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Research Article

Therapeutic Potentials of "Cordyceps Plus" Capsule on Gentamicin Induced Nephrotoxicity in Male Albino Rats

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Abstract

Gentamicin is an aminoglycoside antibiotic commonly used for the treatment of Gram-negative bacterial infection, but limited by its nephrotoxic side effects particularly if multiple doses accumulate over a course of treatment. Nephroprotective agents are substances which possess protective activity against Nephrotoxicity. "Cordycep plus" capsule is said to have therapeutic properties due to the presence of various complex chemical substances. To determine the therapeutic effect of "Cordycep plus" Capsule on gentamicin induced Nephrotoxicity. Twenty eight (28) male Sprague-Dawley rats (120 - 150g) were equally divided into four groups: positive control, negative control (untreated), Treatment 1 (300mg/kg body weight of "Cordycep plus" Capsule), and Treatment 2 (600mg/kg body weight "Cordycep plus" Capsule). Nephrotoxicity was induced by intra peritoneal injection of gentamicin for 8days prior to the commencement of treatment. The rats were sacrificed after 4 four weeks of treatment and their blood samples were collected for biochemical assays. The kidneys and livers were harvested for histological assessments. Compared with the positive control group, Gentamicin induction caused a decrease in body weight of the animals, induced nephrotoxicity and the "Cordycep plus" Capsule reduced the toxic effect. The antioxidant levels of GSH, SOD and MDA were significantly ($p < 0.05$) decreased in the negative control (untreated) group but increased in the "Cordycep plus" treated groups as compared with the positive control (normal) group. Urea, Creatinine, Uric acid, catalase, lipid profile and electrolyte levels were significantly increased ($p < 0.05$) in the negative control group as compared with the positive control group and the two treated groups. Histopathological examinaton of the morphological structure of the kidney and liver in the treated group showed that nephrotoxicity can be reversed before necrosis. Treatment with 600mg/kg body weight of "Cordycep plus" Capsule had pronounced nephroprotective potentials on gentamicin induced nephrotoxicity, thus "Cordycep plus" capsule has a therapeutic effect only on renal damage before total cell death.

Keywords: Gentamicin, Nephrotoxicity, "Cordycep plus" Capsule, Medicinal plants

INTRODUCTION

Nephrotoxicity is one of the most common kidney problems that occurs when body is exposed to a drug or toxin (Porter *et al*., 1981). A number of therapeutic drugs like aminoglycoside antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), and other chemotherapeutic agents can adversely affect the kidney resulting in acute renal failure, chronic interstitial nephritis and nephritic syndrome (Hoitsma, 1991). Exposure to chemical reagents like ethylene glycol, carbon tetrachloride, sodium oxalate and heavy metals such as lead, mercury, cadmium and arsenic also induces nephrotoxicity. Prompt recognition of the disease and cessation of responsible drugs are usually the only necessary therapy (Paller, 1990).

Nephrotoxic substances damage different nephron cell types, although tubular epithelial cell necrosis is the phenomenon most extensively studied. Glomerular injuries and functional alterations have also been described, but they are much less well documented (Koh *et al*., 2002). When some drugs (e.g. cyclosporine, cisplatin, or gentamicin) are administered to experimental animals, there is a marked fall in renal blood flow and glomerular filtration rate (GFR), with a rise in renal vascular resistance (Majid, 2013).

Gentamicin is active against a wide range of human bacterial infections, mostly Gram-negative bacteria including *Pseudomonas*, *Proteus*, *Serratia*, and the Gram-positive *Staphylococcus* (Goljan *et al.*, 2011). It is also useful against *Yersinia pestis* and *Francisella tularensis* (Goljan *et al.*, 2011). Gentamicin is one of the few heat-stable antibiotics that remain active even after autoclaving, which makes it particularly useful in the preparation of some microbiological growth media. It is used during orthopaedic surgery when high temperatures are required for the setting of cements (e.g. hip replacements) (Hendriks *et al.*, 2004).

"Cordyceps plus" capsule is one of the supplements produced by Green World Natural Solutions International Limited, USA. It is made from the fungus *Cordyceps sinensis* which is actually a combination of a parasitic fungus and the larvae of a moth (a caterpillar). The caterpillar is attacked and destroyed from within by the fungus. The remaining structures of the caterpillar along with the fungus are dried and sold as cordyceps (EBSCO CAM Review Board, 2011).

