ORIGINAL ARTICLE

Effects of Aqueous and Methanolic Extracts of Cichorium Intybus Seeds on Gentamicin Induced Nephrotoxicity in Rats

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ABSTRACT

Objective: To evaluate the effects of Aqueous and Methanolic extracts of Cichorium Intybus seeds on Gentamicin induced nephrotoxicity in rats.

Study Design: Experimental Study

Place and Duration of Study: Study was conducted from 15 January to 31 March 2017 at National Institute of Health Sciences (NIH) in collaboration with Riphah Institute of Pharmaceutical Sciences (RIPS).

Materials and Methods: Forty healthy Sprague Dawly rats weighing 300-350gms were randomly divided in four groups with 10 rats each. Group A was the control group that received no medications. Group B was given Gentamicin 80mg/ kg body weight / day for 10 days intraperitoneally (IP). Group C was given Gentamicin 80mg/ kg body weight / day IP and Aqueous Extract of Cichorium Intybus seeds 500mg/kg body weight/ day through gavage tube for 10 days. Group D was given Gentamicin 80mg/ kg body weight / day IP and Methanolic Extract of Cichorium Intybus seeds 500mg/kg body weight / day through gavage tube for 10 days. Serum urea and creatinine were measured at day zero, 6, and 11. The values were compared within and between the groups.

Results: Elevated levels of serum urea and creatinine were found in group B indicating renal damage. Whereas administration of Aqueous and Methanolic extracts of Cichorium Intybus seeds reduced the elevated levels of serum urea and creatinine in group C & D. However more reduction was produced in group C.

Conclusion: Concomitant administration of Aqueous and Methanolic extracts of Cichorium Intybus seeds exerted nephroprotective effect on Gentamicin induced nephrotoxicity in rats. Aqueous extract produced greater protection as compared to Methanolic extract.

Key Words: *Cichorium Intybus Seeds, Gentamicin, Nephroprotective effect, Nephrotoxicity.*

Introduction

Kidneys play a vital role in the body performing important functions for example excretion of metabolic waste, drugs, homeostasis maintenance and extracellular environment regulation.[1] Direct or indirect exposure of drugs and different chemicals to kidneys results in nephrotoxicity. Many therapeutically used drugs may result in acute or chronic renal failure.[2] Across the globe approximately 19-25% cases of nephrotoxicity in critically ill patients results from nephrotoxic drugs.[1] Nephrotoxicity is indicated by the raised levels of serum urea and creatinine.[3] Drug groups which cause nephrotoxicity include immunosuppressant, anticancer drugs, radio contrast media and antibiotics.[4]

Aminoglycosides (AGs) is among one the widely prescribed antibiotic group throughout the world being cost effective.[4] Aminoglycosides are bactericidal antibiotics which irreversibly inhibit protein synthesis by binding to specific 30S-subunit ribosomal protein through interference of initiation of complex of peptide, breaking of polysome into monosomes and misreading of mRNA.[5] The major adverse effects caused by aminoglycosides are ototoxicity and nephrotoxicity, which limits their use. Aminoglycosides are excreted unchanged through glomerular filtration without metabolism.[7] During the process of concentration and reabsorption

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Funding Source: NIL; Conflict of Interest: NIL
Received: July 04, 2018; Revised: October 23, 2018;
Accepted: November 11, 2018

proximal tubular cells are mainly exposed to damage by the drugs. Free radicals generated due to oxidative stress causes mitochondrial damage in tubules resulting in cytotoxicity. Inflammation in glomerulus and tubules is induced by nephrotoxic drugs which ultimately result in fibroses of renal tissue.[8]

From ancient days medicinal plants have been widely used as a source of medication providing a cheap source of treatment for a majority of world's population. Herbal plants have got nephroprotective effect.[9] Several plants along with their extracts have got protective effect against nephrotoxic drugs due to their anti-oxidant, anti-inflammatory and diuretic properties.[10] Cichorium intybus , generally called chicory (Kasni) is vertical woody perennial plant distributed in Europe, Mediterranean region, Northern Asia and Pakistan.[11] All parts of Cichorium intybus is known to have antimicrobial, gastroprotective, hepatoprotective, anti-diabetic, antimalarial, anti-inflammatory and anti-oxidant effects due to the presence of phytocompounds like polyphenols , alkaloids, flavonoids and tannins.[12,13]

