NEPHROPROTECTIVE ACTIVITY OF KATAKA-TAKA (*Kalanchoe pinnata*) ETHANOLIC LEAF EXTRACT ON GENTAMICIN-INDUCED NEPHROTOXICITY IN MALE SPRAGUE-DAWLEY RATS

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ABSTRACT

This research study determined the nephroprotective activity of Kataka-taka (*Kalanchoe pinnata)* ethanolic leaf extract against gentamicin-induced nephrotoxicity in male Sprague-Dawley rats. Ethanolic leaf extract of Kataka-taka (*Kalanchoe pinnata)* was prepared by maceration. Male Sprague-Dawley rats weighing 100-250g were divided into 6 groups; kidney care capsule, normal saline (1ml/kg/day i.p.), gentamicin (80mg/kg/day i.p.) and ethanolic extract of *Kalanchoe pinnata* at 140, 120, 100mg/kg/day plus gentamicin (80mg/kg/day) intraperitoneally for 7 days. Kidney damage was first induced with gentamicin for 5 days. Blood Urea Nitrogen (BUN) and Serum Creatinine (SrCr) was measured at day 0 and day 5 for checking of kidney damage. Administration of the different treatments started on the 6th day of the experiment up to day 12. At the end of the experiments, the rats were sacrificed by exposure to ether then both kidneys were removed. The kidneys were processed for histopathological examination. As evident on the results for BUN, it was found out that there are no significant differences in all the doses of Kataka-taka leaf extract, specifically, 140mg/kg, 120mg/kg and 100mg/kg with p-values of 0.3332, 0.1215, and 0.045, respectively. Moreover, result for serum creatinine (SrCr) also revealed that there are no significant differences in all the doses of Kataka-taka leaf extract, specifically, 140mg/kg, 120mg/kg and 100mg/kg with p-values of 0.4429, 0.4077 and 0.3202, respectively. Histopathological result showed that co-administration of Kataka-taka at 120mg/kg dosage plus gentamicin showed significant decrease in glumerular congestion with few RBC's and lesser edematous tubules Thus, the researchers conclude that statistically, Kataka-taka ethanolic leaf extract in all doses within the scope of this research study possess nephroprotective characteristics without significant difference. However, histopathological result confirmed that 120mg/kg dosage is considered the most effective compared to 140mg/kg and 100mg/kg.

Key words: *Kalanchoe pinnata, ethanolic leaf extract nephrotoxicity, nephroprotective, male Sprague-Dawley rats, blood urea nitrogen, serum creatinine*

INTRODUCTION

Nephrotoxicity is the most common kidney problem that happens when the body is exposed to a drug or toxin that triggers kidney damage. The increase level of ions, serum creatinine, serum urea, blood urea nitrogen (BUN) and serum total protein produces harmful effects in our body which causes pathological effects on the kidneys (Duggal, 2018).

According to WHO, nephrotoxicity is one of the top 10 diseases in the world, specifically ninth leading cause of death. While in the Philippines, it was reported that 20% of nephrotoxicity is caused by the induced drugs, and for elderly patient, medication increases the incidence of nephrotoxicity up to 66%. (Kohli et. al., 2000). This circumstance lead nephrotoxicity as a seventh cause of death in the country. The use of aminoglycosides as an antibiotic is known to be potential nephrotoxic and that remain as a challenge as it will result to kidney problem. (Aiswarya et al., 2018).Kidneys plays an important role in our body in the formation of urine, water and electrolyte balance and production of hormones and enzyme (Reddy et al., 2015).When kidney is poisoned by toxic chemicals and medication, it is very alarming for it will damage the organ with no noticeable symptoms which is the reason why it is called "silent killer".

Kataka-taka which is commonly known as "miracle leaf" belongs to the Crasulaceae family commonly found on plains and temperate region possess many medicinal uses (Rajsekhar, et al., 2016). In folkloric, *K pinnata* is widely used as an astringent, antiseptic and counterirritant against poisonous bites. Various other biological activities attributed to the plant includes antimicrobial, anti-fungal, muscle relaxant, anticonvulsant and hypoglycemic this findings lead the researchers to do further study in *K. pinnata*. In year 2016, a phytochemical study by (Srivastava ,et. al., 2018) of *K. pinnata* leaf extract suggest that it can be use a nephroprotective in gentamicin-induced nephrotoxicity. However, the nephroprotective activity of K. pinnata has not been established and likely with no enough evidence. Hence the researcher now came up with the idea of having Kataka-taka as an object in nephroprotective experimentation. The researchers aim is only limited in determining the nephroprotective activity of Kataka-taka (Kalanchoe pinnata).

From this information, the researcher will then conduct a study regarding the nephroprotective activity of Kataka-taka (*Kalanchoe pinnata*) in gentamicin-induced nephrotoxicity by administering kidney care capsule, normal saline, gentamicin and gentamicin plus *K. pinnata* for 7 days in male Sprague-Dawley rats. Elevated levels of serum creatinine, blood urea nitrogen, and histopathological alterations signifies drug-induced nephrotoxicity in test animals (Dungca, 2016).