Cordyceps dates back to its use in China as a "tonic", a substance said to generally energize the body, especially after an illness. It was also used to treat kidney failure, bronchitis, and tuberculosis (Pegler *et al.*, 1994).

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Although used in the treatment of gram negative bacterial infection, the nephrotoxic side effect of aminoglycosides, especially gentamicin remains a major problem in clinical use mean while, Cordyceps is widely marketed today as treatment for many conditions including kidney failure. However, there is no reliable scientific evidence that it actually provides any medical benefits (EBSCO CAM Review Board, 2011)

This study was therefore designed to investigate the therapeutic potentials of "Cordyceps plus" capsule on gentamicin induced nephrotoxicity in rats.

MATERIALS AND METHODS

Chemicals and Reagents: "Cordycep plus" Capsule (World Tianjin) Nutrition & Health Food Co., Ltd., China), Gentamicin (40mg/kg), Creatinine kit (Biolabo reagents, France), Urea kit (Biolabo reagents, France), Sodium (Na) and Potasium (K) (Biolabo reagents), DPPH (1, 1 – diphenyl – 2 – picrylhydrazyl), chloroform.

Animal preparation and housing: Twenty eight albino male rats weighing 120–180gms, were purchased from Rat matterz ltd in Isolo area of Lagos State for this study. Studies were conducted in compliance with applicable internationally acceptable standard for animal treatment.

The rats were weighed and sorted into four groups with seven animals in each cage. The animals were housed in well airated plastic cages in the animal house of the College of Medicine, University of Lagos for two weeks prior to the experiment; they were fed standard rat chow and tap water *ad libitum*. They were housed in the animal house with 12–12 h light–dark cycle that was maintained at 25°C (Bishayee and Chanterjee, 1994). All animals were weighed every 48hour before the gentamicin induction to calculate the dose to induce based on the body weight. The animals were then treated according to the groupings below and closely observed. Blood samples were collected by cardiac puncture for biochemical investigations like blood urea, uric acid, creatinine, serum Na, K, Ca, lipid profile, and heamatological full blood count analysis. Bilateral periumbilical vertical incisions were made to harvest the two kidneys and the liver and weighed immediately then preserved in 10% formalin for histological analysis and some others rinsed in phosphate buffer to analyse for the anti oxidant markers.

Experimental Design: The animals were divided into four groups of seven animals each and were treated as described in table 1:

Table 1:
Experimental Design

Group I (Positive control)	**Water**
Group II (Negative control)	Gentamicin and Water
Group III (Treatment 1)	Gentamicin and "Cordycep plus" capsules (300mg/kg bw)
Group IV (Treatment 2)	Gentamicin and "Cordycep plus" capsules (600mg/kg bw)

Nephrotoxicity (renal injury) was induced in groups II, III, and IV by intra-peritoneal administration of gentamicin (80mg/kg bw) for eight days (Milind *et al.*, 1996). Subsequently, only groups III and IV were treated by oral administration of 300mg/kg bw and 600mg/kg bw "Cordyceps plus" respectively for four weeks.

Sacrifice of Animals: All the animals were sacrificed by cervical dislocation. Blood samples were collected into plain bottles, and then centrifuged for 10 minutes at 3000 rpm within an hour to obtain the serum for analysis of creatinine, urea, electrolytes (Na, K and Cl) and lipid profile. Bilateral periumbilical vertical incisions were made to harvest the two kidneys for the assay of antioxidant markers and histological analysis.

Biochemical Examination

Determination of Serum Urea: Estimation of serum urea was carried out using respective kits purchased from Biolabo reagents, France by the principle of Searcy, 1967.

Determination of Serum Creatinine: Estimation of creatinine was carried out using diagnostic kit purchased from Biolabo reagents, France by the principle of Fabiny and Ertingshausen, 1971 and Labbe , 1996.

Determination of Lipid Profile: Serum triglyceride was assayed enzymatically using diagnostic kits purchased from Biolabo reagents, France as described by Fossati and Prencipe, 1982. Total cholesterol concentrations were assayed enzymatically using diagnostic kits purchased from Biolabo reagents, France as described by Allain *et al.*, 1974. High density lipoprotein was isolated using diagnostic kits purchased from Biolabo reagents, France as described by Tietz *et al*., 1999 and then the cholesterol concentration of the high density lipoprotein (HDL cholesterol) was determined, as in total cholesterol. Serum LDL-cholesterol concentration was calculated using the Friedewald equation (Friedewald *et al.*,1972)

Determination of serum electrolyte: Sodium (Na), Potassium (K) and Chloride (Cl) analysis were done using the Biolabo reagents, from Biolabo, SA. The method of Tietz, 1995 and Young, 1990 were used.

