

Basic Research

Effect of green tea (*Camellia sinensis*) on gentamicin induced nephrotoxicity in Long Evans male rats

Rahatul Jannat Nishat^{1*}, Mohammad Rabiul Halim^{2*}, Md Motiul Islam², Tarikul Hamid², Kazi Nuruddin Ahmed², Rajib Hasan², Md Mazharul Haque², Md Atiquzzaman², Asfaq Rafed Rahman¹, Tarak Nath Das¹, Rifat Chowdhury¹, Israt Jahan Chowdhury¹, Jakir Mohammed Hossen¹, Injamam Ull Haque², Ehte Shamul Hakim², Sadia Jesmin²

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Abstract:

Background: The kidneys are a pair of essential excretory organs. It can be damaged by poisonous effects of chemicals, toxins, prolonged and uncontrolled use of drugs. Green tea is a popular choice of beverage being increasingly used in recent times which may have nephroprotective effect.

Objectives: To observe the nephroprotective effect of green tea against gentamicin induced nephrotoxicity in Long Evans male rats.

Methods: This study was carried out in the Department of Physiology, Sir Salimullah Medical College (SSMC), Dhaka from 1st July 2019 to 30th June 2020. A total number of thirty (30) apparently healthy Long Evans male rats, 90-120 days old, weighing between 150-200 g were taken for the study. After acclimatization for 14 days, they were divided into two groups, control group (Group A) and experimental group (Group B- green tea pretreated and gentamicin treated group). Control group was subdivided into group A₁ (baseline control group) and group A₂ (gentamicin treated control group). Each of this group was consisted of ten rats. All the rats received basal diet and baseline control group also received normal saline (1 ml/kg/day) for 28 days. Experimental group received ethanolic extract of green tea orally (300 mg/kg/day) for 28 days. Gentamicin treated control group and experimental group also received injection gentamicin intraperitoneally (80 mg/kg/day) for last 3 days (26th to 28th days) of the study period. All the rats were sacrificed on 29th day. After sacrifice blood (from heart) and kidney samples were collected. Serum levels of creatinine, urea and blood urea nitrogen (BUN), renal contents of malondialdehyde (MDA) and histopathology of kidney were done by using standard laboratory method to compare nephrotoxicity among experimental and control group. Statistical analysis was done by using Statistical Package of Social Science (SPSS) for windows version 22. Data were presented as mean±SD. One way ANOVA test, post hoc-Bonferroni test, paired 't' test and Fisher's Exact test were done to compare the data as applicable. *p* value ≤ 0.05 was considered as level of significance.

Results: The mean serum creatinine, urea, blood urea nitrogen (BUN) levels and mean malondialdehyde (MDA) concentration in kidney were significantly lower in green tea pretreated and gentamicin treated group than those of gentamicin treated control group but the levels were significantly higher in comparison to those of baseline control group. Moreover, abnormal histological findings of kidney was observed in 30% of rats in green tea pretreated and gentamicin treated experimental group but the abnormality was 100% and 0% in gentamicin treated and baseline control group of rats respectively.

Conclusion: The present study reveals that green tea has nephroprotective effect against gentamicin induced kidney damage in Long Evans male rats.

Keywords: Nephrotoxicity, green tea, gentamicin, Long Evan male rat.

Introduction

The kidneys are the essential organs of our body. They play a dominant role in maintaining the consistency of the internal environment by filtering the plasma and producing urine to excrete metabolic waste products from the body and conserves necessary substances depending on the needs of the body¹. Kidneys also maintain many homeostatic functions by regulation of water and electrolyte balance, regulation of blood pressure and acid base balance². Kidney disease is increasing across the world. The most important cause of acute kidney damage is destruction of tubular epithelial cells (acute tubular necrosis) by toxin or ischemia or drugs like Aminoglycosides¹.

Gentamicin (GM) is most commonly used aminoglycoside antibiotic against gram-positive and gram-negative aerobic bacterial infection. Its therapeutic efficacy, however, is limited by renal impairment that occurs in up to 5-25% of treated patients receiving gentamicin more than 3-5 days. With traditional dosing, adjustments must be made to prevent accumulation of drug and toxicity in patients with renal insufficiency. For this reason either the dose of drug is kept constant and the interval between doses is increased, or the interval is kept constant and the dose is reduced. Measurement of gentamicin serum levels is also essential³.

