Supplementary Information

Supplementary Method Description

Blood Processing:

Blood for liver function test was collected in a serum separation vacutainer and delivered to the UCLA clinical laboratory. Blood for HPLC analysis was collected in EDTA vacutainers. The blood was centrifuged at 1780xg (3000 rpm) for 15 minutes at room temperature. Plasma was frozen in aliquots of 1.5 ml mixed with 30 µl of ascorbic acid mix1 (20% ascorbic acid, 0.1% EDTA, 0.04 M NaH_2PO4 (pH 3.6), final pH 2) or without ascorbic acid. Buffy coat was separated from red blood cells and frozen at -80ºC. 3 ml of red blood cells were mixed with 6 ml of cold phosphate buffered saline (PBS) followed by centrifugation for 10 minutes at 790xg (2000 rpm). Supernatant was discarded and RBCs were washed two more times with PBS and frozen at -80ºC in aliquots.

Urine processing:

During the screening visit participants provided a spot urine sample. Participants were instructed to collect a morning first void urine sample at home on the morning of the 3 week timed blood draw and day of prostatectomy. Urine collection cups with 0.1 g of ascorbic acid were provided. Prior to freezing 6 ml of urine was mixed with 30 µl of ascorbic acid mix1 (defined above in blood processing). The pH of urine samples was determined and additional ascorbic acid mix1 was added if pH was higher than pH 5.

Prostate tissue processing:

Following radical prostatectomy, the pathologist provided a small aliquot of prostate tissue for research. Prostate tissue was aliquoted into 200-300 mg pieces and flash frozen in liquid nitrogen. Once frozen, tissue samples were transferred to a -80 ºC freezer.

Prostate, blood and urine processing for HPLC and LC-MS/MS analysis.

Tea polyphenols (GTPs) in plasma and prostate were analyzed by HPLC with coularray detection [9]. Urine GTPs and urine, plasma and prostate quercetin (Q) were analyzed by LC-MS/MS[30].

Prostate: 300 mg of fresh frozen prostate tissue was cut into small pieces with a razor blade and homogenized using an Ika ultra-turrax homogenizer (Ika Works, Wilmington NC) in 300 µl of 2% ascorbic acid (AA) in water and 20 µl of catechin gallate (1 ppm) as internal standard. Homogenate was cooled intermittently to avoid overheating. 200 µl of sodium acetate buffer (0.2 M, pH 5), 1,000 units of β-glucuronidase (G8420, Sigma Aldrich, St Louis, MO) and 20 units of sulfatase (S9754, Sigma Aldrich) were added to the homogenate, mixed and incubated at 37°C for 60 min. During incubation the homogenate was mixed every 15 minutes. The homogenate was extracted with 1 ml ethylacetate (EA), vortexed for 5 minutes and centrifuged at 8,600xg (10,000 rpm) for 5 minutes. 800 µl of EA supernatant was transferred to a new glass tube and 20 µl of 2% AA in methanol was added to the glass tube. The homogenate was extracted two more times with 1 ml EA and supernatants combined in the glass tube. The EA extract was dried in a speedVac (Savant, Thermo Fisher, Waltham MA). The pellet was reconstituted in 200 µl reconstitution buffer (7 ml of mobile phase A+3 ml of mobile phase B and 0.5 ml 20% ascorbic acid/0.5% EDTA sol). Mobile phase A consisted of citric acid (75mM) and ammonium acetate (25 mM) pH 2.8 and mobile phase B consisted of 50% mobile phase A and 50% acetonitrile. Reconstituted prostate extract was centrifuged at 16,873xg (14,000 rpm) for 10 min and filtered using 0.45 µm PTFE single use filters (Thomson Instrument Company, Oceanside, CA) prior to HPLC analysis.

