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Dietary Polyphenolics Supplementation with Drinking Black Tea Ameliorates Gentamicin-Induced Nephrotoxicity in Mice

A. Adhikari[1], A. K. Hazra[2], S. Bhattacharya[1], T. Seal[3], T. K. Sur[4*]

[1]*Department of Pharmacology, R.G. Kar Medical College, Kolkata 700 004, India*
[2]*Chemistry Division, R.K.M.A Quality Testing Laboratory, Narendrapur, Kolkata 700 103, India*
[3]*Plant Chemistry Department, B.S.I, Shibpur, Howrah 711 103, India*
[4]*Department of Pharmacology, I.P.G.M.E & R, Kolkata 700 020, India*

ABSTRACT

Black tea (*Camellia sinensis*) supplement on renal disorders has poorly been explored. The present study was aimed to identified essential polyphenols present in black tea and it's the role in gentamicin (GEN) induced nephrotoxicity in mice. The polyphenols present in 2.5% black tea infusion (BT) was determined by HPLC and antioxidant activity was assessed by DPPH radical scavenging. The renoprotective role of BT (125 mg/kg and 250 mg/kg orally for 7 days) was assessed in GEN (80 mg/kg, *i.p*, daily for 7 days) induced mice. BUN and creatinine was estimated in blood and lipid peroxides, glutathione, catalase and protein was determined in renal tissues. Ten polyphenols including catechin, caffeic acid, rutin, sinapic acid, ferulic acid, p-coumaric acid, myricetin, gallic acid, quercetin and kaempferol were identified and quantified in BT by HPLC. Moreover, it also exhibited powerful DPPH radical scavenging property (IC_{50} 74.75µg/mg black tea). Finally, BT not only significantly and dose dependently (p<0.05) lowered BUN and creatinine in blood and reduced lipid peroxides in kidney, but also eventually enhanced the cellular antioxidants, glutathione and catalase in renal tissues. Therefore, black tea could be a good source of polyphenols that may protect kidneys from gentamicin induced oxidative stress.

Keywords: Black tea, HPLC, Gentamicin, Nephrotoxicity, Antioxidant, Catechin.

INTRODUCTION

Black and green teas are the main commercial teas obtained from buds and leave of *Camellia sinensis* L. (family Theaceae) and are the most widely consumed beverage in the world after water. [1] Green tea is rich in flavonoids including catechins, mainly epigallocatechin 3-gallate, while black tea contains two major pigments, theaflavins and thearubigins. [2-3] A large number of *in vitro* and *in vivo* studies supported that tea polyphenols can provide health benefits such as, lowering hypertension, coronary heart disease, diabetes, cognitive dysfunction and cancer. [4-6] It has been reported that black tea extracts are potent scavengers of reactive oxygen and nitrogen species. [7] Although black tea is much more commonly consumed, studies of health benefits have been more focused on green tea. On the other hand, gentamicin (GEN) or aminoglycoside is a very effective antibiotic in treating gram-negative infections, but it causes acute renal

***Corresponding author: Dr. Tapas Kumar Sur,**
Senior Research Associate, Department of Pharmacology, Institute of Postgraduate Medical Education & Research, 244B Acharya J.C Bose Road, Kolkata 700 020, West Bengal, India; **Tel.:** +91-8017575428; **E-mail:** drtapaskumarsur@gmail.com
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failure in 10-20% of therapeutic courses. [8] GEN has been shown to enhance the generation of reactive oxygen species (ROS) in renal tubules, more specifically in the proximal part of the tubule that led to tubular necrosis. [9] Furthermore, GEN induced nephrotoxicity was ameliorated by antioxidant α-tocopherol. [10] Green tea polyphenols (EGCG) have shown beneficial effects on pathological states related to oxidative stress on the kidney and improved renal functions. [11-12] Black tea supplementation improves antioxidant status during oxidative stress [13-14], but unfortunately, the protective role of antioxidants from black tea has been poorly explored in renal diseases. Besides theaflavins and thearubigins, till date it is not apparent about other bioactive components, particularly polyphenols present in black tea for their biological activities. Thus, the aim of the present study was to searching all important polyphenols present in black tea and its role in gentamicin-induced nephrotoxicity in mice.