Research Questions

Generally, the study aimed to determine the nephroprotective activity of *Kalanchoe pinnata* ethanolic leaf extract against gentamicin-induced nephrotoxicity in male Sprague-Dawley rats.

Specifically, this aimed to answer the following questions:

1. What are the phytochemical constituents present in the local variety of the *K. pinnata* extract that attributes in its nephroprotective activity?
2. What is the kidney function of the subjects before and after administration of the different treatments in terms of:
    a. Serum Creatinine
    b. Serum Blood Urea Nitrogen (BUN)
3. What is the histopathological characteristic of the subjects' kidneys after administration of the different treatments?
4. Is there a significant difference in the kidney function of the subjects before and after administration of treatments?
5. Is there a significant difference in the kidney function of the subjects after administration of treatments when grouped according to treatments received?

Hypothesis

- There is no significant difference in the kidney function of the subjects before and after administration of treatments.
- There is no significant difference in the kidney function of the subjects before and after administration of treatments

Significance of the Study

The study provided the public a safe and effective folkloric medicinal plant that they can use in order to prevent drug-induce nephrotoxicity. The study provided benefit in advancing pharmacy development in the Philippines through the use of alternative medicinal plants which are bounteous in our country.

Literature Review

Nephrotoxicity

The kidney is an essential organ which performs some important functions in our body. One of its roles is regulation of the extra cellular cell including detoxification and excretion of toxic metabolites and drugs. Hence kidney can be considered as a major target of exogenous toxicants that may cause nephrotoxicity.

Nephrotoxicity is one of the top 10 causes of death in the whole world; it is kidney-specific feature in which excretion does not go smoothly owing to toxic chemicals or drugs. Nephrotoxicity can be diagnosed through blood test, evaluation of the blood includes the measurement of blood urea nitrogen, serum creatinine, glomerular filtration rate and creatinine clearance. Blood urea nitrogen indicates the amount of nitrogen in the form of waste product called urea present in the body and elevation of creatinine mainly suggest the possible malfunction or failure of the kidneys.

Based on studies, chemotherapy and anticancer drug has seen limited due to nephrotoxicity that it can cause to the human body. Some studies also revealed that approximately 20% cause of nephrotoxicity is triggered by induced drug such as aminoglycosides.

Gentamicin is an aminoglycoside antibiotic which is used to treat bacterial infections. It is used to treat serious infections such as meningitis, septicemia, pneumonia, bacterial endocarditis, acute pyelonephritis, prostatitis, bacterial infections in newborn babies and bacterial infections of the gall bladder. Gentamicin affects the production of bacteria of certain proteins that are required for their survival (Galdino et. al., 2017). The bacteria will cause to produce abnormal and faulty proteins. Gentamicin will eventually penetrate the bacteria and clears up the infection. Aminoglycoside as are nephrotoxic because a small volume of administered dose of gentamicin is retained in the epithelial cells in the lining of the kidney. Thus, small doses of gentamicin leading to nephrotoxicity cause functional alteration of the kidney.

Traditional Alternative Medicine Act of 1997

Using herbal medicine was a practice a long time ago. The community uses their available resources such as herbal plants in order to prevent and manage their diseases. They considered herbal medications effective to prevent and cure such illnesses and infections because of their constant availability in the community. They also believe to witch doctors and folk specialist who makes a diagnosis from concoction and create medicines from herbal plants.

Traditional Alternative Medicine Act of 1997 or the Republic Act No. 8423 is an act by the Philippine Institute of Traditional Alternative Health Care which helps Filipino people for the acceleration of the development in the use of indigenous plants/ traditional plants. This act helps secure the source and knowledge of traditional medicine of indigenous societies and can get acknowledgement and financial income from the permitted users. This policy also serves as guidelines of the researchers in manufacture, quality control and marketing of traditional plants.

This act promotes the use of traditional plants and encourages indigenous people to share their knowledge for others to study about the safety and efficacy of the traditional plants. The health care professionals should be aware of these alternative medications and promote to their patients. By this, our countrymen

would encounter more alternative medicines coming from that cost much lesser than existing drugs. The cheaper the medicines get, the more patients will comply with medication (Nolledo, 2015).

Kataka- taka

Kataka-taka (*Kalanchoe pinnata)* belonging to the family of crassulaceae has high index in therapeutic values (Matthew et.al, 2013). It is a plant native to Madagascar which are commonly found Australia, New Zeland, Malanesia, Hawaii and widely distributed in the Philippines known as Kataka-taka meaning astonishing or remarkable (Pinnata, 2017). Kataka-taka is commonly known as air plant, cathedral bells, life plant, miracle leaf, Goethe plant, curtain plant, floppers, good luck leaf, Mexican love plant and mother in law. The leaves which are very edible, are thick, fleshy, elliptical in shape, curved with a crenate or serrated margin and simple or compound in pairs on reddish stem (Choudhary et. al., 2013).