Plasma: 200 µl plasma was combined with 20 µl AA mix2 (20% AA, 0.5 M NaH2PO4, 0.1% EDTA, pH 5). 500 U of β-glucuronidase from E coli (G8420, Sigma Aldrich) and 14 U of sulfatase from abalone entrails (S9754, Sigma Aldrich) were added and mixture was incubated for 45 minutes at 37ºC. GTPs were extracted with 800 µl of EA and vortex mixing for 5 minutes, centrifugation at 8600xg (10,000 rpm) for 5 minutes. The top layer was transferred to a new glass tube and 20 µl of 2% AA in methanol was added. The remaining plasma mix was extracted two more times with 700 µl EA. Supernatants were combined and dried in speedVac (Savant, Thermo Fisher). Pellet was reconstituted with 200 µl of the same reconstitution solution as used for prostate above. Reconstituted extracts were centrifuged at 16,873xg (14,000 rpm) for 10 min and filtered using 0.45 µm PTFE filters (Thomson Instrument Company) prior to injection in HPLC.

Urine: 250 µl urine was mixed with 50 µl of AA mix3 (10% AA, 0.5 M NaH2PO4, 0.05% EDTA, pH 6), 24 U β-glucuronidase (G8420, Sigma Aldrich) and 27 U sulfatase from abalone entrails (S9754, Sigma Aldrich) and incubated for 2 hours at 37°C. GTPs were extracted using solid phase extraction C18 cartridges Sep-Pak Vac RC, 500 mg (Waters, Milford, MA). The cartridges were prepared by flushing with 3 ml methanol, 3 ml water and 3 ml sodium acetate (0.2 M, pH 5). Urine mix was loaded, washed with 3 ml water and 3 ml 5% methanol in water. Columns were dried and eluted with 4 ml methanol. 20 µl of 2% AA in methanol was added and mixture was dried in the speedVac (Savant, Thermo Fisher). Pellets were reconstituted with 200µL 50% methanol in water. Reconstituted urine extracts were centrifuged at 14,000 rpm for 10 min and filtered using 0.45 µm PTFE filter (Thompson Instrument Company) prior to LC-MS/MS analysis of EGC, EC, 4'-MeEGC, quercetin and isorhamnetin.

HPLC analysis with coularray detection: The HPLC-electrochemical detection system consisted of an Agilent 1100 Series quaternary pump solvent delivery system (Agilent Technology, San Diego), an Agilent 1100 temperature regulated autosampler, an Agilent 1100 Chemstation Software 9.01, an ESA5600A coulometric array electrochemical detector (ESA, Bed-ford, MA) with coularray detection cells (Model 6210), a C18 Alltima guard column (7.5 mm x 4.6 mm, particle size of 5μm; Alltech, Deerfield, IL), and a C18 Alltima Rocket ™ column (53 mm x 7 mm, particle size of 3 μm; catNo 50605, Alltech). The column was eluted with a linear gradient from 100% mobile phase A (75mmol citric acid/25mmol ammonium acetate) to 90% mobile phase A and 10% mobile phase B (75mmol/L citric acid and 25 mmol/L ammonium acetate:acetonitrile, 50:50) in 4 min at a flow rate of 1 mL/min. The gradient was changed from 90% mobile phase A and 10% mobile phase B to 70% A and 30% B (4–12 min), 66%A/34%B (12–17 min), 63% A/37%B (17–20 min), 57% A/43% B (20–29 min), 100% B (29–33min), and 100% A (33–35 min) and maintained at 100% A until analysis of the next sample. The eluent was monitored by coularray detection with potential settings at -90, 10, 70, 150, 230, 310, 400, and 480 mV. The dominant channel for GTP was 230 mV.