Histological Study of the kidneys: The tissues were fixed in 10% formalin solution for ten days. They were then dehydrated through ascending grades of alcohol to absolute alcohol (50%, 70%, 90%, 95%, absolute I, II and III alcohol for 30 minutes each respectively). The tissues were left in xylene overnight. Wax was kept in an electrically heated and thermometrically controlled oven maintained at about 56-57^{0}C to maintain it in molten form. The tissues were then transferred into the molten wax for 30 minutes for impregnation. After this, the tissues were embedded in solidified wax. 5µm thick sections were cut using a microtome and stained with haematoxylin and eosin (Baker and Silverton, 1985). The specimens were evaluated with light microscope. All histopathological changes were examined by pathologist.

Statistical analysis: Data are presented as the mean ± standard error of the mean (S.E.M). All statistical comparisons were made with one-way analysis of variance (ANOVA) followed by Turkey Multiple Comparison Test using Graph Pad Prism 4.0. A value of $p< 0.05$ indicates significant differences in all cases.

RESULTS

Effects of "Cordyceps Plus" Capsules on the serum creatinine and urea.

Table 2 shows the effects of "Cordyceps Plus" Capsule (300 and 600mg/kg body weight) on the serum creatinine and urea of Gentamicin intoxicated rats. A significant ($P < 0.05$) increase in serum creatinine and urea was observed in the negative control group when compared to the positive control (normal) group. Treatment with "Cordyceps Plus" Capsules significantly ($p < 0.05$) reduced the elevated serum creatinine and urea levels.

Table 2:
Effects of "Cordyceps plus" capsules on the serum creatinine and urea of Gentamicin intoxicated rats.

	Positive Control	Negative Control	300mg/kg	600mg/kg
Urea	6.148 ± 0.768	15.49 ± 0.475*	7.678 ± 1.099	5.123 ± 0.345
Creatinine	79.10 ± 4.856	145.4 ± 15.12*	69.28 ± 3.810	57.00 ± 1.346
Uric Acid	211.1 ± 11.26	160.6 ± 10.52*	92.20 ± 10.22*	98.87 ± 4.978*

Result presented as mean ± SEM. Astericks signifies significant difference as compared to the positive control

Table 3:
Effect of "Cordycep Plus Capsules" on the Electrolytes of Gentamicin induced Nephrotoxicity.

	Positive Control	Negative Control	300mg/kg	600mg/kg
Na+	148.8 ± 1.474	141.8 ± 4.016	147.2 ± 1.326	142.6 ± 1.651
K+	5.103 ± 0.300	6.455 ± 0.502*	5.290 ± 0.187	5.255 ± 0.179
Cl-	113.2 ± 0.705	124.9 ± 6.165	113.0 ± 1.345	110.1 ± 1.921
HCO3-	9.925 ± 1.165	10.43 ± 1.285	16.80 ± 1.042*	17.95 ± 0.970*

Result presented as mean ± SEM. Astericks signifies significant difference as compared to the positive control

Effects of "Cordyceps Plus" Capsules on the serum electrolytes

Table 3 shows the effects of "Cordyceps Plus" Capsules (300 and 600mg/kg body weight) on the serum electrolytes of Gentamicin intoxicated rats. Na was significantly ($p < 0.05$) reduced while K and Cl were significantly ($p < 0.05$) increased in the negative control when compared with the positive control (normal) group. The "Cordyceps Plus" Capsules treated groups showed significant ($p < 0.05$) increase in Na with a corresponding decrease in K and Cl when compared with the negative control.

Effects of "Cordyceps Plus" Capsules on kidney antioxidants

Figure 1-4 show the effects of "Cordyceps Plus" capsules (300 and 600mg/kg body weight) on the kidney antioxidant level of Gentamicin intoxicated rats. A significant ($p < 0.05$) decrease in reduced glutathione content, superoxide dismutase and catalase level with a corresponding increase in malodialdehyde content was observed in the untreated rats (negative control) when compared to the positive control. These were significantly ($p < 0.05$) reversed in the "Cordyceps Plus" capsules treated groups.

