Published in the Slovak Republic
European Journal of Molecular Biotechnology
Has been issued since 2013.
E-ISSN: 2409-1332
2021. 9(1): 31-36

DOI: 10.13187/ejmb.2021.1.31
www.ejournal8.com

Evaluation of Protective Potential of Ethyl Acetate Extract of Cocus Nucifera in Gentamycin Induced Nephrotoxicity in Albino Mice

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Abstract

Renal disorders have always remained a major area of concern for physicians since a long time and most are of these are drug induced. Antibiotic are used to treat infections caused by organisms that are sensitive to them, e.g gentamycin which is an aminoglycosides which are ototoxic and nephrotoxic. This study looks at the Nephroprotective activity of ethyl acetate extract of husk fibre of Cocus nucifera for its protective effects on gentamicin-induced nephrotoxicity in albino mice. For studying acute toxicity study, the study groups contained eight rats in each group and oral dosage of 100, 50, 25 mg ethyl acetate husk fibre of Cocus nucifera extract/kg body weights was administered to albino mice. Nephrotoxicity was induced in albino mice by daily intraperetoneal administration of gentamicin 45 mg/kg/day for 10 days. Effect of concurrent administration of ethyl acetate extract of Cocus nucifera at a dose of 100, 50 and 25 mg/kg/day given by oral route for 17 days. The biochemical parameter such as serum electrolytes as indicators of kidney damage was determined by using one way ANOVA the results are significant at P>005. The result shows that at higher concentration of the extract, kidney damage was not seen while lower concentration it was seen.

Keywords: nephrotoxicity, Cocus nucifera, gentamycin, Kidney, Protective, Wistar albino mice, polyphenols, husk fibre, Coconut and ethyl acetate.

1. Introduction

Nephrotoxicity is one of the most common kidney problems and occurs when body is exposed to a drug or toxin. When kidney damage occurs, the body unable to rid of excess urine and wastes from the body and blood electrolytes (such as potassium and magnesium) will all become elevated and Sodium will reduce. A number of therapeutic agents can adversely affect the kidney resulting in acute renal failure, chronic intestinal nephritis and nephritic syndrome because increasing number of potent therapeutic drugs like aminoglycoside antibiotics, chemotherapeutic agents and NSAIDs have been added to the therapeutic arsenal in recent years. Exposure to chemical reagents like ethylene glycol, carbon tetra chloride, sodium oxalate and heavy metals like lead, mercury, arsenic and cadmium also induces nephrotoxicity (Ramya, 2011).

Many plants have been used for the treatment of kidney failure in traditional system of medicine throughout the world. Indeed along with the dietary measures, plant preparation formed the basis of the treatment of the disease until the introduction of allopathic medicine. Traditional knowledge will serve as a powerful search engine and most importantly, will greatly facilitate intentional, focused and safe natural products research to rediscover the drug discovery process.

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Therefore, search of nephroprotective herbs from medicinal plants has become important and need of the day (Bharti et al., 2012).

Nephroprotective agents are the substances which possess protective activity against nephrotoxicity. Medicinal plants have curative properties due to the presence of various complex chemical substances. Ancient literature has prescribed various herbs for the cure of kidney disease (Chan, Elevitch, 2006).

Coconut tree has been eulogised as 'Kalpavriksha' (the all giving tree) in Indian classics for is all round usefulness (Adebayo et al., 2013), Husk fibre of a coconut tress has been reported to have antibacterial, antifungal, antiviral, antiparasitic, antidermatophytic, antioxidant, hypoglycemic, hepatoprotective, immunostimulant, antiblenorrhagic, antibronchitis, febrifugal, and antigingivitic properties (Adebayo et al., 2013), and antimalarial activity (Terri, Sesin, 1958). Very few studies were reported in literature regarding histopathology of gentamicin induced renal failure in Albino mice. So, the present study is taken up to see the effect of ethyl acetate extract of *cocus nicifera, it nephroprectective properties* by investigating the biochemical and histopathological changes of mice.

2. Materials and methods

Wistar albino mice weighing 20-26 g, are utilized for the present study. Experiments were performed with the permission of the institutional ethics committee. In the present study, albino mice were used and are grouped as follows:

Group A: (Control Positive): Administered appropriate volume of 5 % DMSO solution.

Group B: (Control Negative): Administered appropriate volume of 5 % DMSO solution + 200µl Gentamycin.

