

# Effect of Chinese Green Tea on Glomerular Filtration Function of Albino Rats Treated with Gentamicin

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Received: 05 May 2024/ Revised: 16 May 2024/ Accepted: 21 May 2024/ Published: 31-05-2024

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**Abstract**— Acute kidney injury (AKI) results from reduction in glomerular filtration rate (GFR) causing the passage of little or no urine. This study investigated the effects of Chinese green tea on glomerular filtrate functions of albino rats treated with gentamicin in view to evaluate its nephroprotective effect. Animals were procured from the animal house of Veterinary Medicine Department University of Nigeria Nsukka. The rats were transported to Biochemistry laboratory, acclimatize for 7 days and randomly assigned into five groups. The rats were fed with commercially prepared pellets (vital feed) and watered ad libitum throughout the duration of the study. Nephrotocicity was induced with gentamicin at 80mg/kg for 7 consecutive days with co-administration of camellia sinensis extract. Blood samples were drawn from rats in each groups and were analyzed for biochemical parameters. Results obtained are; from urea (13.08-6.12mg/dl), creatinine (70.08-42.25mg/dl), cystatin C (0.084-0.027mg/dl) and GFR (2.20-1.30ml/min). Findings reveal that Gentamicin induces nephrotoxicity through induction of oxidative stress and generation of Reactive Oxygen species (ROS). Nephrotoxicity is an acute kidney injury marked by induced GFR and serum accumulation of urea, creatinine and cystatin C. Camellia sinensis contains powerful antioxidants catechins which counters the oxidant effect of gentamicin, thereby ameliorating its nephrotoxicity. Therefore crude extract of Camellia sinensis has nephro-protective effect and ability to maintain a relatively normal GFR.

**Keywords**— Gentamicin, nephrotoxicity, GFR, green tea.

## I. INTRODUCTION

Acute Kidney Injury (AKI) is a condition where kidney's glomerular filtration rate (GFR) is reduced and as a result, there is little quantity of urine passed out, this condition is known as oliguria (Schrier et al., 2014). When the glomerulus at the Bowman's capsule is tempered, its filtration function is impaired. This reduced GFR leads to accumulation of certain nitrogenous substances within the body which alters the osmolalic concentration of the blood leading to inflammation (oedoma) which is seen as a common symptom of kidney problem (Mizota et al., 2017).

There are various causes of AKI which include infectious diseases, drugs, heavy metals and other toxicants. Some infections, such as septicemia and acute pyelonephritis, can directly injure ones kidneys. Drugs that cause acute kidney injuries include aminoglycoside family, this is a family of antibiotics whose members include streptomycin, clindamicin, gentamicin etc. (Bellomo et al., 2014).

Gentamicin is an aminoglycoside member known potentially to cause AKI when administered consecutively for 7 days (Ratliff et al., 2016). The kind of AKI caused by gentamicin is characterised by oliguria, preturia and inflammation which sometimes

leads to oedema of the limbs. All of these are as a result of low or reduced ultrafiltration or alternatively the reduced Glomerular Filtrate Rate (GFR) (Fenoglio *et al.*, 2019).

GFR is an index used in measuring or indicating AKI. It can be done with the following parameters: urea, creatinine, cystatin C (which are endogenous) and inulin (exogenous) which is only found in plants and is indigestible (Dan *et al.*, 2011). Since animals do not metabolise the inulin, whenever an animal takes it into the body, it stays in the body only to be passed out through urine. It is used to check the glomerular filtrate rate (Wołyniec *et al.*, 2018).

*Camellia sinensis* is a plant native in Asia, China precisely, but now cultivated all over the whole world for its medicinal and beverage importances. It is known to contain certain Phytochemicals especially the antioxidant phytochemicals as polyphenols, and catechins (Prasanth *et al.*, 2019). As a result of this reach antioxidant phytochemical constituents, *Camellia sinensis* is now seem important in the prevention and management of the diseases: diabetes, hypertension anti-ulcer etc (Kaushal *et al.*, 2019, Ngobidi *et al.*, 2016 ana Kanlaya *et al.*, 2019).