MATERIALS AND METHODS

Chemicals and Reagents

The standard chemicals like ascorbic acid, glutathione, catalase, BSA, DPPH, phenolic acids (gallic, methyl gallate, caffeic, syringic, p-coumaric, ferulic, and sinapic), flavonoids (catechin, rutin, myricetin, quercetin, apigenin and kaempferol) were purchased from Sigma Chemical Co. (St. Louis, MO , USA), gentamicin from Piramal Healthcare (Mumbai, India), biological kits (urea, creatinine) from Span Diagnostics Limited (Surat, India) and the HPLC-grade solvents such as chloroform, methanol, water, acetic acid and other reagents were purchased from Merck (India).

Animals

Swiss mice of either sex weighing 25-30 g were used in this study. The animals were kept in colony cages under identical housing conditions, *i.e.,* 12h light: 12h dark cycle, 50-60% humidity and 22-25ºC temperature. They were fed with commercial pellet diet made for rodents and water *ad libitum*. The care and maintenance of the animals were as per approved guidelines. [15] The Institutional Ethics Committee (Regd. 1443/PO/b/11/CPCSEA) approved the study.

Preparation of Tea Sample

Branded TATA Premium CTC (curl, tear and crush) black tea was obtained from the local market in Kolkata. Tea infusions were prepared according to previous studies. [16] In brief, accurately 1.25 g of black tea was transferred into a conical flask and 25 ml of deionized water previously brought to boiling point (90°C) and stirred slowly with magnetic stirrer for 15 min, cooled and the supernatant was filtered on a 0.45µ nylon filter. The tea leaves was extracted a second time with 25 ml boiling water and filtered. The two filtrates were combined (maximum 2.5% or 25 mg/ml aqueous tea extract) and used for study (BT). It was renewed every day during the experimental period.

In Vitro Antioxidant Activity

The radical scavenging activity of BT was determined using the stable radical DPPH (1, 1-diphenyl-2-picrylhydrazyl). In brief, 0.1 ml of BT at different known concentrations was mixed with 3.9 ml of DPPH solution (0.135 mM) and read after 30 min at 517 nm. The result was expressed as IC_{50}. [17]

HPLC Estimation of Phenolics

HPLC analyses of BT was performed with Dionex Ultimate 3000 liquid chromatograph (Germany) with four solvent delivery system quaternary pump (LPG 3400 SD) including a diode array detector (DAD 3000) with 5 cm flow cell, a manual sample injection valve equipped with a 20µl loop and Chromeleon 6.8 system manager as data processor. The separation was achieved by a reversed-phase Acclaim™ 120 C_{18} column (5µm particle size, i.d. 4.6 × 250 mm). The mobile phase contains 1% aqueous acetic acid solution (Solvent A) and acetonitrile (Solvent B), the flow rate was adjusted to 0.7 ml/min, the column was thermostatically controlled at 28°C and the injection volume was kept at 20µl. A gradient elution was performed by varying the proportion of solvent B to solvent A. The gradient elusion was changed from 10% to 40% B in a linear fashion for duration of 28 min, from 40 to 60% B in 39 min, from 60 to 90% B in 50 min. The mobile phase composition was put back to initial condition (solvent B: solvent A: 10: 90) in 55 min and allowed to run for another 10 min, before the injection of another sample. Total analysis time per sample was 59 min. HPLC Chromatogram was detected using a photo diode array UV detector at 272 nm according to absorption maxima of analyzed compounds. The individual standard solution was prepared separately at the concentration 1 mg/ml with methanol and mobile phase (1:1v/v). Each compound was identified by its retention time and by spiking with standards under the same conditions. The quantification of the sample was done by the measurement of the integrated peak area and the content was calculated using the calibration curve by plotting peak area against concentration of the respective standard. [18] The data were reported with convergence limit in six times. The high recovery rate in the range of 96-103% for the samples is indicative of efficacy and consistency. According to the USP and ICH guidelines, there are various parameters to validate the reproducibility of the method viz. the effectiveness, the limit of detection (LOD), the limit of quantization (LOQ), the linearity, the precision and the accuracy. [19]