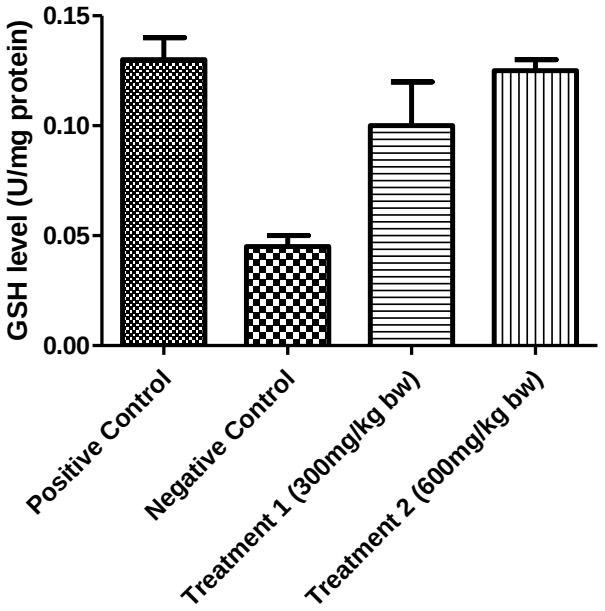

Figure 1:
Effects of "Cordyceps plus" capsules on the kidney reduced glutathione (GSH) level of Gentamicin intoxicated rats. Values are Mean ±SEM, n=7 in each group. *Significant change in comparison with the positive control group at $p < 0.05$. ªSignificant change in comparison with the negative control group at $p < 0.05$.

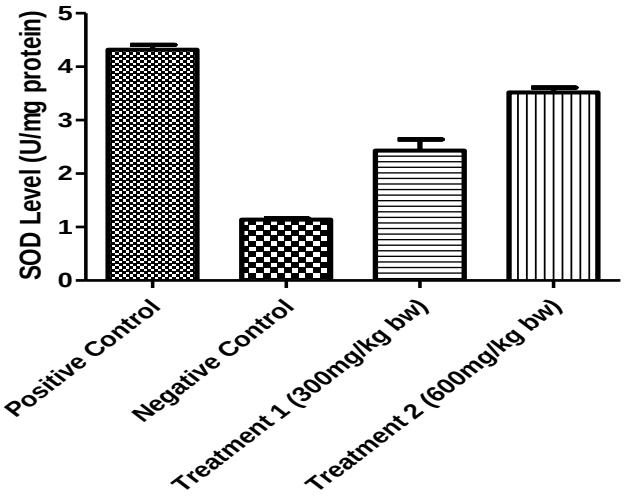

Figure 2:
Effects of "Cordyceps plus" capsules on the kidney superoxide dismutase (SOD) level of Gentamicin intoxicated rats. Values are Mean ±SEM, n=7 in each group. *Significant change in comparison with the positive control group at $p < 0.05$. ªSignificant change in comparison with the negative control group at $p < 0.05$

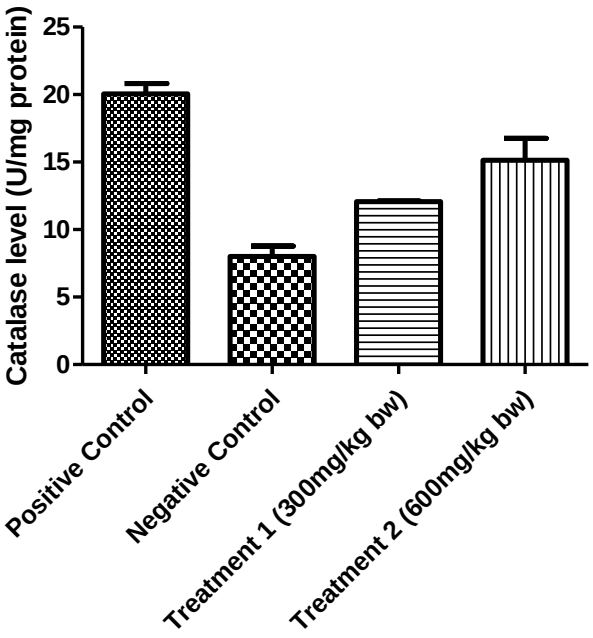

Figure 3:
Mean of Catalase level (U/mg of protein) in the kidney of rats administered with 80mg/kgbw of Gentamicin and treated with "Cordyceps plus capsules" as compared with the control group