But there are few information available on how the oxidant in *Camellia sinensis* affect the GFR in ameliorating AKI. And this leads us to the current study (Prasanth *et al.*, 2019). This study aimed at determining the effect of effect of chinese green tea on glomerular filtrate functions of albino rats treated with gentamicin.

## II. MATERIALS AND METHOD

### 2.1 Plant material source:

The *Camellia sinensis* leaves was sourced from shop Rite Enugu Nigeria.

### 2.2 Preparation of plant extracts:

The plant leaves was ground into powder by using a mortar and pestle. Known mass of the powder was macerated in Known volume of ethanol for 48hrs. After then, the extract was first sieved with cheese cloth and later filtered using a Buckner funnel and Whatman NO 1 filter paper. The filtrate was concentrated to dryness by air drying and then stored in an air tight container under refrigeration at 40°C until required.

### 2.3 Experimental Animals:

Adult albino rats weighing between the range of 180-200g were obtained from Veterinary Medicine Department University of Nigeria Nsuka. The rats were kept in cages in the Biochemistry laboratory of Science Laboratory Technology Department Akanu Ibiam Federal Polytechnic, Unwana and allowed to acclimatise for 7 days before the study. All rats were fed with commercially prepared pellets (vital feed) and watered *ad libitum* throughout the duration of the study.

### 2.4 Study Design:

Induction of Nephrotoxicity: It was induced by intraperitoneal injection of gentamicin (80mg/ Kg body weight) for seven consecutive days.

### 2.5 Grouping of animals:

- GROUP 1: Normal control (no Induction and treatment)
- GROUP 2: Negative control (receive gentamicin only )
- GROUP 3: No Induction and treated with 200mg/kg/day of *Camellia sinensis*
- GROUP 4 : Induced and treated with 200mg / kg/ day of *Camellia sinensis*
- GROUP 5: Induced and treated with 400mg/kg/ day of *Camellia sinensis*

All groups received test agents via oral route using a gavage needle once daily for seven days. Both the Induction and administration of test agents took place simultaneously for seven consecutive days.

At the end of the administration chloroform anesthesia was used to sacrifice the animals. Blood sample was collected directly through the cardiac puncture, about 5ml was put into heparinised container and about 4ml in container free from anticoagulant and allowed to clot for 20 minutes and centrifuged at 4000rpm for 15 minutes. Sera was collected using micropipettes for onward biochemical analysis

### III. BIOCHEMICAL ANALYSIS

#### 3.1 Determination of Serum Urea:

Serum Urea was determined Using Urease Enzymatic method as described by Fawcett (1960). Principle Urea in serum is hydrolysed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot reaction.



#### 3.2 Determination of serum creatinine:

Serum creatinine was determined using colorimetric method as described by Henry (1974). Principle Creatinine reacts with picric acid in alkaline conditions to form a color complex, which absorbs at 510nm. The rate of formation of color is proportional to the creatinine concentration in the sample. In the endpoint method the difference in absorbance measurements after color formation yielded a creatinine value corrected for interfering substances.

#### 3.3 Serum cystatin C:

Serum cystatin C assay was made by latex particle enhanced turbidimetric immunoassay. PET kit (13,14) (Dako, Glostrup, Denmark). Concomitant change in the absorbance signal with rabbit antibody against human cystatin C. A reaction between these immunoparticles. The cystatin C concentration of the patient particles of uniform size, chemically coupled and cystatin C in a patient specimen results. Cystatin C PET kit contains polystyrene in the formation of agglutinates and a specimen is determined by interpolation on a calibration curve.

### IV. STATISTICAL ANALYSIS

Data obtained was presented as  $M \pm SD$  (Standard Deviation). Statistical analysis was done using SPSS version 20 for one way ANOVA followed by LSD post hoc test.  $P < 0.05$  was considered significant.