Liquid Chromatography Mass Spectrometry (LC-MS/MS) analysis of quercetin in prostate, plasma and urine and EGC, EC and MeEGC in urine:

The HPLC with electrospray ionization mass spectrometry (ESI/MS) system consisted of an LCQ Advantage Finnigan system (ThermoFinnigan, San Jose, CA), equipped with a Surveyor LC system consisting of an autosampler/injector, quaternary pump, column heater, and diode array detector (DAD) with Xcalibur 1.2 software (Finnigan Corp., San Jose, CA) as described previously [30] . The HPLC column was an Agilent Zorbax Sb-C18 3.5μm (150 x 2.1 mm). The

flow rate was 0.2 mL/min and gradient elution was used. The gradient started at 5% solution A (acetonitrile) and 95% solution B (1% acetic acid/water) increasing over 40 min to 60% solution A and 40% solution B. MS parameters: Ionization negative mode ESI^-; spray voltage, 5kV; sheath gas, 40; and capillary temperature, 275 ºC. Peak identities were obtained by matching their most intense fragment ion 301/179 (for quercetin), 315/300 (for methyl quercetin), 289/245 (EC), 319/301 (methyl EGC) and 457/287 (EGCG).

RTqPCR of COMT, DNMT in prostate tissue:

30 mg of prostate tissue was homogenized in a bead tube with 600 µl of Qiagen RLT buffer in the Beadmill Homogenizer (Fisher Scientific). RNA was extracted using the RNeasy extraction kit (Qiagen, Germantown, MD) according to manufacturer's instructions. RNA concentration was determined using the Nanodrop (Fisher Scientific). C-DNA synthesis was performed by heating 1 ug RNA with oligoDT(12-18) (Invitrogen) and dNTP mix (Promega) for 5 minutes at 65 ºC and placed on ice. Superscript III reverse transcriptase (Invitrogen), DTT 100 mM) and first strand buffer were mixed and incubate at 50 ºC for 1 hr and 70º C for 15 min according to Invitrogen protocol. RTqPCR was performed using Taqman® gene expression assay kits (ID: Hs02511558_s1 for COMT, and ID: Hs00154749_m1 for DNMT1) (Applied Biosystems, Foster City, CA). cDNA, 2X TaqMan universal PCR master mix, 20X gene expression assay mix and water were incubated in the 7900HT Fast Real-Time System (Applied Biosystems) for 40 cycles of 95ºC for 0.15 min and 60ºC for 1 min according to Applied Biosystem protocol. The $2^{-(\Delta\Delta Ct)}$ method was used to normalize the expression of COMT and DNMT1 in each sample to GAPDH expression and to compare to the average ΔCt value.

Supplementary Table 1. Liver function test at baseline and 3-week visit in participants from the GT+PL and GT+Q groups.

	GT+Placebo		p-value	GT+Quercetin		p-value
	BL	3 week		BL	3 week	
AP	68.6±16	69.9±18	0.46	65.8±19	67.0±17	0.51
AST	21.5±4	24.3±6	0.08	25.4±8	26.3±6	0.54
ALT	21.4±5	21.9±6	0.67	25.1±8	27.7±10	0.17

AP alkaline phosphatase, AST aspartate aminotransferase, and ALT alanine aminotransferase. Number of participants: GT+PL =16, GT+Q =15.

Supporting Table 2. List of adverse events experienced by participants in the GT+PL and GT+Q group.

Adverse Event	**Group assignment** GT+PL GT+Q	**Severity** 1-Mild 2-Moderate 3-Severe 4-Life-threatening or disabling 5-Death
Headache	GT+Q	2
Weight Gain	GT+Q	1
Left Sided Flank Pain	GT+Q	1
Constipation	GT+Q	1
Paroxysmal Atrial Fibrillation	GT+PL	1
Bilateral Lower Extremity Edema	GT+Q	1
Numbness and Tingling in the Left Thumb, Middle and Index fingers	GT+Q	1
Weight gain	GT+Q	1
Headache	GT+Q	1
Dry Mouth	GT+Q	1
Nausea	GT+PL	1
Insomnia	GT+Q	1
Cholecystitis	GT+PL	1

Number of participants: GT+PL =16, GT+Q =15.