment of malaria and cancer.

The use of herbal plants to treat illnesses has been an accepted practice among indigenous people throughout the world. In some traditional practices, extracts from different parts of a plant were used for therapeutic purposes (Galia & Galia, 2016). Several cultures have distinct uses of plants for the treatment of various diseases. These herbals play an important role for cancer treatment due to their multiple chemical compound in discovering new active materials against cancer (Ahmad, Jantan & Bukhari, 2016).

The use of Tinospora crispa traditionally is to treat numerous health conditions namely, fever, malaria, diabetes and in maintaining good health (Kooti et al., 2017). However, there are adverse drug effects in taking up some medications. Cisplatin is regularly prescribed in the treatment of cancer and while they are effective, their use is limited by their severe, dose-limiting side effects. The dose limiting side effect for cisplatin is nephrotoxicity (Oun, Wheate & Moussa, 2018).

Cancer is one of the major causes of death in the world, and it is the second leading cause of mortality after cardiovascular diseases (Kooti et al., 2017). According to Dasari & Tchounwou (2014) cisplatin is a well-known chemotherapeutic drug used for treatment of numerous human cancers including bladder, head and neck, lung, ovarian, and testicular cancers. Unfortunately, cisplatin is renally excreted and can produce nephrotoxicity (Pinzani et al., 1994). In addition, according to Calvo et al. (2004), cisplatin or its metabolites are eliminated through the kidneys and hence cause renal toxicity.

In Malaysia, a study of Noor & Ashcroft (1989) proved that an aqueous extract of Tinospora crispa stems is taken orally to treat diabetes mellitus. Moreover, acute intravenous treatment with the extract caused an increase in plasma insulin levels. This data supports the traditional belief could improve diabetic conditions virtue of its action on the endocrine pancreas. Likewise, in Kuala Lumpur, Malaysia a study of Kadir, Othman, Abdulla, Hussan & Hassandarvish (2011) showed that traditional folklore attributes various therapeutic uses to its stem for treatment of fever, jaundice, hyperglycemia, hypertension, wounds, intestinal worms and skin infections. It is also used to treat tooth and stomach aches, cough and asthma.

Published literatures shows that nephrotoxicity caused by cisplatin administration is among the burden of cancer patients in their fight for life. This study would help them lessen kidney damage secondary to cisplatin treatment.

III. METHODOLOGY

Methods were patterned from Lakshimi, Neelima, Umarani & Sudhakar (2009) and Adikwu & Bokolo (2018) but with modifications due to various limitations.

Drugs and Chemicals

Cisplatin (brand name: platin) and sterile water used for this study were procured from Eastern Visayas Regional Medical Center (EVRMC) Pharmacy. The barks of makabuhay were sourced from Barangay Sta. Elena, Tacloban City, Philippines. Tinospora crispa species was identified and authenticated by the Department of Agriculture.

Preparation of Makabuhay Bark Decoction

The bark of makabuhay were collected, washed, air dried, blended using an electric blender. Blended makabuhay barks (250 grams) were boiled in 500 mL distilled water at 1000C for thirty minutes (30min), macerated for twenty-four hours (24hrs) and filtered using a muslim cloth for eight (8) times. The filtrate was centrifugated using a centrifuge machine at 5000 revolutions per minute (rpm). The supernatant was separated and placed in a clean vial for storage.

Experimental Animals

Nine (9) adult albino mice, (all male, all white, 25-35g) in weight and has no observable physical abnormalities were procured from the Visayas State University, College of Veterinary Medicine, Baybay City, Leyte, Philippines. The mice were housed at the Northern Tacloban City National High School (NTCNHS) Science Research Laboratory and were grouped in plastic colony cages with three mice per cage in a well-ventilated standard room condition. Test animals were identified using tail tags with free access to food and water ad libitum in 12-hour day-night cycle. The mice underwent acclimatization for 7 days to adjust to its new environment and overcome stress during the transit. Apparent changes in behavior and motor coordination were monitored daily.

The researchers attended a seminar on Animal Welfare Laws, Rules and Regulations conducted by the Bureau of Animal Industry (BAI) and were trained intensively on Animal Handling Procedures and Techniques by Dr. Johdel S. Ty, a licensed veterinarian of the City Veterinary Office, Tacloban City, Philippines.

Dose Selection

Cisplatin was induced in male albino mice to develop nephrotoxicity using 7mg/kg repeated low dose cisplatin intraperitoneally. Makabuhay bark decoction and sterile water were administered through oral gavage with a dose of 10 mg/kg and 0.5ml respectively using appropriate crop

needled feeding tubes. The dosing method was guided by a licensed pharmacist and approved by a licensed veterinarian.