Acute Oral Toxicity Study

Single dose toxicity study was done on female Swiss albino mice according to Organisation for Economic Co-operation and Development (OECD) guidelines No. 423 (2001) adopted for acute toxicity in animals up to 2 g/kg (limit test). [16, 20]

Gentamicin-induced Nephrotoxicity

Male mice were randomly assigned to five groups in 6 animals each as follows:

(1) Control group: these mice were received a daily oral dose of deionised water (10 ml/kg) for 7 consecutive days;

(2) BT group (*per se*): these mice were received a daily oral dose BT (10 ml/kg) for 7 consecutive days;
(3) GEN group: these mice were treated intraperitoneally with the GEN (Piramal Healthcare, Mumbai, India), 80 mg/kg daily for 7 days and received a daily oral dose of deionised water (10 ml/kg); [21]
(4) Simultaneous treatment with BT and GEN group: these mice were treated by BT daily orally (5 ml/kg ~ 125 mg/kg) after the GEN injection for 7 days;
(5) Simultaneous treatment with BT and GEN group: these mice were treated by BT daily orally (10 ml/kg ~ 250 mg/kg) after the GEN injection for 7 days.
These doses were selected on the basis of pilot studies. On day 8, after euthanizing all animals, blood samples were withdrawn by cardiac puncture and urea and creatinine were estimated spectrophometrically (Span Diagnostics Limited, Surat, India). Kidney tissues were dissected out and a portion was homogenized in cold phosphate buffer (0.1M, pH 7.2), centrifuged and used for estimation of lipid peroxides [22], glutathione [23], catalase [24] and protein. [25]

Statistical Analysis

The data were expressed as mean ± S.E.M. and analyzed by paired test using statistical software SPSS version 17 (IBM, Chicago, USA). For comparing means *p* value <0.05 was considered as statistically significant.

RESULTS

***In vitro* Antioxidant Activity**

BT exhibits strong DPPH radical scavenging action in dose dependant manner. The IC_{50} of BT is 74.75µg/mg of dry black tea and regression coefficient (r^2) is 0.993 (Fig. 1).

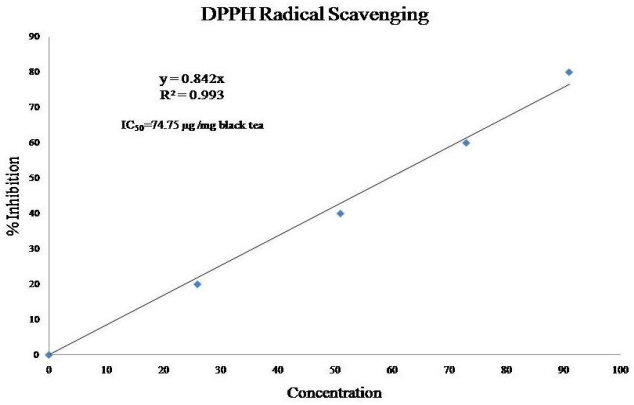

Fig. 1: DPPH radical scavenging activity of black tea

HPLC Estimation of Phenolics

HPLC chromatogram shows black tea contains 10 phenolics (Fig. 2). As shown in the chromatogram most of the investigated compounds had response at 272λ where they were successfully separated and quantified (Table 1). The quantification of phenolics obtains from integrated peak area and calibration curve by plotting peak area against concentration of the respective standard. Black tea has maximum catechin (1946.6µg/g), followed by caffeic acid (174.2µg/g), rutin (78.4µg/g), sinapic acid (71.1µg/g), ferulic acid (61µg/g), p-coumaric acid (39.45µg/g), myricetin (38.4µg/g), gallic acid (33.55µg/g), quercetin (29.05µg/g) and kaempferol (28.95µg/g).