Kalanchoe pinnata finally known as wonder of the world- due to its medicinal uses is one of the chief herbs used as an astringent, antiseptic and counter irritant against poisonous bites. In tradition medicine, the leaf extract of Kataka-taka *(K. pinatta)* is used in the treatment of kidney stone, gastric ulcer, pulmonary infection and many more (Paari et.al, 2014). In the French West Indies, Kalanchoe pinnata called zeb maltet, is also use against headaches.

Research Paradigm

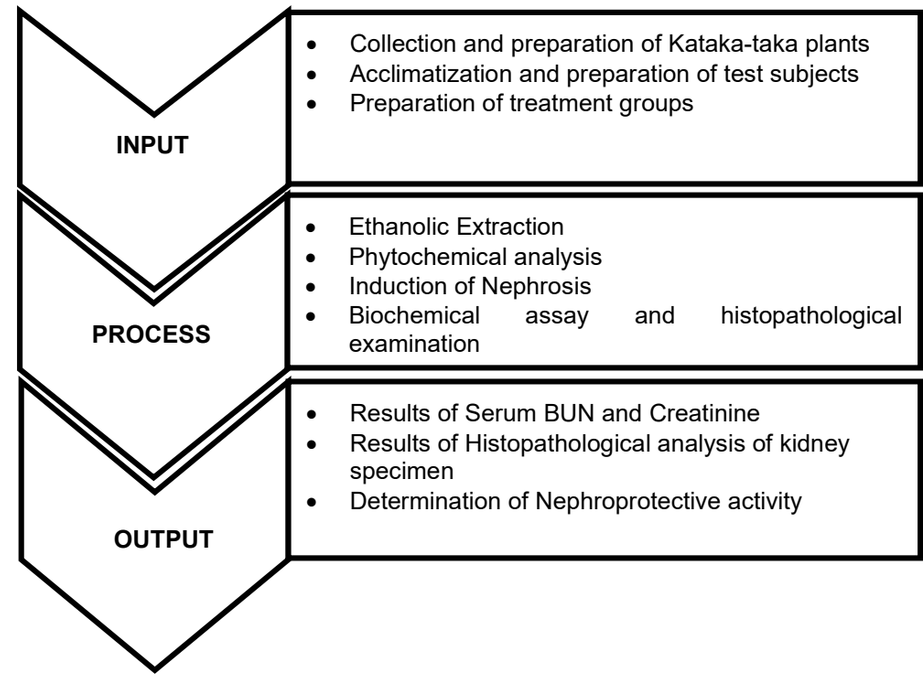

Figure 1. *Research Paradigm*

The figure shows how the modifying variable, which is the gentamicin alone and extracted Kataka-taka (*Kalanchoe pinnata)* leaves induced with gentamicin as the experimental control, and the positive control which is the kidney care capsule and negative control which is the normal saline, can affect the dependent variables when used as a nephroprotective in male Sprague-Dawley rats and to determine their significant difference.

METHODS

This includes different equipment, methods and procedures on the determination of the nephroprotective activity of the extract of Kataka-taka (*Kalanchoe pinnata)*. The research methodology, subject of the study and statistical tool that was used are shown below.

Research Design

The study utilized the experimental method of research that determined the nephroprotective activity of Kataka-taka (*Kalanchoe pinnata).* The researchers conducted an experiment and the data obtained were analyzed using statistical tools. Furthermore, the parameters that were measured were Blood Urea Nitrogen (BUN), Serum Creatinine and the result of histopathological examination