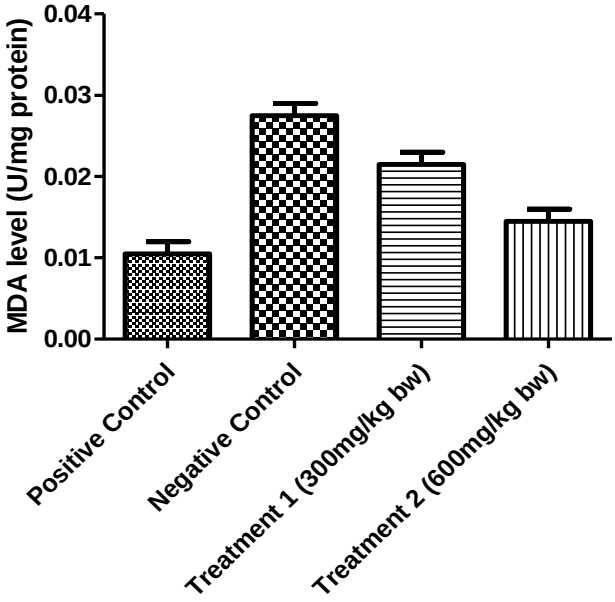

Figure 4:
Mean of Malondialdehyde (MDA) level (U/mg of protein) in the kidney of rats administered with 80mg/kgbw of Gentamicin and treated with "Cordyceps plus capsules" as compared with the control group

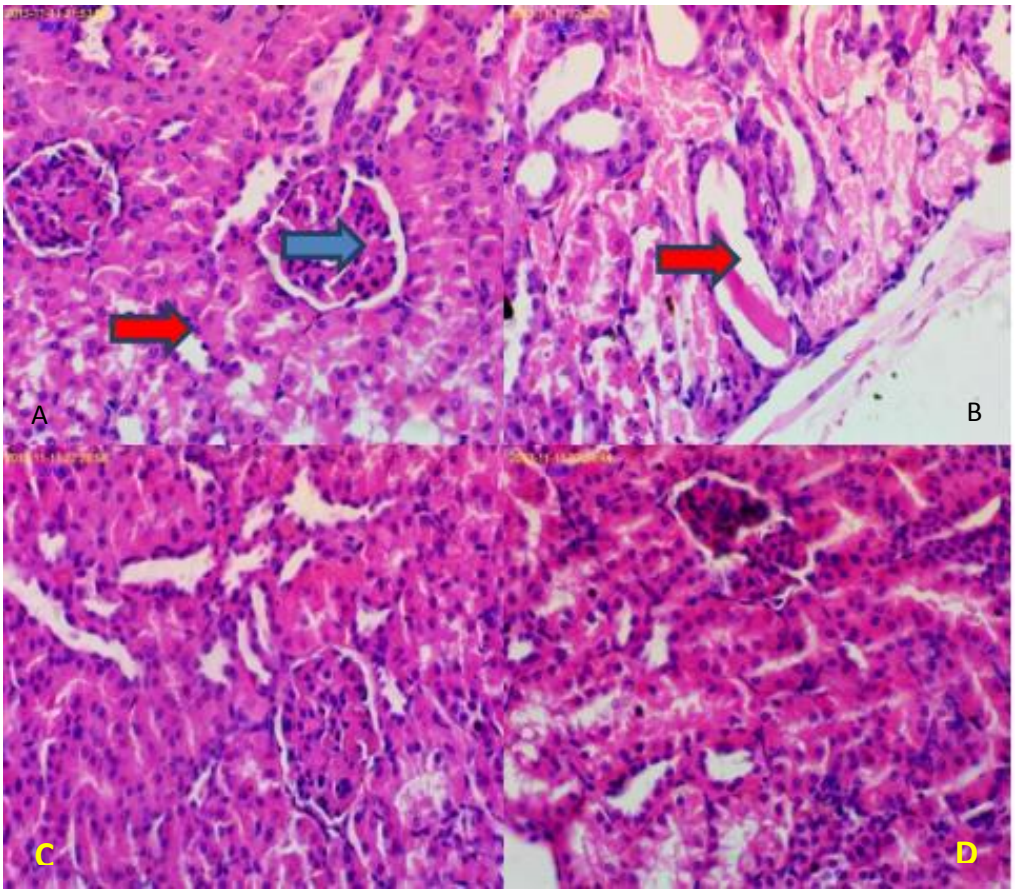

Plate 1:
Histological photograph showing the kidney of (a) Positive Control; shows normal glomerulus and tubules, (b) Negative Control; shows tubular cast and tubular necrosis, (c) Treatment 1; shows a healing glomerulus and tubule (d) Treatment 2; shows a more rapid healing glomerulus and tubule