The presence of diabetes or hypotension and the use of other nephrotoxic drugs and iodinated contrast were independent risk factor for the development of gentamicin-induced

nephrotoxicity⁴. Gentamicin induced nephrotoxicity occurs as a result of increased serum creatinine and blood urea nitrogen and decreased glutathione, super oxide dismutase, catalase and increased thiobarbituric acid reactive levels (oxidative stress biomarker)⁵.

On the basis of these facts researchers across the world are now showing interest in the use of alternative medicines for the treatment of kidney disease. Several experimental studies have been employed for preventing and treating gentamicin induced nephrotoxicity such as kalajira⁶, alovera⁷, curcumin⁸.

Green tea derived from the leaves of the *Camellia sinensis* plant. Originally cultivated in East Asia, this plant grows as large as a shrub or tree. Green tea is made from unfermented leaves⁹. Fresh green tea leaf contains the highest concentrations of powerful antioxidants due to presence of vitamin C¹⁰ and the flavonol group of polyphenols also known as green tea catechin which may constitute up to 30% of the dry leaf weight¹¹. Among them epigallocatechin gallate possessed greater antioxidant activity, which had a free radical scavenging rate of 72.2%¹² and especially singlet oxygen scavenging activity¹³. In addition, it also contains chlorophylls and carotenoids¹⁴, minerals such as chromium, manganese, selenium and zinc¹⁵.

Green tea theanine, theobromine, catechin, caffeine and gallic acid contents are among the more active constituents related to the quality of tea leaves and to the degree of fermentation during manufacturing¹⁶. Epidemiological studies have shown that drinking green tea is beneficial to people's health by the prevention of cancer¹⁷, anti-atherosclerosis¹⁸, anti-inflammatory¹⁹, antibacterial²⁰, anti-angiogenesis²¹, antiviral²², neuroprotective activities²³. Instead of drinking, green tea plant and its extract are very important raw material for cosmetic industry²⁴. Again, some researchers have focused on the incorporation of green tea extract into food, such as bread²⁵, biscuits²⁶.

Green tea has revealed polyphenolic compounds, vital for the nephroprotective activities in experimental study against chemotherapeutic agent- cisplatin²⁷ and environmental toxin-bisphenol²⁸ etc. Beneficial effects of green tea extract improved gentamicin induced functional and histopathological kidney damage²⁹. Another study showed green tea extract prevent gentamicin induced nephrotoxicity

and oxidative damage by improving antioxidant defense and tissue integrity³⁰. In this context, it is necessary to find out the nephroprotective effect of green tea to prevent and treat gentamicin induced kidney damage.

Methods: This experimental study was carried out in the Department of Physiology, Sir Salimullah Medical College (SSMC), Dhaka from 1st July 2019 to 30th June 2020. Before conducting the study, ethical permission was taken from the Institutional Ethics Committee (IEC) of Sir Salimullah Medical College, Dhaka. A total number of thirty (30) apparently healthy Long Evans male rats, 90-120 days old, weighing between 150-200 g were taken for the study were purchased from animal house of Department of Pharmacology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka.

Inclusion criteria: Apparently healthy Long Evans male rats. Age: 90 to 120 days old. Weighing between 150 to 200 grams.

Exclusion Criteria: Unhealthy and diseased rats.

They were kept in the animal house of Institution of Nutrition and Food Science, University of Dhaka, where the experiment was carried out. All the animals were acclimatized for 14 days prior to intervention at 27 to 28 °C room temperature³¹. They were kept under 12 hours dark-light cycle. During this period, the animals had free access to standard food pellets and allowed drinking water as desired. After 14 days acclimatization the total study period was twenty eight (28) days. At the beginning of the study period (day 1) initial body weight of all the rats were measured and at the end of the study period their final body weight was also measured.