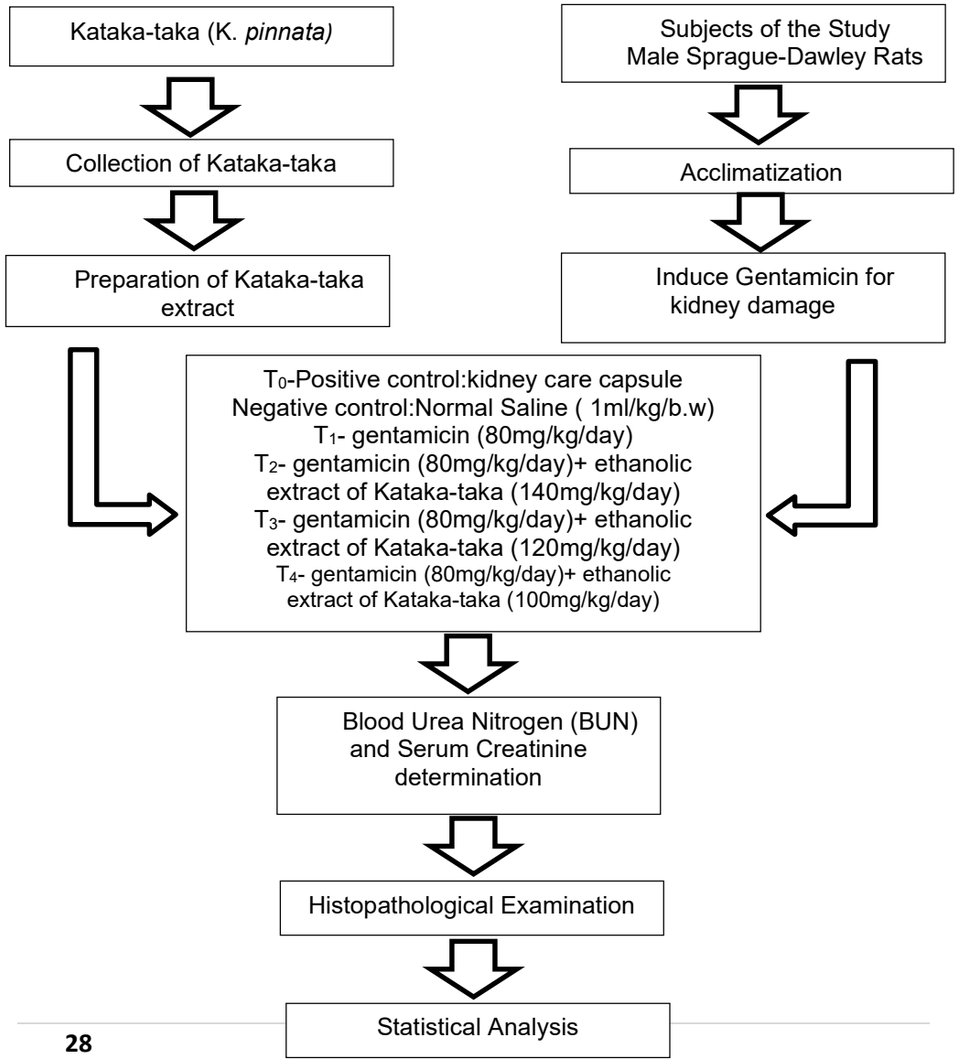

Figure 2. *Methodological Framework*

Procedures of Data Gathering

1. Collection and Preparation of Plant Control

1.1 Plant Authentication

Prior to the conduct of the experiment, authentication of the plant was accomplished at Department of Agriculture, Regional Office, Carig Sur, Tuguegarao City. Department of Agriculture authenticated our plant sample as Kataka-taka *(Kalanchoe pinnata).*

1.2 Collection of Plant Sample

The leaves of Kataka-taka (*Kalanchoe pinnata)* was collected from the local area of Camalaniugan, Cagayan and authenticated in the Department of Agriculture (DA) Tuguegaro City, Cagayan.

1.2 Preparation of Extract for Administration

1.3.1 The preparation of the plant leaf extract for phytochemical analysis was based on (Rajsekhar et. al., 2016).

1.3.2 The collected leaves were dried for 2 weeks under shade and ground.

1.3.3 The 250g of ground leaves were soaked in 500 mL erlenmeyer flask with sufficient amount of ethanol for one week at room temperature with occasional stirring.

1.3.4 The solvent was completely removed by rotatory evaporation and filtered with filter paper.140, 120, 100 mg/kg doses were prepared.

1.3.5 Erlenmeyer flask was sealed with cork and wrapped with aluminium foil to prevent from spilling. The extract was then preserved for further use.

1.4. Preparation of Kataka-taka leaf extract for the phytochemical analysis

1.4.1. The preparation of the plant leaf extract for phytochemical analysis was based on the textbook of (Guevarra, 2005) on Phytochemical Analysis of Plants.

1.4.2. Ethyl alcohol, 80% was prepared by diluting 80 mL of ethyl alcohol with distilled water 100mL.

1.4.3. Ground dried plant material was weighed at around 100g in an Erlenmeyer flask and was treated with sufficient 80% ethyl alcohol to completely submerge the plant material. The volume of alcohol used was noted (Guevara, 2005).

1.4.4. The plant suspension was macerated for 48 hours.

1.4.5. After maceration, the plant material was washed with fresh portions of alcohol in a Buchner filtration. The plant material residue was discarded.

1.4.6. The collected filtrate from the Buchner filtration process was filtered again using a filter paper.

1.4.7. Then it was subjected to the rotatory evaporator for 1 hour to completely remove the ethanolic component and concentrate.

1.4.8. After an hour, a sample extract was subjected to a flame test to confirm traces of ethanol. If positive, it will be subjected again to rotatory evaporation for 10 minutes. If the flame test is negative, the final extract will then be weighed using an analytical balance.

1.4.9. The weight and the final concentration of the plant extract were noted.

1.4.10. It was stored and labeled in a clean Erlenmeyer flask.

2. Preliminary Phytochemical Screening of Constituents

The Kataka-taka (*Kalanchoe pinnata)*_leaf extract was screened of its phytochemical constituent to confirm the presence of phytosterol and flavonoids in the extracted Kataka-taka (*Kalanchoe pinnata).* The test was conducted at the Pharmacy Laboratory of University of Saint Louis Tuguegarao.

2.1. Detection of Alkaloids: extracts were dissolved individually in dilute hydrochloric acid and filtered.

2.1.1. Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric iodide). Formation of a yellow colored precipitate indicated the presence of alkaloids.

2.1.2. Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicated the presence of alkaloid.