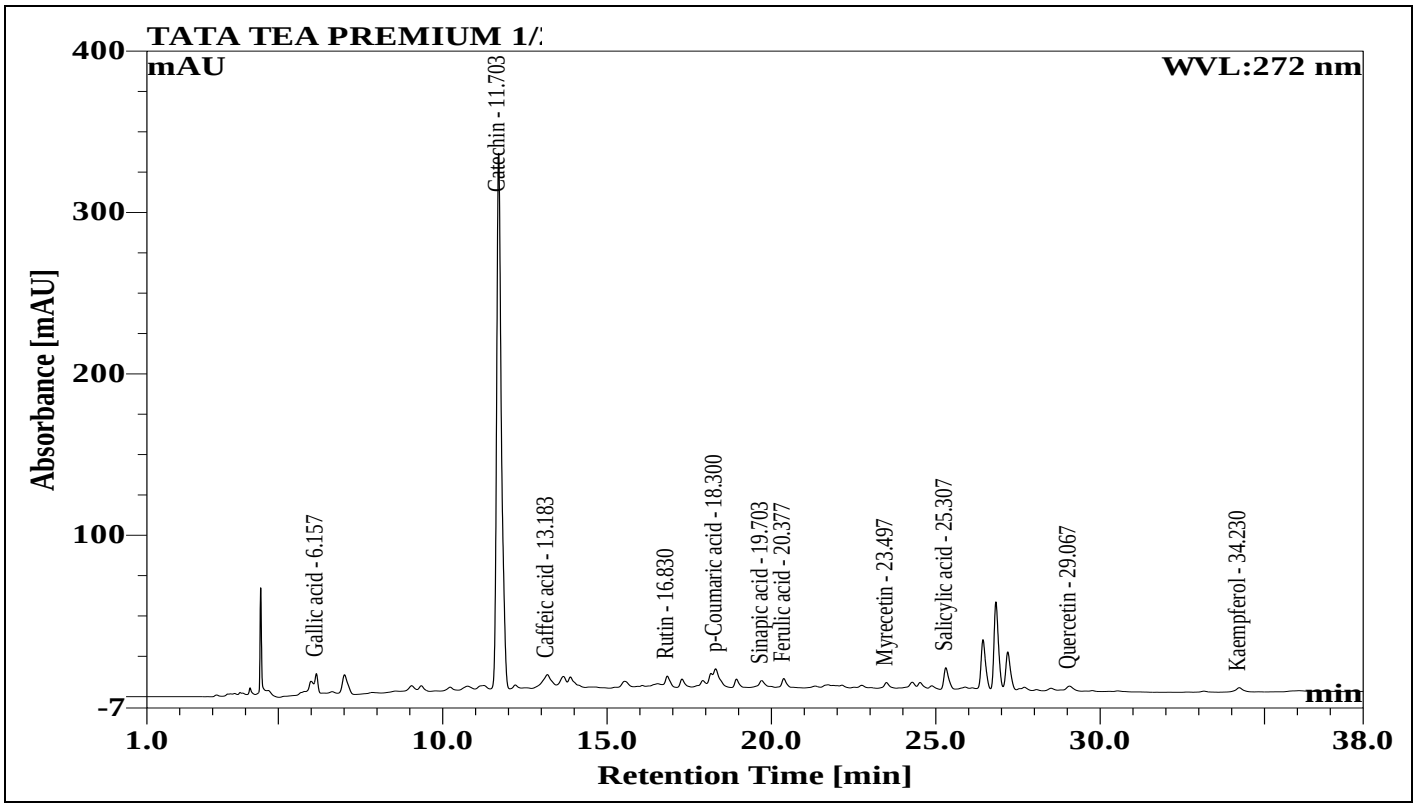

Fig. 2: HPLC chromatogram of black tea

Table 1: Integration and quantification of polyphenols present in black tea by HPLC