Table 4
Effects of "Cordyceps plus" capsule on the serum lipid profile of Gentamicin intoxicated rats

	Positive Control	Negative Control	Treatment 1(300mg/kg bw)	Treatment 2 (600mg/kg bw)
Total Cholesterol	1.560 ± 0.037	2.910 ± 0.221*	2.170 ± 0.092[a]	2.045 ± 0.099[a]
Triglyceride	0.970 ± 0.147	2.205 ± 0.097*	1.738 ± 0.238 [a]	1.378 ± 0.106 [a]
HDL-Cholesterol	2.448 ± 0.177	1.260 ± 0.142*	1.688 ± 0.051 [a]	1.935 ± 0.049 [a]
LDL-Cholesterol	0.267 ± 0.033	1.150 ± 0.090*	0.795 ± 0.037 [a]	0.450 ± 0.037 [a]

Values are Mean ±SEM, n=7 in each group. *Significant change in comparison with the positive control group at $p < 0.05$. [a]Significant change in comparison with the negative control group at $p < 0.05$.

Effects of "Cordyceps Plus" capsules on lipid profile
Table 4 shows the effects of "Cordyceps Plus" capsules (300 and 600mg/kg body weight) on the lipid profile of Gentamicin intoxicated rats. A significant ($p < 0.05$) increase in total cholesterol, triacylglycerol and LDL-cholesterol with a corresponding decrease in HDL-cholesterol was observed in the untreated rats (negative control) when compared to the positive control. These were significantly reversed ($p < 0.05$) in the "Cordyceps plus" capsule treated groups.

Effects of "Cordyceps plus" capsules on the kidney histology
Plate 1 shows the effects of "Cordyceps plus" capsule on the kidney histology. The histological photomicrograph of the kidney showed glomeruli casts and tubular necrosis in the negative control group while the treated groups (300 and 600 mg/kg bw "Cordyceps plus" capsules) showed a rapidly healing glomerulus and tubule.

DISCUSSION

The incidence of renal dysfunction following amino-glycoside administration had been observed by many workers (Garetz and Schacht., 1996; Baliga *et al*., 1997 and Abdel Naim *et al*., 1999). The results for the biochemical assays showed significant increase in the lipid profile of the negative control group as compared with the positive control group while the treatment 1 group showed no significant difference when compared with the negative control group, there was significant difference in the treatment 2 group as compared with the negative control group thus, the high dosage reverts increase in lipid profile as reviewed by Heibashy & Abdel Moneim (1999).

The administration of gentamicin into rats induced impairment of renal function through liberation of oxygen free radical (Heibashy & Abdel Moneim, 1999; Heibashy *et al.*, 2009). Acute renal failure is characterized by disorders in some biochemical parameters in gentamicin treated rats as shown in the results for biochemical assays in Tables 1-4. Rats induced with 80mg/kg bodyweight gentamicin showed a significant increase in the concentration of serum urea, creatinine and uric acid in the kidney function assays in table 2. These results confirmed that gentamicin produced nephrotoxicity as previously reported by Ali *et al.*, 2003; Goto, 2004; and Heibashy *et al.*, 2009. The 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 which 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 (Swan, 1997). More than half of the proximal tubules showed desquamation of necrosis but involved tubules easily found, complete or almost complete tubular necrosis and tubular casts. Some animals were lost in the process of induction especially from day 5 to day 8 and there was a significant reduction in the body weight of the negative control group while the groups treated with "Cordycep plus" Capsule has a significant increase in body weight as compared to the treatment 2 group in figure 1.

Table 2 showed no level of significance between the control group and the treated groups especially the level of urea and creatinine but there was a level of significant increase in the level of significance between the negative control group and the positive control group, while uric acid showed no level of significance when compared with the negative control group.