Group C: Administered 100mg/Kg body weight of Cocus nucifera extract fraction + 200 µl Gentamycin.

Group D: Administered 50mg/Kg body weight of Cocus nucifera extract fraction + 200 µl Gentamycin.

Group E: Administered 25 mg/Kg body weight of Cocus nucifera extract fraction + 200 µl Gentamycin.

Method

All rats were kept under observation for 2week prior to the experiments to acclimatize with environment. All animals were fed standard rat chow and were provided tap water to drink *ad libitum*. They were housed in a facility with 12-12 h light-dark cycle that is maintained at 25°C. All animals were weighed before the injections. The animals were anaesthetized with diethyl ether inhalation. Blood samples were collected with cardiac puncture for biochemical investigations (serum) Na, K, Cl and HCO_3 were determine, the effect of body ratio and the Histological analysis

Administration of the sample: daily intraperitonial injection of gentamycin was given to each group for 10 days and the daily oral administration of ethyl acetate extract of Cocus nucifera are given to each group for 17 days.

Collection of Blood Sample: At the end of the 17-day experimental period, the Mice were sacrificed by slight diethyl ether anaesthesia, the neck area was quickly cleared of fur and the jugular veins exposed, from which blood was collected into EDTA bottle to prevent clotting. The EDTA blood sample was centrifuged at 3000 rpm for 10 minutes and the serum pipetted out. This was stored frozen at -20°C until needed for analysis.

Determination of serum electrolytes:-Sodium (Na) and Potassium (K) analysis were carried out using Randox Laboratory kit according to the method of Terri et al 1958 (Garetz, Schacht, 1996). Serum calcium was determined colorimetrically using commercial kits (Erba, Germany) according to the method of Moorehead W R et al 1974 (Baliga et al., 1997).

3. Results

Effect of polyphenols of Ethyl Acetate Extract of *Cocos nucifera* Husk Fibre on kidney/Body Weight Ratio

Gentamicin caused a significant increase ($p < 0.05$) in kidney/body weight ratio compared to control (Table 1). The administration of polyphenols of ethyl acetate extract of *Cocos nucifera* husk fibre (WAT) at various doses investigated in this study was not able to reverse the significant increase ($p < 0.05$) in kidney/body weight ratio caused by gentamicin (Table 1).

Table 1. Effect of polyphenols of *Cocos nucifera* Husk Fibre (WAT) on kidney-Body Weight Ratio of mice with gentamicin-induced kidney damage

Treatment (mg/Kg body weight)	Organ Body Weight (%)
Control (5 % DMSO)	0.98±.016[a]
Gentamicin	1.34±0.08[b]
Gentamicin + 25mg/kg body weight of polyphenols	1.15±0.09[ab]
Gentamicin + 50 mg/kg body weight of polyphenols	1.27±0.06[b]
Gentamicin + 100mg/kg body weight of polyphenols	1.33±0.07[b]

Values are expressed as mean of 5 replicates ± SEM. Values with the same superscript are not significantly different at $p < 0.05$.

Table 2. Effect of polyphenols extract of *Cocos nucifera* husk fibre (WAT) on some selected electrolytes of Gentamycin induced renal impairment in Mice

Group	Na^+(Meq/L)	K^+(Meq/L)	Cl^-	HCO_3
Control (5 % DMSO)	156.08±4.42[a]	4.50±0.33[a]	91.25±2.5[b]	26.15±1.62[a]
Gentamycin	147.85±2.64[b]	7.50±0.32[b]	82.00±6.58[b]	23.02±2.33[a]
Gentamycin + 25mg/kg body weight of extract	151.05±0.78[a]	5.37±0.21[a]	78.00±5.59[a]	24.45±1.67[a]
Gentamycin + 50mg/kg body weight of extract	147.95±4.09[a]	5.40±0.23[a]	90.00±3.92[b]	22.00±1.22[a]
Gentamycin + 100mg/kg body weight of extract	153.25±5.47[a]	5.68±0.32[a]	95.00±3.74[b]	20.95±1.34[a]