Previous studies have shown anti-oxidant effects of either Aqeous or methanolic extracts of Cichorium intybus roots, leaves and stem against gentamicin induced nephrotoxicity or hepatotoxicity in animal model.[10] Evaluation of both aqueous and methanolic extracts of Cichorium intybus seeds against gentamicin induced nephrotoxicity in literature is lacking. Present study was designed to evaluate the effect of both aqueous and methanolic extracts of Cichoriumintybus seeds against gentamicin induced nephrotoxicity in rat's model.

Materials and Methods

This experimental randomized control trial was conducted from 15th January 2017 to 31st March 2017 in the department of Pharmacology and Therapeutics, Islamic International Medical College, Rawalpindi in collaboration with the Riphah Institute of Pharmaceutical Sciences and Animal house at National Institute of Health (NIH), Islamabad. Forty healthy adult male Sprague Dawly rats were included through simple random sampling and the study was conducted after approval from Ethical Review Committee of Islamic International Medical College, Riphah International University (RIU), Islamabad.

Rats weighing between 300-350 grams with normal Serum urea and Creatinine levels were included in the study. Rats were first allowed to get acclimatized for one week in the NIH Animal house in 50-70% humidity at a room temperature of 24$\pm$2°C with a 12 hour light and dark cycle. [14] Rats were randomly divided into four groups of ten rats each (n=10). Group A was normal control, given diet and water ad libitum for ten days. Group B was administered Gentamicin at 80 mg/kg intraperitoneally once daily for ten days. Group C was administered Gentamicin 80mg/kg intraperitoneally along with Aqueous extract of Cichorium intybus seeds orally mixed in 2ml distal water through gavage tube at a dose of 500mg/kg once daily for ten days.[10] Group D was administered Gentamicin 80mg/kg body weight once daily intraperitoneally along with Methanolic extract 500mg/kg orally mixed in 2ml distilled water through gavage tube once daily for ten days.[15]

Dried seeds of Cichorium intybus were purchased and authenticated from herbarium department of National Agriculture and Research Centre (NARC) Islamabad. Aqueous extract of Cichoriumintybus seeds was prepared at RIPS, Islamabad by using fine homogenized powder of chicory seeds which were mixed with distilled water, the whole solution was boiled for 2 hours and after cooling was filtered through filter paper what man no 3. The aqueous extract was formed by using vacuum rotary evaporator and was frozen dried.[16] To obtain the methanolic extract, 1 kg of Cichorium Intybus seeds powder was soaked in 8 L of methanol (95%) for 24 h. The extract was filtered using what man paper No 3 and the filtrate was evaporated (78 °C) to dryness using a rotary evaporator; 25.64 g of dried methanolic extract were obtained giving an extraction yield of 2.56% (w/w based on the dried starting weight).[17] Sampling at day 0 and day 6 were done via lateral tail vein. Sampling at day 11th was done through cardiac puncture. Biochemical analysis of serum urea and serum creatinine was estimated through commercially available kits by Merk and auto analyzer Micro lab 300 on photometric system.

Statistical analysis of collected parametric data was done by using SPSS version 21 and Mean ± Standard Error of Mean was calculated. One way ANOVA and Post Hoc Tuckey tests were applied to compare the mean difference between control and rest of the groups and mean difference in between the groups.

p value of <0.05 was considered statistically significant.

Results

Mean initial Serum Urea for Group A (Normal Control Group) was 23.70 ± 1.521 mg/dl. Mean initial Serum Urea for Group B (Disease Control Group) was 24.00 ± 1.732 mg/dl. Mean initial Serum Urea for Group C (Aqueous extract Experimental group 1) was 25.56 ± 1.744 mg/dL. Mean initial Serum Urea for Group D (Methanolic extract Experimental group 2) was 22.10 ± 1.912 mg/dl. There was no significant difference in the serum urea levels among these groups on day 0 (p Value .589). Serum Urea on day 6 for Group A (Normal Control Group) was 24.70 ± 1.633 mg/dl, for Group B (Disease Control Group) was 83.30 ± 2.119 mg/dl, for Group C (Aqueous extract Experimental group 1) was 67.10 ± 1.703 mg/dl& for Group D (Methanolic extract Experimental group 2) was 84.00 ± 1.592 mg/dl. There was significant difference in the serum urea levels among these groups on day 6 (p Value .000). This suggests that nephrotoxicity was induced by Gentamicin.