2.1.3. Hager's test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow colored precipitate.

2.2. Detection of Carbohydrates: Extracts were dissolved individually with 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

2.2.1. Molisch's test: Filtrates were treated with 2 drops of alcoholic o- naphthol solution in a test tube. Formation of the violet ring at the junction indicated the presence of Carbohydrates.

2.2.2. Fehling's test: Filtrates were hydrolysed with diluted HCl, neutralized with alkali and heated with Fehling's A&B solutions. Formation of red precipitate indicated the presence of reducing sugars.

2.3. Detection of Glycosides: Extracts were hydrolyzed with diluted HCl, and were subjected to test for glycosides.

2.4. Modifies Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonium solution. Formation of rose-pink color in the ammoniacal layer indicated the presence of anthranol glycoside.

2.5. Detection of Saponins

2.5.1. Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicated the presence of saponins
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2.5.2. Foam Test: 0.5 gram of extract was shaken with 2 ml of water. If foam produced persists for 10 minutes, it indicated the presence of saponins.

2.6. Detection of Phenols

2.6.1. Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of blush black color indicated the presence of Phenols.

2.7. Detection of Tannins

2.7.1. Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitates indicated the presence of Tannins.

2.8. Detection of Flavonoids

2.8.1.Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color,

which becomes colorless on addition of dilute acid, indicated the presence of Flavonoids.

2.9. Detection of Proteins and Amino Acids

2.9.1 Xanthoproteic Test: The extracts were treated with few drops of concentrated Nitric acid. Formation of yellow color indicated the presence of Proteins.

2.10. Detection of Sterols

2.10.1. Salkowski Test: Few milligrams of residue of each extract were taken in 2 ml of chloroform and in it 2 ml of concentrated sulfuric acid was added from the side of the test tube. The test tube was shaken for few minutes. The development of red color in the chloroform layer indicated the presence of sterols.

2.10.2. Liebermann-Burchard Reaction: Few milligrams of residue was dissolve in chloroform. To this, few milliliter of acetic anhydride was added. Then two drops of concentrated sulfuric acid was added from the side of the test tube. The greenish transient color indicated the presence of sterols.

3. Biological Assay

3.1 Experimental Animals

The researchers brought 30 pieces of healthy male Sprague-Dawley rats weighing 100-250g in Isabela, Philippines. The rats were housed in polypropylene cages in a controlled room temperature and were fed with standard rodent diet and water as needed. Animals were acclimatized for one week before the beginning of experiment. The animals were fed on basal diet and were provided with water *ad libitum* during the experimental period (Kanna, 2014).

3.2 Induction of Nephrosis

Male Sprague-Dawley rats weighing 100-250 grams will be divided into six (6) groups each with 5 animals. Kidney damage was produced by the intraperitoneal administration of gentamicin (80mg/kg b.w.) with the help of 25 G needle for 5 days (Reddy et. al., 2015).

3.3. Experimental Procedure

After acclimatization, thirty male Sprague-Dawley rats was assigned to 6 groups of 5 animals each and was placed in separate cages. The animals were grouped as follows: receiving kidney care capsule orally was used as positive control and normal saline (1ml/kg body weight) intraperitoneal was used as negative control. The third group, received daily intraperitoneal injections of gentamicin (80mg/kg body weight) served as disease control. The animals in group four, five and six received 80mg/kg of gentamicin intraperitoneal and in addition also of the test drug, ethanolic extract of Kataka-taka (*Kalanchoe pinnata)* leaves intraperitoneally at doses 140mg/kg/day, 120mg/kg/day, 100mg/kg/day for 7 days (Kushwaha et. al., 2015). The gentamicin leaves an hour before the administration of the test drug, ethanolic extract of *Kalanchoe pinnata* for seven days. Blood samples were collected via tail tipping at the start of the experiment (before the administration of gentamicin, Day 0 and before the administration of treatments (Day 6) and immediately before the test animals were sacrificed (Day 13) through cardiac puncture. At the end of the experiments, the rats were sacrificed by exposure to ether and both kidneys will be removed. The kidneys from all the treatments were processed for histopathological examination (Dungca, 2016).

3.4 Blood sample collection and Assessment of Biochemical Parameters

Twenty-four hours after the last injection, the rats were anesthetized with ether and blood samples were collected by cardiac puncture. The blood sample collected, after a standing of 30 minutes, was centrifuged at 3000 rpm for 5 minutes (Srivastava et.al, 2016) and was then submitted to BEST DIAGNOSTICS Tuguegarao City, Cagayan for determination of serum creatinine and blood urea nitrogen as an indicator of kidney damage.

3.5 Histopathological Examination

The rats were sacrificed immediately. The kidney was examined on the 13th day, cut longitudinally into two halves and was preserved into 10% neutral formalin solution, embedded in paraffin and used for histopathological examination. Sections were taken using a microtome about 2µm thick, stained with hematoxylin and eosin and were examined under a microscope. Stained with hematoxylin and eosin and were examined under a microscope (Sule et.al, 2016). The renal sections were

examined for the extent of damage to glomeruli, tubules as well as glomerular congestion, cellular and hemorrhage.