All the rats were divided into two groups, control group (Group A) and experimental group (Group B- green tea pretreated and gentamicin treated group). Control group was subdivided into group A₁ (baseline control group) and group A₂ (gentamicin treated control group). Each of this group was consisted of ten rats. All the rats received basal diet for 28 days. In addition to basal diet, baseline control group also received normal saline (1 ml/kg/day) for 28 days. Gentamicin treated control group received injection gentamicin intraperitoneally (80 mg/kg/day) for last 3 days (26th to 28th days) of the study period. Again experimental group received ethanolic extract of green tea orally (300 mg/kg/day) for 28 days and injection gentamicin intraperitoneally (80 mg/kg/day) for last 3 days (26th to 28th days) of the study period. All the rats were sacrificed on 29th day.

The ethanolic extract of green tea was prepared. Green tea was collected from local market of Dhaka city. The fresh green tea sample from the commercial packet was dried overnight in a hot air oven to remove any additional moisture. Then they were crushed into fine powder with an electrical grinder. A glass jar was taken and washed thoroughly. Then dried powdered sample of green tea was taken in the jar. After that ethanol was poured into jar up to 1 inch height above the sample surface as it can cover the sample surface. Aluminium foil was used to close properly to resist the entrance of air into jar. It was kept for four (4) days with frequent shaking every day. After the extraction process, the green tea extract was

1. Physiology Department, Sir Salimullah Medical College, Dhaka 1206, Bangladesh.

2. Critical Care Medicine, Asgar Ali Hospital, Dhaka 1204, Bangladesh.

*These authors contributed equally to this work

***Corresponding Author:**

Rahatul Jannat Nishat
Flat 13B, 102 Elephant road, New market, Dhaka 1205
E-mail: dr.rahatulnishat@gmail.com
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filtered by Whatman no.1 filter paper in a funnel. The filtrate was concentrated by evaporating the solvent using a rotator evaporator. The concentrated extract was dissolved in distilled water for administration to rats²⁹.

On the day 31, at the end of the study period, all the rats were sacrificed on day 29 (after 24 hours of last dose of gentamicin injection administration on day 28). They were anesthetized with the help of chloroform (30%). Then blood samples (approximately 3 ml) were collected from the heart by using disposable syringe and were taken in separate clean and dry test tubes with proper identification numbers and were kept in standing position till formation of clot. Then blood was centrifuged at a rate of 3000 rpm for 10 minutes. After that supernatant serum were collected in labeled Eppendorf test tube and preserved in the refrigerator for estimation of all the biochemical parameters.

Kidney was also removed from each rat and weighed and washed in ice-cold saline, then it was wiped in tissue paper and their weight was measured by electric balance analyzer. A small part of kidney tissue (approximately 300 mg) was cut and taken in foil paper with proper labeling and placed on an ice bath and was kept in deep freeze until homogenized. This portion was used for MDA measurement. The Rest of the kidney tissue was preserved and fixed in 10% formalin solutions for subsequent histological processing. Histological findings were categorized by normal and abnormal changes⁵.

Serum levels of creatinine (Jaffe method), urea, and blood urea nitrogen (BUN) were measured by Roch-Ramel enzymatic method in the Department of Biochemistry and

Molecular Biology in BSMMU. Assessment of malondialdehyde (MDA) content of kidney tissue homogenate was done by Ohkawa method in the laboratory of Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka. To find out the histopathological changes of kidney tissue, histological slides were prepared, observed under microscope and photomicrographs were taken by using standard laboratory procedure in the Department of Pathology, SSMC.

Statistical analysis: Data were expressed as mean±SD (standard deviation). Statistical analysis was done by using Statistical Package for Social Science (SPSS) for windows version 22. For statistical analysis, one way ANOVA test was performed for comparison among the groups and then post hoc-Bonferroni test was done to compare between the groups. Paired ‘t’ test and Fisher’s Exact test were done as applicable. p value ≤0.05 was considered as level of significance.

Results:

The mean (±SD) initial body weight of group A₁, group A₂ and group B was almost similar and the difference was not statistically significant. Final body weight and mean (±SD) percent (%) change of body weight was higher in group B than that of group A₁ and A₂, although the differences were not statistically significant (Table I).