### V. RESULTS

**TABLE 1:**  
**GROUP WISE COMPARISON OF UREA, CREATININE, CYSTATIN AND GFR**

Group	Urea (mg/dl)	Creatinine (mg/dl)	Cystatin C (mg/L)	GFR (ml/min)
1	6.12 ± 0.93	42.25±2.28	0.027	2.20 ± 0.1
2	13.08 ± 1.68	70.08 ± 3.76	0.084	130 ± 0.06
3	6.46 ± 1.12	48.02 ± 1.36	0.041	1.90 ± 0.03
4	6.81 ± 0.56	52.88 ± 3.27	0.045	1.70 ± 0.02
5	6.29 ± 1.62	49.30 ± 4.20	0.049	1.70 ± 0.09

Serum urea, creatinine and cystatin C increase significantly at  $P < 0.05$  in group 2 compare with other groups. Conversely, GFR decreases significantly in group 2 compare to other groups. There was slight increment in urea, creatinine and cuystatine C in group 3, 4, and 5 when compared with group 1 (normal control). Conversely, there was slight decrement in GFR of group 3, 4, and 5 compared with group 1.

### VI. DISCUSSION

Gentamicin is still considered as an important amino glucoside antibiotic against life threatening infections regardless of it's nephrotoxic effects. This is because of it's high chemical stability in the body. Gentamicin induced nephrotoxicity has been widely used in animal model to study acute renal failure in experimental research (Avila-Carrasco et al., 2021). Regeneration of reactive oxygen specie and oxidative stress has been suggested as the mechanism of Pathogenesis of gentamicin Induced nephrotoxicity. Regeneration of reactive oxygen species induces necrosis of both glomerular filtration rate and impaired reabsorption function. (Weyker *et al.*, 2012).

We found that gentamicin caused significant elevations of serum cystatin C, Urea, creatinine concentrations. The increase in these three parameters, indicates accumulation in the blood stream due to produced glomerular filtration rate. Treatment with *Camellia sinensis*, however, presented very slightly elevation in serum concentration of cystatin C, Urea, creatinine with no statistical significant when compared with the normal control. This finding from *Camellia sinensis* treatment reviews no

accumulation of the parameters quantifier and also could stand as an indication of unaffected glomerular filtration rate (Kaushal *et al.*, 2019).

This could further suggest the inhibition of generation of ROS and oxidative stress. *Camellia sinensis* is known to be rich in beneficial polyphenols. Mainly catechins and their derivatives which are potent antioxidants and could be the reason for the renoprotective anti-diabetic, anti-mutagenic, neuroprotective and anti carcinogenic effects (Prasanth *et al.*, 2019). The findings of this study is in agreement with the studies conducted by where gentamicin also leads to increased urea, uric acid, ROS, MDA and creatinine (Dan *et al.*, 2011).

GFR which is the rate at which the nephron filters the blood in order to excrete waste out from it is also a veritable tool in prediction and monitoring of acute renal failure. Reduction in GFR value is associated with acute renal failure (Weykerv *et al.*, 2016).

From our study, there was a significant reduction in GFR in the gentamicin control compared with other groups. This suggested that an acute injury to the nephron especially at the glomerulus where ultrafiltration takes place. This observation was further strengthened by the significant increased serum concentration of urea, creatinine and cystatin C indicating the accumulation due to insufficient ultrafiltration. It could also be observed that the *Camellia sinensis* in the coadministration with gentamicin prevents the suppression of GFR by some margin which is also significant statistically. *Camellia sinensis* is known to contain antioxidant that counters the oxidant and reactive oxygen species generation which leads to nephron damage and nephrotoxicity (Irazabal *et al.*, 2020, Ostermann *et al.*, 2020 and Meng *et al.*, 2019).

## VII. CONCLUSION

Gentamicin induces nephrotoxicity through induction of oxidative stress and generation of Reactive Oxygen species (ROS). Nephrotoxicity is an acute kidney injury marked by induced GFR and serum accumulation of urea, creatinine and cystatin C as a result of reduced GFR. *Camellia sinensis* contains powerful antioxidants catechins which counters the oxidant effect of gentamicin, thereby ameliorating its nephrotoxicity. We therefore conclude that crude extract of *Camellia sinensis* has nephroprotective effect and ability to maintain a relatively normal GFR.

## RECOMMENDATION

Further Studies are recommended to purify, isolate, characterise and optimize the lead compound in *Camellia sinensis* leaf extract towards producing a solution against gentamicin nephrotoxicity.

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