Data Analysis

Data results were presented by paired t-test and comparison within groups which was done using Kruskal wallis test and Dunn's test was used to calculate the statistical significance among the different groups. A value of $p<0.05$ was considered statistically significant.

Ethical Consideration

The researchers asked a permission from the Associate Dean, Academic Dean, University of Research Ethics Board, Vice President for Academics, and the University President to conduct the research study. The researchers were given an ethical clearance no.117711.

Prior to the experimentation phase, the researchers requested a certificate of authentication of the plant and animal subjects that were utilized in the study. Authentication of the animal model was done by the Bureau of Animal Industry and authentication of plant model by the Bureau of Plant Industry.

The researchers asked permission from Philippine Institute of Traditional and Alternative Heath Care as regards to the housing of the animal models, in which prior cages and disposal of test subjects were taken into consideration to avoid contamination and spread of diseases.

The researchers were assisted by the Registered Veterinarian during the extraction of blood samples to the test subject taking into consideration the laws that are being implemented in properly utilizing rat models.

After the experiment, the rats used in this research were disposed according to the Disposal Procedures and Protocols of the Cagayan Valley Herbal Processing Plant PTAHC in Carig, Tuguegarao City, Cagayan.

RESULTS

All the end results of every experiment performed on the duration of this research endeavor are stated in this section.

Table 1. *Phytochemical Screening Result for Kataka-taka (Kalanchoe pinnata) Ethanolic Leaf Extract*

Constituent	Result
Alkaloids	(-)
Carbohydrates	(+)
Glycoside	(+)
Saponins	(+)
Phytosterol	(+)
Phenolic Compound	(+)
Flavonoids	(+)
Proteins	(+)

Legend: Presence (+), Absence (-)

Table 2.1. *Kidney Function (BUN and Serum Creatinine) of Test Subjects before and after Administration of the different Treatments*

Treatment	Before Administration of Treatments (Day 5)		After Administration of Treatments (Day 13)	
	Blood Urea Nitrogen	Serum Creatinine	Blood Urea Nitrogen	Serum Creatinine
Positive Control (Kidney care capsule)	16.010	91.592	10.192	68.198
Negative Control (1mL/kg NSS)	14.956	91.084	15.164	94.240
Treatment 1 (Gentamicin 80mg/kg/day)	14.230	91.594	17.640	94.594
Treatment 2 (Gentamicin 80mg/kg/day + ethanolic extract of Kataka-taka 140mg/kg/day)	14.550	29.950	10.664	63.380
Treatment 3 (Gentamicin 80mg/kg/day + ethanolic extract of Kataka-taka 120mg/kg/day)	14.744	89.412	11.434	70.610
Treatment 4 (Gentamicin	14.540	90.422	12.1380	72.614

80mg/kg/day + ethanolic extract of Kataka-taka 100mg/kg/day)				

The table above presents the levels of serum creatinine and Bun which are measures of kidney function. A decrease in both enzymes during the post treatment was observed in the positive in the positive control and in the treatments 2 to 4. An increase in the enzymes was observed in the negative control and in treatment 1 (Gentamicin only).

Table 2.2. *Result of Histopathological Examination of Subjects' Kidney Specimens after administration of different Treatments*

Treatments	Histopathologic Description
Positive Control (Kidney care capsule)	Minimal damage
Negative Control (1mL/kg NSS)	Hyper cellular, dilated blood vessel filled with numerous RBC's , glomerular necrosis, hemorrhage, kidney parenchymal, edematous
Treatment 1 (Gentamicin 80mg/kg/day)	Extensive necrosis, severe glomerular congestion
Treatment 2 (Gentamicin 80mg/kg/day + ethanolic extract of Kataka-taka 140mg/kg/day)	Cellular with moderate glomerular congestion showing presence of numerous RBC's in edematous tubules.
Treatment 3 (Gentamicin 80mg/kg/day + ethanolic extract of Kataka-taka 120mg/kg/day)	Minimal glomerular congestion filled with few RBC's and lesser edematous tubules
Treatment 4 (Gentamicin 80mg/kg/day + ethanolic extract of Kataka-taka 100mg/kg/day)	Hyper cellular with severe congestion showing presence of numerous RBC's and severe damage on tubular structure (edematous) was seen.

The table above shows the results of the histopathological examination done to the kidney specimens of the subjects. The examination further concluded that the Kataka-taka at dose 120 mg/kg/day plus gentamicin shows minimal kidney damage as compared to the other treatments except the positive control. Furthermore, the dose 120mg/kg/day has comparable nephroprotective activity with the positive control.