The mean (p<0.01) (±SD) kidney weight was significantly higher in group B in comparison to that of group A₁ whereas this level was significantly lower in comparison to that of group A₂ (p<0.001) (Table 1)

Table I: Body weight and kidney weight in different groups of rats (N=30)

Group	Body weight (gm)		% change of body weight from final (F) to initial (I) [(F-I)/Ix100]	Kidney Weight (gm)
	Initial (I)	Final (F)		
A1 (n=10)	175.80 ± 9.82 (165 - 192)	212.30 ± 7.36 (201 - 224)	21.06 ± 6.33 (13.90 – 35.76)	0.87 ± 0.07 (0.76 - 0.98)
A2 (n=10)	182.00 ± 13.23 (161 - 200)	211.80 ± 9.23 (197 - 224)	16.66 ± 5.38 (11.86 - 27.91)	1.32 ± 0.18 (0.99 - 1.56)
B (n=10)	179.10 ± 7.82 (170 - 195)	220.20 ± 9.09 (203 - 230)	23.10 ± 6.28 (14.04 - 34.12)	1.14 ± 0.20 (0.84 - 1.32)
Multiple comparisons	Initial body weight	Final body weight	% change of body weight	Kidney weight
	p value	p value	p value	p value
A ₁ vs A ₂ vs B	0.431 ^{ns}	0.067 ^{ns}	0.068 ^{ns}	<0.001 ^{***}
A ₁ vs A ₂	0.597 ^{ns}	1.000 ^{ns}	0.335 ^{ns}	<0.001 ^{***}
A ₁ vs B	1.000 ^{ns}	0.150 ^{ns}	1.000 ^{ns}	0.004 ^{**}
A ₂ vs B	1.000 ^{ns}	0.114 ^{ns}	0.072 ^{ns}	0.047 ^{**}

Data are expressed as mean ± SD. For statistical analysis, one way ANOVA test was performed for comparison among the groups and then post hoc-Bonferroni test to compare between groups. Figures in parentheses indicate ranges. **Group A₁**: Baseline control group, **Group A₂**: Gentamicin treated control group, **Group B**: Experimental group (Green tea pretreated and gentamicin treated group). N = Total number of rats; n= number of rats in each group; ns = not significant; *=significant at p <0.05; **= significant at p <0.01; *** = significant at p <0.001.

Table II: Mean initial and final serum creatinine levels in different groups of rats (N=30)

Group	Serum creatinine (mg/dL)		p-value
	Initial	Final	
A ₁ (n=10)	0.72±0.10 (0.57 - 0.87)	0.76 ± 0.15 (0.58 - 1.00)	0.555 ^{ns}
A ₂ (n=10)	0.84 ± 0.11 (0.67 - 1.00)	3.08 ± 0.20 (2.78 - 3.34)	0.001 ^{***}
B (n=10)	0.84 ± 0.15 (0.58 - 1.09)	1.70 ± 0.29 (1.27 - 2.11)	0.001 ^{***}

Data are expressed as mean ± SD. For statistical analysis, Paired 't' test was done to compare between groups. Figures in parentheses indicate ranges. **Group A₁**: Baseline control group, **Group A₂**: Gentamicin treated control group, **Group B**: Experimental group (Green tea pretreated and gentamicin treated group), N = Total number of rats; n= number of rats in each group; ns = not significant; * = significant at p-value <0.05; ** = significant at p-value <0.01; *** = significant at p-value <0.001.

The mean (±SD) initial and final serum creatinine levels were almost similar in group A₁ and the differences were not statistically significant, whereas mean final serum creatinine level was significantly higher in group A₂ (p<0.001) and group B (p<0.001) in comparison to that of group A₁ (Table II).

The mean (±SD) serum creatinine level was significantly higher in group A₂ (p <0.001) and group B (p<0.001) in comparison to that of group A₁, whereas this level was significantly lower in group B (p<0.001) than that of group A₂.

The mean (±SD) serum urea was significantly higher in group A₂ (p<0.001) and group B (p <0.001) than that of group A₁, whereas serum urea level was significantly lower in group B (p <0.001) than that of group A₂.

The mean (±SD) serum BUN was significantly higher in group A₂ (p <0.001) and group B (p <0.001) in comparison to that of group A₁, whereas serum BUN level was significantly lower in group B (p <0.001) than that of group A₂ (Table III).