Retention Time (min)	Peak Name	Height (mAU)	Area (mAU*min)	Relative Area (%)	Regression coefficient (r^2)	Amount (µg/ml)	Absolute Amount (µg/g dry black tea)
6.16	Gallic acid	8.852	0.763	0.61	0.9956	1.342	33.55 ± 0.28
11.70	Catechin	332.032	55.561	44.27	0.9970	77.864	1946.6 ± 0.81
13.18	Caffeic acid	8.381	2.685	2.14	0.9969	6.968	174.2 ± 0.69
16.83	Rutin	5.973	0.904	0.72	0.9970	3.136	78.4 ± 0.48
18.30	p-Coumaric acid	5.397	0.794	0.63	0.9967	1.578	39.45 ± 0.31
19.70	Sinapic acid	4.261	0.971	0.77	0.9967	2.844	71.1 ± 0.46
20.38	Ferulic acid	5.434	0.833	0.66	0.9970	2.44	61 ± 0.52
23.50	Myricetin	3.525	0.618	0.49	0.9947	1.536	38.4 ± 0.39
29.07	Quercetin	2.842	0.679	0.54	0.9957	1.162	29.05 ± 0.17
34.23	Kaempferol	2.710	0.710	0.57	0.9906	1.158	28.95 ± 0.12

Detection wavelength 272λ; absolute value represents as Mean ± SEM; N=3 in each test

Table 2: Effect of black tea on gentamicin-induced nephrotoxicity in mice

	Control (10 ml/kg)	BT (10 ml/kg)	GEN (10 ml/kg)	GEN+BT (5 ml/kg)	GEN+BT (10 ml/kg)
Blood Urea (mg/dl)	16.50 ± 0.64	16.75 ± 0.47(a) [1.51]	73.25 ± 1.93(a)*** [343.9]	52.50 ± 3.32(b)** [-28.32]	33.50 ± 2.21(b)*** [-54.26]
BUN (mg/dl)	7.66 ± 0.30	7.76 ± 0.24(a) [1.3]	34.16 ± 0.90(a)*** [345.95]	24.48 ± 1.55(b)** [-28.33]	15.62 ± 1.03(b)*** [-54.27]
Blood Creatinine (mg/dl)	0.33 ± 0.017	0.34 ± 0.018(a) [3.03]	2.37 ± 0.16(a)*** [921.22]	1.76 ± 0.07(b)*** [-47.77]	1.51± 0.06(b)*** [-55.19]
Renal tissue lipid peroxide (nM/mg protein)	0.026 ± 0.0017	0.027 ± 0.0018(a) [3.84]	0.051 ± 0.0025(a)*** [96.15]	0.036 ± 0.0022(b)** [-29.41]	0.032 ± 0.0019(b)** [-62.74]
Renal tissue glutathione (nM/g wet tissue)	20.51 ± 0.85	21.46 ± 0.49(a) [0.23]	8.53 ± 0.25(a)*** [-60.34]	12.17 ± 0.63(b)*** [42.67]	15.10 ± 0.31(b)*** [77.02]
Renal tissue catalase (µmoles of H_2O_2 decomposed/min/mg protein)	0.55 ± 0.031	0.52 ± 0.030(a) [-5.45]	0.26 ± 0.019(a)*** [-52.72]	0.38 ± 0.012(b)*** [46.15]	0.43 ± 0.017(b)** [65.38]

Values represent the mean ± SEM; N=6; BT means black tea and GEN means gentamicin; all values are statistically analyses by paired test using SPSS v17 (IBM, Chicago, USA); (a) indicates compares with control and (b) with GEN; asterisks denote the significance level, ** means $p<0.01$ and *** means $p<0.001$

Acute Toxicity

No adverse action of BT shows 2.0 g/kg, *p.o* (limit lest) in mice.