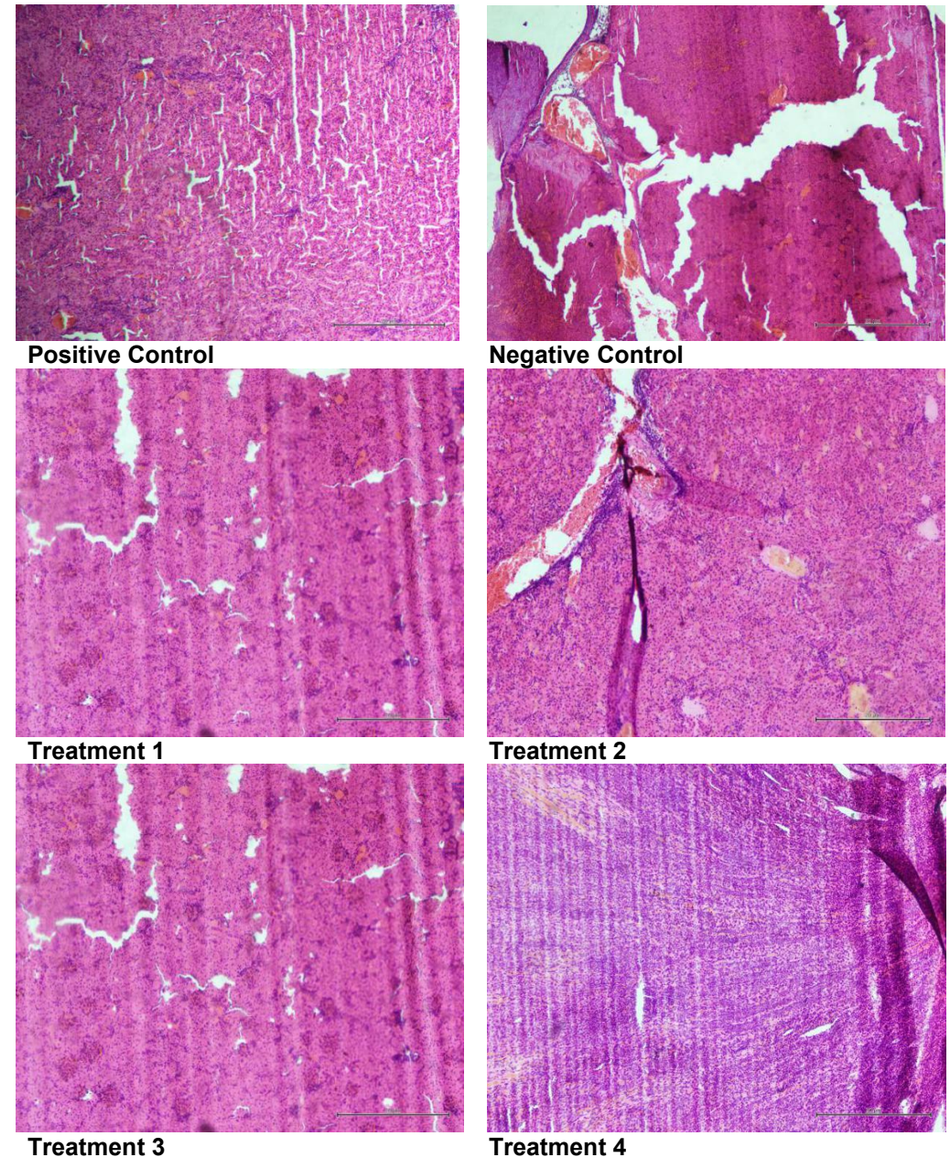

Figure 3. *Microscopic View of the Histopathological Characteristics of the Kidney Specimen of Test Subjects after Administration of Different Treatments*

Table 3. *Test of Significant Difference in the Kidney Function of Test Subjects Before and After Administration of the Different Treatments*

Kidney Function Test	Treatment	t-value	p-value	Decision
Blood Urea Nitrogen	Positive Control	18.646	.000	Reject Ho
	Negative Control	-1.037	.358	Accept Ho
	Treatment 1	-5.226	.006	Reject Ho
	Treatment 2	7.436	.002	Reject Ho
	Treatment 3	3.576	.023	Reject Ho
	Treatment 4	5.319	.006	Reject Ho
Serum Creatinine	Positive Control	3.087	.037	Reject Ho
	Negative Control	-.783	.477	Accept Ho
	Treatment 1	-3.662	.002	Reject Ho
	Treatment 2	4.317	.012	Reject Ho
	Treatment 3	4.267	.013	Reject Ho
	Treatment 4	5.911	.004	Reject Ho

The table shows significant decrease in kidney enzyme levels from the positive control, treatment 2, 3 and 4 (Gentamicin with different doses of Kataka-taka extract) which signifies a nephroprotective effect. A significant increase was also observed in the subjects treated with Gentamicin only (Treatment 2) signifying the nephrotoxic effect of the drug.

Table 4.1. *Test of Significant Difference in the Kidney Function of Test Subjects according to Treatments Received*

Kidney Function Test	F-value	p-value	Decision
Blood Urea Nitrogen	12.509	.000	Reject Ho
Serum Creatinine	7.290	.000	Reject Ho

The table shows significant difference in the BUN and creatinine levels of the rats subjected to the different treatments.