Serum electrolytes were altered in rats induced with gentamicin as compared with the positive control animals. Lower value of serum sodium in the negative control group as compared with the positive control group indicated inability of kidney to conserve sodium and chloride though not statistically significant. Haemo-dilution too may be involved in the fall of sodium value via excess intake of water and (or) increased production of endogenous water.

Increase in the level of Potassium in the negative control group may be due to reduced excretion of K aggravated by leakage of intracellular potassium into the blood stream as a result of gentamicin induced lesions in renal tubular epithelium. There was no significant difference between the treated groups and the positive control as compared with the negative control group. The results in this research study are in harmony with the data obtained by Heibashy & Abdel Moneim 1999; and Heibashy *et al*., 2009).

There was a increase in the level of Chlorine for the rats induced with gentamicin as compared with the positive control group and the treated groups though it was not statistically significant.

Apoptosis plays a major role in kidney embryogenesis, resulting in large-scale cell death during development (Coles *et al*., 1993). 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; Davis *et al.,* 1998).

Thus, apoptosis is clearly involved in ischemic renal atrophy (Gobe *et al*., 1987) gentamicin treated rats show tubular eptithelial damage with intense granular degeneration involving > 50% of renal cortex. Some of the tubular epithelium contains tubular casts observed in the histological photomicrograph results in Figures 6-13 which is in correlation with the results observed by Vijay Kumar *et al*., 2000.

For Heamatological parameters, the WBC and Neutrophiles results showed a significant ($p< 0.05$) difference between the negative control group and the positive control group, while there was no significant ($p< 0.05$) difference between the positive control group and the treatment 1 group, there was a significant ($p< 0.05$) increase in the WBC and neutrophiles of the treatment 2 group as compared with the positive control group. All other results in Heamatology were not statistically significant.

The antioxidant results showed a significant ($p< 0.05$) decrease in the level of GSH for the negative control group as compared with the positive control group and the treated groups in Figure 2 For the SOD level, the negative control group was also the lowest then the treatment 1 group then the treatment 2 group as compared with the control group in Fig 3.

For the Catalese level, the negative control group also showed a significant ($p< 0.05$) decrease as compared with the positive control group but there was no significant difference between the postive control group the treated groups. The MDA for the negative control group was significantly ($p< 0.05$) increased as compared with the positive control group and the treated groups. Renal and liver tissue specimens obtained after the animals were fasted and sacrificed four weeks after administration were examined histologically as discussed by Yoshiyama *et al.,* 1992 with similar findings as observed by other authors.

Gentamicin renal cell damage as induced by tubular necrosis i.e., marked congestion of the glomeruli with glomerular atrophy, degeneration of tubular epithelial cells with casts in the tubular lumen and infiltration of inflammatory cells in the interstitium was confirmed on histopathological examination as proposed by Shirwaikar *et al.,* 2003 in Figures 6-7 i.e Histological photomicrograph results. The liver was also examined histologically and the result showed a slightly inflammed hepatocytes for the negative control group as compared with the positive group with normal hepatocytes and surrounding portal tracts. It was observed that the inflammed hepatocytes showed a rapid healing process and reformation of the inflammed hepatocytes in the treated groups (300mg/kg and 600mg/kg body weight) although the high dose treated group showed a faster inprovement.

In this present study also for the kidney tissue, glomeruli casts and tubular necrosis was observed in the negative group as compared with the positive control group, and the groups treated with the low (300mg/kg) and high (600mg/kg) dose of "Cordycep Plus" Capsule as compared with the works of others.

In conclusion, daily intraperitorial injection of rats with 80mg gentamicin /kg body weight for 8days can cause serious harmful effect and itis evident on renal function and other biochemical tests. Thus it is suggested that gentamicin should be administered in the lowest therapeutic doses in patients with normal kidney function, administratin should be followed by antioxidant administration and renal function test.

A variety of medicinal plants and plants extracts have been reported for its significant nephroprotective activity in animal models probably due to the presence of Flavanoids in all the few medicinal plants. "Cordycep plus" capsule is a valued traditional Chinese medicine and experiments have shown that cordyceps has several bioactivities, such as antitumor, immunomodulatory, antioxidant, sexual and reproductive function enhancement, hypoglycemic, and antifatigue activities, and have a protective effect on the kidney and liver hence meeting the manufacturers claims. From this study, it can be concluded that "Cordycep Plus" has the therapeutic effect on cell apoptosis but cannot reverse necrosis or total cell death. It was also observed that continous use for a longer time could allivate the therepeutic process.

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