Drug Administration

Nine (9) male albino mice were divided into three groups (T1, T2, T3) each treatment was replicated thrice with 1 mouse per replication. Treatment 1 (T1) mice were treated with oral treatment of 0.5ml sterile water daily for 5 days that served as the normal control group. Treatment 2 (T2) were treated with cisplatin single dose 7mg/kg daily intraperitoneally following 0.5ml sterile water for 5 days. Treatment 3 (T3) mice were treated with cisplatin single dose 7mg/kg daily intraperitoneally following 10mg/kg makabuhay decoction orally daily for 5 days.

Sacrifice of Animals

Mice were weighed and sacrificed under anesthesia on the 6th day after an overnight fast using cervical dislocation method. Blood were collected through cardiac puncture, placed in a microtube and sent to RTR Hospital Laboratory for blood test. The mice were dissected, weighed and rinsed in normal saline solution and fixed in ten percent (10%) formalin for twenty-four hours (24hrs). After the experiment, euthanized mice were wrapped in a newspaper, sealed in a garbage bag and disposed properly.

Hematological Test

Blood urea nitrogen (BUN) and serum creatinine (Scr) are standard measures of kidney function parameters as their levels increase in the blood when the filtering capacity of the kidney is significantly reduced (Sharp, 2016). Blood urea nitrogen (BUN) and serum creatinine were measured using Biochemistry Calibrator (Biosystems cod. 18011) at RTR Hospital Laboratory. The normal values for mice BUN are 10-33 mg/dL and 0.5-2.2 mg/dL for serum creatinine.

Histopathological Evaluation of the Kidneys

Kidney samples were rinsed normal saline and fixed in 10% formalin for 24hrs and transported to the testing laboratory. Kidney tissues were processed routinely and embedded in paraffin blocks. Slides were prepared by a registered medical technologist of RTR Hospital Laboratory, Tacloban City, Philippines. Prepared slides were interpreted and analyzed by Dr. Gerry A. Camer, a veterinary pathologist of the University of Eastern Philippines, Catarman, Northern Samar, Philippines.

Statistical Analysis

Data were presented in mean and subjected to a one-way ANOVA at $p<0.05$, using SPSS software. Means were compared by Fisher`s Least Significant Difference (LSD).

IV. RESULTS AND DISCUSSION

Table 1. Effects of makabuhay bark decoction in the initial and final body weight of the cisplatin-induced nephrotoxic mice

Treatments		Weight (g)	
		Initial body weight	***Final body weight***
T1	Normal Control (NC)	29.83	31.83
T2	Cisplatin (CIS)	32.33	28.33
T3	Cisplatin + Makabuhay (CIS+MKB)	32.50	29.50

Table 2: Effects of makabuhay bark decoction in kidney function parameters of the cisplatin-induced nephrotoxic mice

Treatments		Kidney function parameters	
		BUN (mg/dL)	***SCr (mg/dL)***
T1	Normal Control (NC)	21.56	1.29
T2	Cisplatin (CIS)	61.73	3.31
T3	Cisplatin + Makabuhay (CIS+MKB)	33.19	2.52

**normal values: BUN= 10-33 mg/dL, SCr= 0.5-2.2 mg/dL*

Discussion

Table 1 shows body weights of the three treatments namely the T1, T2 and T3. Treatment 1 has initial and final body weight of 29.5 g and 31.38 g, treatment 2 has 32.33 g and 28.33 g and treatment 3 with 32.50 g and 29.5 g, respectively.

Although, the results showed difference in the initial and final weight measurement in the experiment, the initial and final body weight were not statistically significant with p-value of 0.41 and 0.26 which is greater than the 0.05 level of significance. Thus, the experiment did not lead to drastic weight loss that is an indication of stress. No mortality was recorded. There was no significant loss of fur and skin, nose and eyes appeared clear and normal throughout the duration of administration. There was no convulsion, salivation, respiratory distress or coma which are signs commonly associated with oral toxicity. The animals were not aggressive and did not exhibit any unusual behaviour during handling.

Table 2 shows the haematological test results of the three treatments namely T1, T2 and T3. The administration of makabuhay bark decoction to T3 mice showed significant difference in Blood Urea Nitrogen (BUN) levels as compared to T1 and T2 with a p-value of 0.000 respectively, which is less than the p-value of 0.05 level of significance.

On the other hand, the serum creatinine level of the makabuhay group did not show significant difference as compared to T1 and T2 mice.

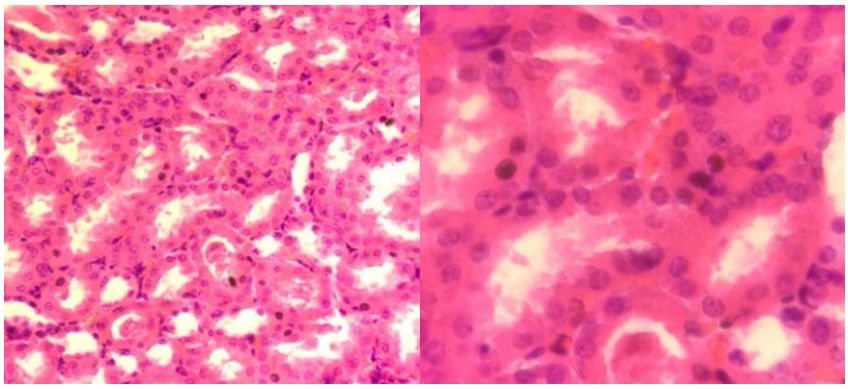

Figure 1. Kidney sections of treatment 1 mice (normal control group) in 40X and 100x magnification using a compound light microscope.