Table III: Mean serum creatinine, urea and BUN levels in different groups of rats (N=30)

Group	Serum creatinine (mg/dL)	Serum urea (mg/dL)	Serum BUN (mg/dL)
A ₁ (n=10)	0.76 ± 0.15 (0.58 - 1.00)	35.29 ± 6.54 (24.05 - 44.85)	16.49 ± 3.06 (11.24 - 20.96)
A ₂ (n=10)	3.08 ± 0.20 (2.78 - 3.34)	114.10 ± 8.06 (102.33 - 125.45)	53.32 ± 3.77 (47.82 - 58.62)
B (n=10)	1.70 ± 0.29 (1.27 - 2.11)	69.96 ± 5.39 (62.53 - 78.39)	33.16 ± 3.55 (29.22 - 41.30)

Multiple comparisons

	Serum creatinine p value	Serum urea p value	Serum BUN p value
A ₁ vs A ₂ vs B	<0.001 ^{***}	<0.001 ^{***}	<0.001 ^{***}
A ₁ vs A ₂	<0.001 ^{***}	<0.001 ^{***}	<0.001 ^{***}
A ₁ vs B	<0.001 ^{***}	<0.001 ^{***}	<0.001 ^{***}
A ₂ vs B	<0.001 ^{***}	<0.001 ^{***}	<0.001 ^{***}

Data are expressed as mean ± SD. For statistical analysis, one way ANOVA test was performed for comparison among the groups and then post hoc-Bonferroni test to compare between groups. Figures in parentheses indicate ranges. **Group A₁**: Baseline control group, **Group A₂**: Gentamicin treated control group, **Group B**: Experimental group (Green tea pretreated and gentamicin treated group), N = Total number of rats; n= number of rats in each group; ns = not significant; * = significant at p-value <0.05; ** = significant at p-value <0.01; *** = significant at p-value <0.001

The mean (±SD) MDA level was significantly higher in group A₂ and group B (p <0.001 and p<0.05 respectively) in comparison to that of group A₁, whereas MDA level was significantly lower in group B (p <0.001) than that of group A₂ (Table IV, Figure 1).

Table IV: Mean MDA level in different groups of rats (N=30)

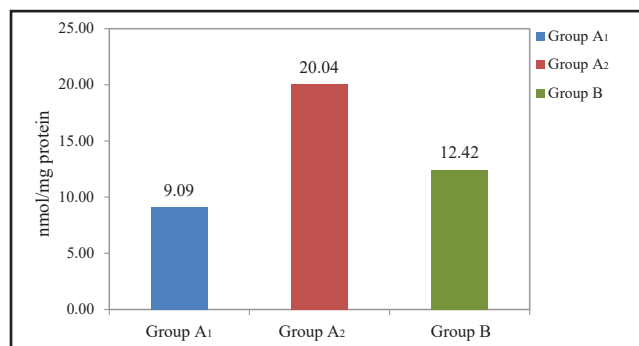
Group	MDA (nmol/mg protein)
A ₁ (n=10)	9.09 ± 2.34 (5.54 - 12.70)
A ₂ (n=10)	20.04 ± 3.04 (15.98 - 24.70)
B (n=10)	12.42 ± 2.60 (8.98 - 16.11)

Multiple comparisons

	MDA p value
A ₁ vs A ₂ vs B	<0.001 ^{***}
A ₁ vs A ₂	<0.001 ^{***}
A ₁ vs B	0.029*
A ₂ vs B	<0.001 ^{***}

Data are expressed as mean ± SD. For statistical analysis, one way ANOVA test was performed for comparison among the groups and then post hoc-Bonferroni test to compare between groups. Figures in parentheses indicate ranges. **Group A₁**: Baseline control group, **Group A₂**: Gentamicin treated control group, **Group B**: Experimental group (Green tea pretreated and gentamicin treated group). N = Total number of rats; n= number of rats in each group; ns = not significant; * = significant at p-value <0.05; ** = significant at p-value <0.01; *** = significant at p-value <0.001

Figure 1 : Mean MDA level in different groups of rats (N=30)