Mean Serum Urea on day 11 for Group A (Normal Control Group) was 24.70 ± 5.165 mg/dl , for Group B (Disease Control Group) was 97.90 ±4.332 mg/dl, for Group C (Aqueous extract Experimental group 1) was 51.30 5.755 mg/dl& for Group D (Methanolic extract Experimental group 2) was 73.60mg/dl ±4.695. There was significant difference in the values of group C & D (p Value .000). This suggests that nephrotoxicity in Group C (Aqueous Extract Treated Group) was improved more than Group D (Methanolic Extract Treated Group).

Mean initial Serum Creatinine for Group A (Normal Control Group) was 0.750 ± 0.0654 mg/dl. Mean initial Serum Creatinine for Group B (Disease Control Group) was 0.740 ± 0.0562 mg/dl. Mean initial Serum Creatinine for Group C (Aqueous extract Experimental group 1) was 0.770 ± 0.0559 mg/dL. Mean initial Serum Creatinine for Group D (Methanolic extract Experimental group 2) was 0.770 ± 0.0667 mg/dL. There was no significant difference in the serum Creatinine levels among these groups on day 0. (p Value .980).

Mean Serum Creatinine on day 6 for Group A (Normal Control Group) was 0.750 ± 0.0619 mg/dL, for Group B (Disease Control Group) was 2.490 ± 0.0849 mg/dl, for Group C (Aqueous extract Experimental group 1) was 1.450 ± 0.0601 mg/dl& for Group D (Methanolic extract Experimental group 2) was 1.730 ± 0.0517 mg/dl. There was significant difference in the serum Creatinine levels among these groups on day 6 (p Value .000). This suggests that nephrotoxicity was induced by Gentamicin.

Mean Serum Creatinine on day 11 for Group A (Normal Control Group) was 0.750, ± 0.0619 mg/dl, for Group B (Disease Control Group) was 2.490, ± 0.0849 mg/dl, for Group C (Aqueous extract Experimental group 1) was 1.450 0.0601 mg/dl& for Group D (Methanolic extract Experimental group 2) was 1.730 ± 0.0517 mg/dl. There was significant difference in the values of group C & D (p Value .024). This suggests that nephrotoxicity in Group C (Aqueous Extract Treated Group) was improved more than Group D (Methanolic Extract Treated Group).

Table I: Comparison of Serum Urea and Creatinine Levels Among Groups By ANOVA at Day 0

		Sum of Squares	df	Mean Square	F	Sig.
Serum Urea	Between Groups	58.275	3	19.425	.648	.589
	Within Groups	1079.500	36	29.986		
	Total	1137.775	39			
Serum Creatinine	Between Groups	.007	3	.002	.060	.980
	Within Groups	1.351	36	.038		
	Total	1.358	39			

Table II: Comparison of Serum Urea and Creatinine Levels Among Groups By ANOVA at Day 06

		Sum of Squares	Df	Mean Square	F	Sig.
Serum Urea	Between Groups	23241.875	3	7747.292	246.141	.000
	Within Groups	1133.100	36	31.475		
	Total	24374.975	39			
Serum Creatinine	Between Groups	10.714	3	3.571	116.668	.000
	Within Groups	1.102	36	.031		
	Total	11.816	39			

Table III: Comparison of Serum Urea and Creatinine Levels Among Groups By ANOVA at Day 11

		Sum of Squares	Df	Mean Square	F	Sig.
Serum Urea	Between Groups	29290.875	3	9763.625	388.173	.000
	Within Groups	905.500	36	25.153		
	Total	30196.375	39			
Serum Creatinine	Between Groups	15.539	3	5.180	119.531	.000
	Within Groups	1.560	36	.043		
	Total	17.099	39			

Table IV: Multiple Comparison of Serum Urea and Creatinine levels Among all Groups By Post Hock Tuckey Test