Nephroprotective Activity

Per se administration of BT at the dose of 10 ml/kg/day (250 mg/kg/day), p.o in mice shows any significant change compare to normal control. But intraperitoneal administrations of gentamicin (GEN) in 7 successive doses alter all blood and tissue antioxidant profile related to renal injuries. GEN not only enhances BUN up to 345.95%, blood creatinine 921.22% and renal tissue lipid peroxides 96.15% but also reduces glutathione 60.34% and catalase 52.72% in renal tissue. Administration of BT at the dose of 5 ml/kg/day (~125 mg/kg/day), *p.o* with GEN for 7 consecutive days improved all these parameters significantly and effectively compared to GEN control mice. It lowers BUN up to 28.33%), blood creatinine 47.77%, renal lipid peroxides 29.41% and also enhances glutathione 42.47% and catalase 46.15%. Similar results ($p<0.001$) are observed after administration of BT at the dose of 10 ml/kg/day (~250 mg/kg/day), p.o treatment with GEN (Table 2). It lowers BUN 54.27%, blood creatinine 55.19%, renal lipid peroxides 62.74% and enhances glutathione 77.02% and catalase 65.38% (Table 2).

DISCUSSION

India is a major producer, consumer and exporter of tea accounting for one third of the total global production of tea. In Western world green tea is much preferable than black tea. Green tea contains mainly four catechins like epicatechin, epigallocatechin, epicatechin 3-gallate and epigallocatechin 3-gallate which have potent therapeutic values. [2] However, in India green tea accounts for only 1% of total production of tea and others are black tea, white tea, oolong tea, herbal tea, pu-erh etc. [16] Since there are very few studies which document the chemical identities and antioxidant potentialities of Indian black tea, an attempt is made to indentify other polyphenols and antioxidant capabilities of black tea and also the possible correlation between antioxidant and renoprotective activity. Nevertheless, diverse renal injury could have been taken place due to adverse drug reactions related to kidney function. Indeed, it is estimated that ~25% of the 100 most used drugs in intensive care units are potentially nephrotoxic, and that nephrotoxicity is responsible for 10-20% of acute renal failure cases. [26] A central aspect of gentamicin (aminoglycoside) nephrotoxicity is their tubular cytotoxicity. Treatment of experimental animals with gentamicin results in apoptosis as well as necrosis of tubular epithelial cells. [27-28] Another hypothesis suggests that renal damage is due to various apoptotic signals activated within the renal tissue due to free radical generation. [29] It has been reported that diet rich in polyphenolic substances reduces the risk of diseases associated with an increase in oxidative stress. [30-31] The beneficial effect of red wine polyphenols have been shown to reduce cyclosporine A induced nephrotoxicity. [32]

In this perspective polyphenols present in black tea has been studied. HPLC chromatogram of black tea

identifies and quantifies eleven polyphenols, including six phenolic acids *e.g.*, gallic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid and sinapic acid and five flavonoids *e.g.*, catechin, rutin, myricetin, quercetin, and apigenin. More than half of the polyphenols present in black tea are catechins. Earlier it has been observed that chatechins present in green tea ameliorates glucose toxicity and renal injury, thus alleviating renal damage caused by abnormal glucose metabolism-associated oxidative stress involved in renal lesions of diabetic nephropathy. [33] Furthermore, green tea catechin supplementation in diabetic rats also appear to inhibit the production of leukotriene B_4 based on regulating the activity of 5'-lipoxygenase, thereby potentially reducing renal oxidative damage and inflammatory reactions. [34] In the present study black tea confirmed its antioxidant activity by scavenging DPPH radical that possibly to presence of high content of polyphenols on it. Moreover, a concomitant administration of black tea for a week, to gentamicin receiving rats, markedly prevented the generation of thiobarbituric acid-reacting substances and significantly attenuated gentamicin-induced renal dysfunction as assessed by estimating serum creatinine and blood urea nitrogen. A considerable improvement in terms of reduced glutathione content and activity of antioxidant enzyme catalase in the kidney homogenate was observed.

From the above findings it may conclude that supplementation of black tea - a rich source of polyphenols could be helpful in reducing gentamicin-nephrotoxicity. Further studies are necessary to confirm its underlying mode of action.

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