Group A₁: Baseline control group, **Group A₂:** Gentamicin treated control group, **Group B:** Experimental group (Green tea pretreated and gentamicin treated group)

In this study, histological examination of kidney revealed normal findings in 100% of rats in group A₁. Whereas abnormal histological finding were observed in 100% of rats in group A₂. Again, 70% of rats in group B showed almost normal structure whereas 30% of them showed mild histological changes in kidney. There were significant differences in histopathological findings of kidney among group A₁ vs group A₂ and group A₂ vs group B. whereas there was no statistically significant difference in the histopathological findings of kidney between group A₁ and group B (Table V, Figure 2).

Table V: Mean distribution of rats by the histopathological findings in kidney (N=30)

Group	Histopathological finding	
	Normal	Abnormal
A ₁ (n=10)	10 (100.0)	0 (0.0)
A ₂ (n=10)	0 (0.0)	10 (100.0)
B (n=10)	7 (70.0)	3 (30.0)

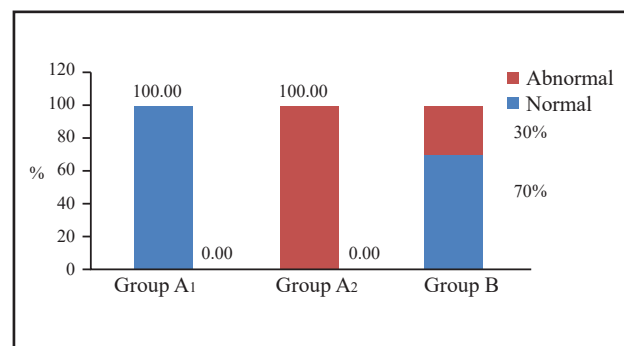
Multiple comparisons

	p-value
A ₁ vs A ₂	<0.001***
A ₁ vs B	0.210 ^{ns}
A ₂ vs B	0.003**

Statistical analysis was done by Fisher’s exact test. Figures in parentheses indicate percentage.

Group A₁: Baseline control group, **Group A₂:** Gentamicin treated control group, **Group B:** Experimental group (Green tea pretreated and gentamicin treated group). N = Total number of rats; n= number of rats in each group; ns = not significant; * = significant at p-value <0.05; ** = significant at p-value <0.01; *** = significant at p-value <0.001

Figure 2: Mean distribution of rats by histopathological changes in kidney (N=30)



Group A₁: Baseline control group, **Group A₂:** Gentamicin treated control group, **Group B:** Experimental group (Green tea pretreated and gentamicin treated group)

Discussion: The present study was carried out to evaluate the nephroprotective effect of green tea on gentamicin induced nephrotoxic rats. For the purpose of the study, serum levels of creatinine, urea, blood urea nitrogen, malondialdehyde (MDA) in kidney homogenate were measured to assess renal function. Moreover, histopathological examination of kidney was also done to observe the microscopical findings of the kidney.

In the present study, percent change of body weight was higher in green tea pretreated and gentamicin treated experimental group than that of gentamicin treated control group and baseline control group, although the differences were not statistically significant. Almost similar finding was observed by some other researchers³²⁻³³. Significant (p <0.001) reduction of rat body weight was found after induction of nephrotoxicity by insecticide (cyromazine and chlorpyrifos) in comparison to that of baseline control group³⁴. The researchers also observed significant (p<0.001) increase of rat body weight after green tea administration. The researchers suggested that this effect was may be due to toxic dose of insecticide and antioxidant effect of green tea.

In this study, kidney weight was significantly (p<0.001) higher in both gentamicin treated control group and green tea pretreated and gentamicin treated group (p<0.01) in comparison to that of baseline control group and also supported by different researchers³⁵. Kidney weight of EGCG (epigallocatechin-3-gallate) pretreated and fluoride treated group was found higher (p<0.01) than control group experimented by Thangapandiyan and Miltonprabu. This may be due to nephrotoxic effect of EGCG on the fluoride induced oxidative damage of the renal tubular cells³⁶.