Parameter	Groups	Mean difference	P Value
Serum Urea	Group A (control group) Vs Group B (Disease Control Group)	-73.200(*)	.000
	Group A (control group) Vs Group C (Experimental group 1)	-26.600(*)	.000
	Group A (control group) Vs Group D (Experimental group 2)	-48.900(*)	.000
	Group B (Disease Control Group) Vs Group C (Experimental group 1)	46.600(*)	.000
	Group B (Disease Control Group) Vs Group D (Experimental group 2)	24.300(*)	.000
	Group C (Experimental group 1) Vs Group D (Experimental group 2)	-22.300(*)	.000
Serum Creatinine	Group A (control group) Vs Group B (Disease Control Group)	-1.7400(*)	.000
	Group A (control group) Vs Group C (Experimental group 1)	-.7000(*)	.000
	Group A (control group) Vs Group D (Experimental group 2)	-.9800(*)	.000
	Group B (Disease Control Group) Vs Group C (Experimental group 1)	1.0400(*)	.000
	Group B (Disease Control Group) Vs Group D (Experimental group 2)	.7600(*)	.000
	Group C (Experimental group 1) Vs Group D (Experimental group 2)	-.2800(*)	.024

Discussion

Gentamicin is widely used to induce nephrotoxicity in animal models for research purposes and has been stated by numerous researchers for analyzing nephroprotective effects of natural plants. Key phenomenon elaborating the GM induced nephrotoxicity is damage by reactive oxygen species.[18] Review of literature showed that production of ROS lead to lipid peroxidation causing necrosis and denaturation of proteins. Various studies have concluded that the nephroprotective effects of natural plants are due to the presence of antioxidants.[19,20]

The current study was designed to investigate the protective effects of Aqueous and Methanolic extracts of Cichorium Intybus seeds in Gentamicin induced nephrotoxicity in rats via biochemical parameters. It was found that Gentamicin induced nephrotoxic changes were improved by both Aqueous and Methanolic extracts of Cichorium Intybus seeds but aqueous extract showed better nephroprotective effects against Gentamicin induced nephrotoxicity. In present study, GM induced nephrotoxicity is indicated by raised level of serum creatinine and urea as concluded previously by Nitha and Janardhanan.[21] The process glomerular filtration causes the accumulation of gentamycin in the proximal tubules of kidney. GM Produced ROS causes oxidative stress and tubular injury and is indicated by raised serum creatinine and urea.

Our study in accordance with findings of study by Tanweer khaliq who induced Nephrotoxicity in Albino rabbits with Gentamicin Intraperitoneally in a dose of 80 mg/kg and observed raised levels of biochemical markers.[10] Similarly our study is in correlation with another study performed by V. Chinnapa Reddy showing the nephrotoxic effects of Gentamicin in Rats which were indicated by raised levels of serum urea and creatinine.[16]

Current study is consistent with the study of Tanweer Khaliq who compared the nephroprotective effect of aqueous extract of Cichorium Intybus seeds against Gentamicin induced nephrotoxicity in which he used aqueous extract of Cichorium Intybus seeds in doses of 250 mg/kg administered via gavage tube. His study also evidenced the nephroprotection due to presumed antioxidant properties of Cichorium Intybus seeds.[10]

Review of literature shows that studies have been done on exploring nephroprotective effect of Cichorium intybus in combination with other herbal extracts and medical compounds like silymarin.[10] No individual study was done on aqueous and methanolic extract of Cichorium intybus seed indicating dosage use regarding submaximal, ceiling effect and toxicity. Current study shows the nephroprotective effect of both aqueous and methanolic extract of Cichorium intybus seeds. Further studies are needed to elaborate the mechanism of higher response of methanolic extract as compare to aqueous extract of Cichorium intybus seeds for reducing elevated renal markers. In addition administration through different routes and higher dosage can be tried to see the effect.

Conclusion

Aqueous and Methanolic extracts of Cichorium Intybus seeds exhibited protective effects against Gentamicin induced nephrotoxicity in rats. Aqueous extract Cichorium intybus seeds have exerted greater nephroprotective effect in comparison to Methanolic extract.

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