Table 4.2. *Multiple Comparisons of the Difference in BUN of the different Treatments*

	Positive Control	Negative Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Positive Control						
Negative Control	.000*					
Treatment 1	.000*	.045*				
Treatment 2	.690	.001*	.000*			
Treatment 3	.298	.004*	.000*	.516		
Treatment 4	.109	.016*	.000*	.219	.552	

It can be gleaned from the table above that all treatments with Kataka-taka extract exhibited significantly the same nephroprotective effect in terms of decreasing BUN levels as the positive control. Moreover, no significant difference was seen in the effect of the different doses of Kataka-taka extract.

Table 4.2. *Multiple Comparisons of the Difference in Serum Creatinine of the different Treatments*

	Positive Control	Negative Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Positive Control						
Negative Control	.011*					
Treatment 1	.000*	.071				
Treatment 2	.901	.014*	.000*			
Treatment 3	.800	.019*	.000*	.897		
Treatment 4	.643	.031*	.000*	.734	.833	

It can be gleaned from the table above that all treatments with Kataka-taka extract exhibited significantly the same nephroprotective effect in terms of

decreasing serum creatinine levels as the positive control. Moreover, no significant difference was seen in the effect of the different doses of Kataka-taka extract.

DISCUSSION

The research study determined the nephroprotective activity of Kataka-taka (Kalanchoe pinnata) ethanolic leaf extract in gentamicin-induced nephrotoxicity in male Sprague-Dawley rats. For the determination of its nephroprotective activity, an animal model was used and Sprague-Dawley rats weighing 100-250 grams were selected. Gentamicin induced causing nephrotoxicity is often used for the evaluation of nephroprotective activity of a drug (Kushwaha, et,al, 2016) and male Sprague-Dawley rats were mostly used subject animal for gentamicin-induced nephrotoxicity (Gautier et.al., 2014).

Based on the phytochemical test conducted by the analyst of Saint Louis University Baguio, the result had shown that carbohydrates, glycosides, saponins, phytosterol, phenolic compound, flavonoids and proteins were present while alkaloids and antraquinone glycosides were absent. Konda et. al (2014), the possible mechanism for the nephroprotective activity of a plant could be due to the presence of antioxidants. Paari et. al. (2014), suggest that Kataka-taka possess antioxidant activity such as flavonoids, glycosides, triterpenes and phenolic compounds which play an important role in protection of kidney from damage. It was also reported from the study of Mathew et. al, (2013) that Kataka-taka possess significant antioxidant potential. Thus, the findings suggest that the phytochemical present in the ethanolic leaf extract of Kataka-taka (*Kalanchoe pinnata)* contributed to the nephroprotective actions against gentamicin-induced nephrotoxicity.

Gentamicin has shown an induced nephrotoxicity when administered for more than 5-10 days (Nale et. al. 2012). Blood Urea Nitrogen and serum creatinine were used as a parameter in this study. Poblete et. al. (2017), noted that the biochemical parameters were limited to serum creatinine and blood urea nitrogen since these are most accessible. Result of this study showed that gentamicin at dose 80mg/kg produces significantly nephrotoxic as evidenced by the increase in blood urea nitrogen with a p-value of 0.002187 and serum creatinine with a p-value 0.001778 from Day 0 to Day 5. Increased levels of BUN and serum creatinine indicate kidney damage (Duggal, 2018). With the result, this further means that there is kidney damage on the fifth day of the experiment.

Based on the histopathological examination, positive control showed minimal kidney damage whereas normal saline and gentamicin alone showed severe damage to the kidney structure as seen in Figures 5 and 6 respectively, which correlate with the previous study of Srivastava et. al (2018). Co-administration of Kataka-taka at dosage of 120mg/kg plus gentamicin showed

significant decrease in glumerular congestion with few RBC's and lesser edematous tubules, Figure 7 indicates the nephroprotective action of *K. pinnata* leaves against getamicin-induced nephrotoxicity. On the other hand, the dosage at 100mg/kg/day had no nephroprotective activity as compared to 120mg/kg/day dose ethanolic leaf extract of Kataka-taka leaves since histopathological result showed hyper cellular with severe congestion showing presence of numerous RBC's and severe damage on tubular structure (edematous) at 100mg/kg. In the previous study of Kataka-taka against gentamicin-induced nephrotoxicity, it was observed that the *K. pinnata* leaves at dose 125mg/kg significantly protects rat kidney from gentamicin-induced histopathological changes and it also normalizes the increase of Blood Urea Nitrogen and serum creatinine (Harlalka et.al., 2007).

CONCLUSION

The researchers conclude that statistically, Kataka-taka ethanolic leaf extract in all doses within the scope of this research study possess nephroprotective characteristics without significant difference. However, histopathological result confirmed that 120mg/kg dosage is considered the most effective compared to 140mg/kg and 100mg/kg.

RECOMMENDATIONS

In line with this research study, the following recommendations are given:

1. Semi-purification should further be performed in order to determine the specific phytochemical constituent/s of Kataka-taka (*Kalanchoe pinnata*) that have nephroprotective characteristics.
2. Preformulation should be conducted using 120mg/kg dosage since it was already proven by this research study to be effective in protecting the kidney.

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