In this study, serum creatinine level was significantly (p<0.001) higher in gentamicin treated control group and also in green tea pretreated and gentamicin treated group in comparison to that of baseline control group. Almost similar finding was observed by different researchers^{32, 37-39}. Furthermore, serum creatinine level was significantly (p <0.001) lower in green tea pretreated and gentamicin treated

group than that of gentamicin treated control group. Similar finding was observed by different researchers⁴⁰⁻⁴³. On the contrary, significant ($p < 0.01$) increase in serum creatinine level after administration of 1% green tea polyphenol (GTPs) in comparison to that of baseline control group. The researchers suggested that this may be due to reactive oxygen species generated by high dose of EGCG (epigallocatechin-3-gallate) that may have resulted in renal injury⁴⁴.

In this study, serum urea was significantly ($p < 0.001$) higher in gentamicin treated control group and also in green tea pretreated and gentamicin treated group than that of baseline control group. Almost similar finding was observed by different researchers⁴⁵⁻⁴⁸. Again, serum urea level was significantly ($p < 0.001$) lower in green tea pretreated and gentamicin treated group than that of gentamicin treated control group. Almost similar finding was observed by different investigators^{30, 33, 38, 49}.

In this study, serum BUN was significantly ($p < 0.001$) higher in gentamicin treated control group and also in green tea pretreated and gentamicin treated group in comparison to that of baseline control group. Almost similar finding was also observed by different researchers⁵⁰⁻⁵¹. Again, serum BUN was significantly ($p < 0.001$) lower in green tea pretreated and gentamicin treated group than that of gentamicin treated control group. Almost similar finding was observed by other researchers^{41, 52}.

In this study, Malondialdehyde (MDA) is one of polyunsaturated fatty acids peroxidation in the renal cells. An increase in free radicals causes overproduction of MDA is a marker of oxidative stress and the antioxidant status. Gentamicin induces the release of iron from renal mitochondria causes formation of gentamicin-iron complex that leads to lipid peroxidation which is a potent source for generation of reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical in cortical mitochondria of kidney. These reactive oxygen species are considered to be important mediator in the mechanism of gentamicin induced nephrotoxicity which lead to tubular necrosis⁵³⁻⁵⁴.

Furthermore, reactive oxygen species produced by gentamicin causes activation of poly (ADP-ribose) synthase (PARS) and mesangial cell contraction occurs in renal proximal tubular cells result in both inflammatory cell infiltration and activation which ultimately causes renal dysfunction and injury⁵⁵⁻⁵⁶.

Again, significant increase of lipid peroxidation which is evidenced by increased MDA level and decrease the levels of antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione (GSH) after the treatment of gentamicin which indicate oxidative stress. As a result serum urea, serum creatinine and serum blood urea nitrogen levels were also increased^{32, 48}.

MDA concentration in kidney tissue homogenate was significantly higher in both gentamicin treated control group ($p < 0.001$) and green tea pretreated and gentamicin treated group ($p < 0.05$) in comparison to that of baseline control

group. Almost similar finding was observed by different other researchers^{29, 57-58}. Again, MDA concentration in kidney tissue homogenate was significantly lower in green tea pretreated and gentamicin treated group ($p < 0.001$) than that of gentamicin treated control group. Almost similar finding was observed by different researchers^{48, 59-60}. On the contrary, significant elevation of kidney MDA level after 1% GTPs (green tea polyphenol) administration. The researchers suggested that this effect was may be due to induction of oxidative stress by high dose of epigallocatechin-3-gallate⁴⁴.

Moderate histological changes such as presence of tubular necrosis, infiltration of lymphocytes, dilatation of tubules, loss of lining epithelium and numerous hyaline casts were observed in this study in gentamicin treated rats. Almost similar findings were also observed by some researchers^{36, 61-62}. On the other hand, only minimal histological changes of kidney were observed in 30% rats of green tea pretreated and gentamicin treated rats. These findings were also in agreement with those of different researchers of other countries^{5, 27, 63}.

Limitation

- Different doses of green tea were not used to find out the best effective dose.
- Anti-oxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) levels were not studied.
- Smaller sample size.

Conclusion

From this study it may be concluded that green tea has nephroprotective effect on gentamicin induced kidney damage in Long Evans male rats.

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