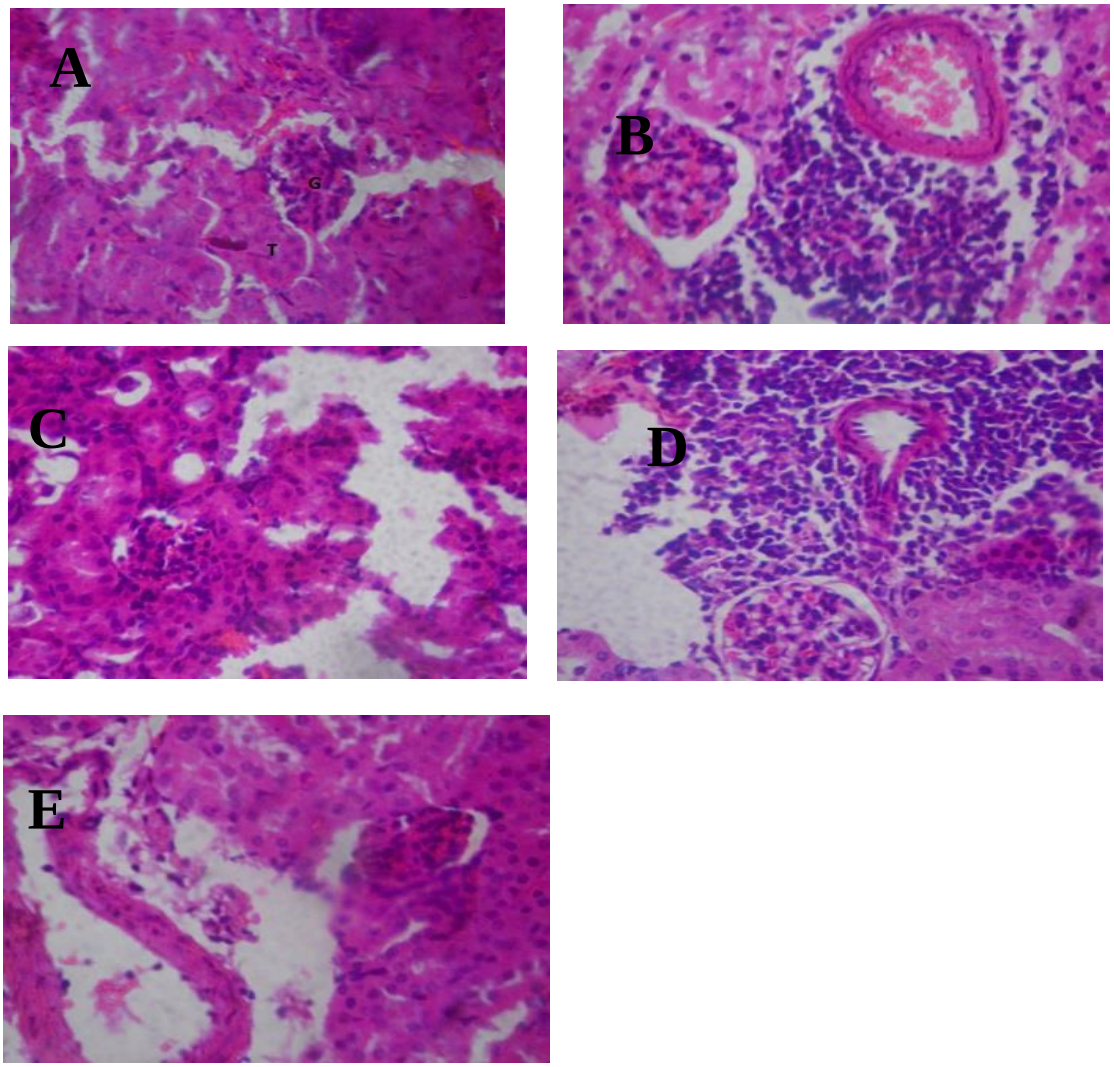
Data are mean ±SEM of five determinations. Values with the same superscript are not significantly different at $p < 0.05$

Protective Effect of polyphenols extract of *Cocos nucifera* husk fibre (WAT) on Histology of kidney of Gentamycin induced renal impairment Mice

Histological analysis revealed that the histo-architectural changes of the kidneys of all the treated animals and the glomerulus Corpuscles reveals the obliteration of the bowman space by the gentamycin (Figure 1).

4. Discussion

The incidence of renal dysfunction following amino-glycoside administration was detected by many workers Garetz and Schacht, 1996; Baliga et al., 1997 and Abdel Naim et al., 1999 (Abdel-Naim et al., 1999; Heibashy et al., 2009; Baliga et al., 1997). The administration of gentamycin into mice induced impairment of renal function through liberation of oxygen free radical (Heibashy, Abdel Moneim, 1999 and Heibashy et al., 2009). Renal failure is characterized by disorders in some biochemical parameters and Kidney function indices. It can be seen that gentamycin produce Nephrotoxicity in the mice by seen the increase in Potassium and lowering the Sodium. Renal function tests are required either to demonstrate the presence or absence of active lesion in the kidney, or to assess the normal functioning capacity of different parts of nephron (Panda, 1989).