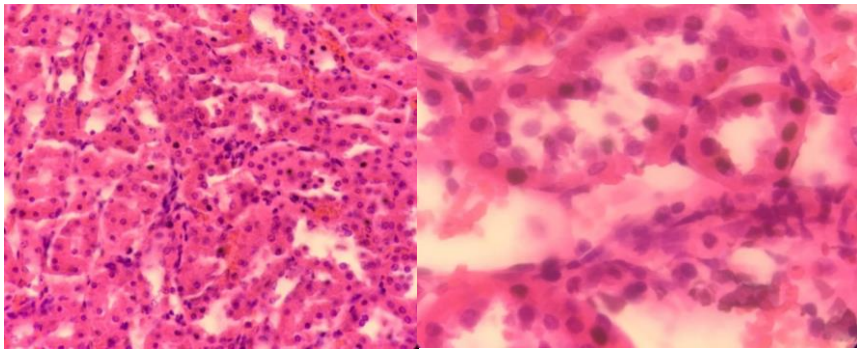

Figure 2. Kidney sections of treatment 2 mice (cisplatin group) in 40x and 100x magnification using a compound light microscope.

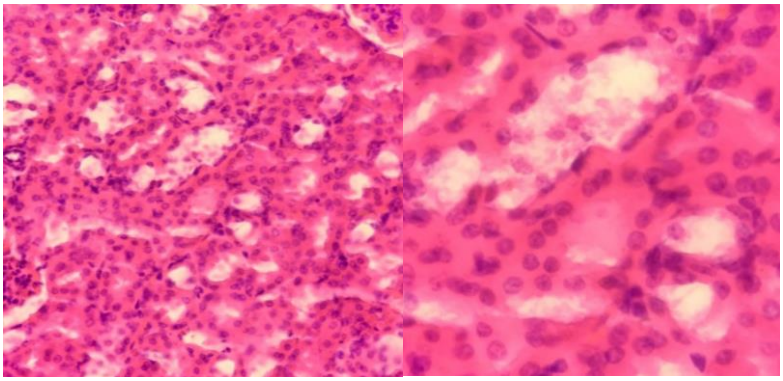

Figure 3. Kidney sections of treatment 3 mice (cisplatin + makabuhay group) in 40x and 100x magnification using a compound light microscope.

Figure 1 shows the photomicrographs of the kidney sections in treatment 1 mice or the normal control group. These shows normal kidney histology with evident intact renal parenchyma. On the other hand, Figure 2 shows the photomicrographs of the kidney sections in treatment 2 mice or the cisplatin group. Repeated low-dose cisplatin was induced intraperitoneally for 5 consecutive days and showed severe renal degeneration and necrosis with congestion. Furthermore, the renal tubular cells are swollen and the nuclei of the most cells and condensed. Figure 3 shows the photomicrographs of the kidney sections in treatment 3 mice or the cisplatin + makabuhay group. repeated low-dose cisplatin was induced intraperitoneally followed by oral makabuhay administration for 5 consecutive days. This shows moderately congested renal parenchyma. Moreover, the renal tubular cells appear cloudy and the glomerulus is intact. The kidney sections present a subacute moderate renal congestion and degeneration.

V. CONCLUSION AND FUTURE SCOPE

Upon conducting this study, the researchers conclude that makabuhay bark decoction has cytoprotective properties in cisplatin-induced nephrotoxicity. This study used an established nephrotoxic mouse model through intraperitoneal injection of low-dose cisplatin. The results showed significant difference in blood urea nitrogen (BUN) levels. While, the serum creatinine (SCr) levels showed no significant difference in makabuhay-treated mice compared to the normal control and cisplatin group. Lastly, kidney

histopathology interpretation showed normal kidney tissues for the normal control group. On the other hand, necrosis or death of tissues, severe degeneration and cell congestion were evident in mice treated with cisplatin only. Furthermore, nephrotoxic mice treated with makabuhay showed subacute moderate renal congestion and degeneration.

Provided with more funding and equipment, the proponents of this study recommend future researchers to use other extraction methods of makabuhay. Multiple concentrations of makabuhay extract and use of other test animals would also be an important point of comparison. Further studies can explore other nephrotoxicity models in animals to understand the cytoprotective effects of makabuhay and utilize non-animal models to minimize use of experimental animals.

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