Representative photomicrographs of the kidney of albino mice, following administration of varied dosages of polyphenols of *Cocos nucifera* Husk fibre (H&E × 100). A served as control and received DMSO. B received polyphenols and gentamicin while C, D, and E were the experimental groups which received 25 mg/kg, 50 and 100 mg/kg body weights of polyphenols for 17 days and gentamicin for 7 days

Fig. 1. Histological observation of the kidney

Organ-body weight ratios are normally investigated to determine whether the size of the organ has changed in relation to the weight of the whole animal and high Organ to body weight ratio has been associated with inflammation while otherwise is constriction (Ali et al., 2003). The increase in kidney/body weight ratio caused by gentamicin (an aminoglycoside) in this study suggests inflammation. Aminoglycosides have been reported to cause nephrotoxicity through oxidative stress and forming free radicals (Goto, 2004). However, the polyphenols at all doses used in this study were not able to prevent the observed inflammation in the kidney, suggesting that doses used ere not sufficient enough to mop up the free radicals generated by gentamicin.

These results confirmed that gentamicin produced nephrotoxicity as previously reported by Ali et al., 2003, Goto, 2004 and Heibashy et al., 2009. Serum electrolytes were disturbed in gentamycin treated mice as compared with control animals and the ethyl acetate of Cocus nucifera extract was seen to be able to attenuate this trend by trying to correct the damages done. Those group that receive 100 and 50 mg/kg b.wt of the extract are found to be doing well and the

effectiveness of the extract was seen while those that receive the 25 mg/kg b.wt, the renal function was seen to be serious in them.

Lower value of serum sodium indicated inability of kidney to conserve sodium and chloride. Haemodilution too may be involved in the fall of sodium value via excess of water intake and or increased production of endogenous water. Increase of Potassium may be due to reduced excretion of K aggravated by leakage of intracellular potassium into blood stream as a result of gentamicin induced lesions in renal tubular epithelium. The present results are in harmony with the data obtained by Heibashy, Abdel Moneim, 1999 and Heibashy et al., 2009.

Apoptosis plays a major role in kidney embryogenesis, resulting in large-scale cell death during development. By contrast, in the adult and under normal circumstances, evidence of apoptosis is seldom found in the kidney, where the rate of cell turnover is very low. However, there are a number of documented cases related to kidney insult in both pathology and toxicology where the renal tissue, in particular the tubular epithelium, exhibits a substantial increase of apoptotic cells (Conaldi et al., 1998).

These results confirmed that gentamicin produced nephrotoxicity as previously reported by Ali et al., 2003, Goto, 2004 and Heibashy et al., 2009. These changes reflected the severity of renal insufficiency which occurred in association with the sudden fall in glomerular filtration rate because of the majority of administrated gentamicin enters specifically the proximal tubular epithelial cells, binds to anionic phospholipids in the target cells inducing abnormalities in the function and metabolism of multiple intracellular membranes and organelles then developed injury in the proximal tubular epithelial cells of kidney that caused acute renal failure. More than half of proximal tubules showing desquamation of necrosis but involved tubules easily found, complete or almost complete tubular necrosis. The polyphenols administered were able to prevent the observed architeratural structure of the kidney at dose dependant manner though perivascular inflammations were seen in group B and D.

5. Conclusion

Daily administration of ethyl acetate extract of Cocus nucifera for 17days was seen to be able to attenuate the renal dysfunction cause by daily intraperitonial injection of gentamicin 45 mg/kg b.w for 10 days is evident on renal function tests. Thus, it could be suggested that gentamicin must be given in the lowest effective therapeutic doses in patients with normal kidney function. Also, gentamicin therapy should be preceded by antioxidant administration and also it could be suggested that the husk fibre of ethyl acetate extract of Cocus nucifera can be modified into drug so that it can be given before administration of gentamycin because it is seen from this presence studies that it has nephroprotective properties

6. Acknowledgements

I really appreciate Dr. Adebayo, who took his time to go through this piece of work, also to the Professors and all the staff of the Department of Biochemistry, University of Ilorin, Kwara State, Nigeria.

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