The effect of green tea on opposing toxicity of acrylamide on kidney function

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Abstract

Purpose – The purpose of this paper is to investigate the effects of green tea extract on kidney function tests, in male rats that received different doses of acrylamide (AA).

Design/methodology/approach – Animals were dispensed at random to one of the following treatments: group 1 served as control, whereas groups 2, 3 received seven, 14 mg/100 g B.W/day of AA, respectively, in drinking water for 15 and 30 days. Group 4 received green tea 1.5 percent concentration and groups 5, 6 received seven, 14 mg/100 g B.W/day in a mixture with green tea for 15 and 30 days.

Findings – Serum urea and creatinine significantly increase with AA. However, Total protein, albumin and A/G ratio showed significant drop in all treated groups when compared with control. Supplementation of rats with antioxidant (green tea) enhanced the general health condition, reduced the severity of genotoxic effect and the alteration in blood and serum parameters produced by AA.

Practical implications – The authors suggest that green tea may deliver a cushion for long therapeutic option against toxins-induced nephrotoxicity without damaging side effects.

Originality/value – The study uses green tea as a natural antioxidant source. Epigallocatechin-3 gallate is the most plentiful catechin preserved in green tea and a high source of flavonoids. Flavonoids are a group of phenolic products of plant metabolism with high antioxidant properties to reduce nephrotoxicity without side effects.

Keywords Urea, Acrylamide, Green tea, Albumin, Creatinine, Globulin

Paper type Research paper

Introduction

Acrylamide (AA) (2-propenamide) monomer might constitute in some foodstuffs prepared at elevated temperatures (Surdyk *et al.*, 2004). The highest concentrations of AA have been recognized in grain-based foods, which are cooked at high temperatures such as baking, frying or grilling (Tareke *et al.*, 2002). AA is assumed to produce in foodstuffs mainly from the reaction of asparagines amino acid with some carbohydrates like glucose (Taubert *et al.*, 2004). AA is found to have important binding power to kidney, liver, erythrocyte and brain. After absorption glutathione-S-transferase (GST), conjugated to N-acetyl-S- (3-amino-3-oxopropyl) cysteine (Sumner *et al.*, 1997). Based on the international agency for research on cancer (IARC, 1994), AA is considered as a group 2A carcinogen. Calleman *et al.* (1990) revealed that AA toxic effect production, could be elucidated by its metabolism into glycidamide (2E1) through the P450 (CYP450) cytochrome route to give 2E1, forming a DNA-reactive epoxide. Johnson *et al.* (1986)

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reported that reaction with receptors or enzymes could induce changes in signal pathways and cellular functions, leading to carcinogenesis. AA triggers oxidative injury by helping making the reactive oxygen species, which boosted the generation of lipid peroxidase lessening the antioxidant protection system (Dobrzynska *et al.* (2004)). Oxidative stress is known as one of the significant means of AA toxic effects.

Free radicles that are produced through oxidation reactions start chain effects resulting in cell injury or necrosis. The antioxidants cease these adverse effects by eliminating the intermediates of free radical, in addition to stopping further oxidatative reactions. Antioxidants do this by being oxidized themselves, as they are reducing agents (Sies (1997)). According to Graham (1992) Epigallocatechin-3 gallate antioxidant is the greatest catechin found in large and preservative amounts in green tea, which is considered as a one of the rich flavonoids' sources. Flavonoids are compounds that considered "as a big gathering of phenolic stuffs of plant metabolism with high antioxidants properties" (Schmdit *et al.*, 2005). This study was done to study the green tea extract effects on some kidney functions in rats received altered acute AA doses.

Materials and methods

Materials

Green tea: obtained from homegrown market. It was freshly made and gives for rat tow times at concentration of 1.5 percent of as the only source of drinking water (Mohamdin *et al.,* 2005).

AA: AA was purchased from Sigma chemical Co. with 99.9 purity. Two dissimilar concentrations AA were given to rat (7 and 14 mg/100 g B.W.) (Lafferty *et al.,* 2004).

Animal: in this study, 60 mature albino male rats (two to four months) of body weight range 120-140 g body weight were used. Normal ration for rats was fed for the control group.

Methods

Experimental design: different doses were distributed to six rats' groups each of ten. The control group (G1) is the first one. The (G2) second group and the (G3) third one were received seven, 14 mg/100 g B.W./day of AA, respectively, in drinking water for a time of 15 plus 30 days. Whereas the fourth group (G4) rats had 1.5 percent green tea as the only source of water from the initial day-to-day 30, the fifth (G5) plus sixth (G6) groups had seven, 14 mg/100 g B.W./day AA, respectively, for 15 and 30 days and 1.5 percent green tea from the first day-to-day 30.

Blood sampling: at 15 and 30 days, five blood samples were collected from eye plexus of the rats from each group, in sterile, dry labeled and centrifuge tubes. Separation of serum was done via centrifugation for ten minute at 3,000 r.p.m.

Ration analysis: the ration was analyzed to determine crude protein and crude fiber content of ration according to Association of Official Agricultural Chemist (AOAC) (1995). Fat content described by Horwitz (1980), Aflatoxin content described by Nabney and Nesbitt (1965) Deleated and moisture and Ash determination described according to Horwitz (1980).

Green tea extract preparation: it was done in consistent with Maity *et al.* (1998) and this was done by socking 5 g of green tea powder in one liter of boiling distilled water for five minutes then filtered to provide solution of green tea (1.5 percent). Green tea extracted was supplied as the only drinking water source to rats.

Biochemical analysis: analysis of serum samples was done for determining the quantities of total protein according to Weichselpaum (1946), protein fractions

according to Redman (1969), serum urea according to Beale and Croft (1961), creatinine according to Faulkner and King (1976) and uric acid according to Fossatti and Prencippe (1982).

Urea was determined on fresh sample while other parameters were estimated within seven days of sampling.

Statistical analysis was carried out according to Snedecor (1946).

Results

Kidney function

Urea. It was observed from Table I that the rats received AA showed significant increase in serum urea on both doses (seven, 14 mg/100 g B.W./day) (G2 & G3), respectively, at 15 days with the mean values of 46.49 ± 3.60 ($p < 0.05$) and 51.62 ± 4.07 ($p < 0.01$), respectively, in comparison with (control) non-treated rats (G1) with the mean values of 34.10 ± 3.35. Whereas a significant increase on serum urea levels displayed also at 30 days with the rats that received AA on dose 14 mg/100 g B.W./day (G3) with the mean values of 49.54 ± 3.06 ($p < 0.05$) in comparison with control rats (G1) with the mean value of 35.90 ± 3.80. Rats, which had green tea extract merely (G4) does not showed effect on activities of enzymes next to 15 and 30 days. The mean values were 30.46 ± 2.44 and 32.12 ± 3.21compared to non-treated (control) rats (G1) (34.10 ± 3.35 and 35.90 ± 3.80), respectively, which indicated that the antioxidant green tea had effects of such enzymes, however it was less than that had AA. The (G5) group that had (1.5 percent) green tea extract with 7 mg/100 g B.W./day AA for 15 and 30 days, decreased the AA effect on injuring cell with mean values 40.02 ± 2, 17 and 38.28 ± 3.91, respectively. The rats of (G6) which had green tea extract of (1.5 percent) with 14 mg/100 g B.W./day AA, respectively, for 15 and 30 days similarly decreased the effect of AA on destructing cell with mean values of 45.33 ± 3.90 and 42.11 ± 4.41, however it was less than (G5) that received (1.5 percent) green tea extract with 7 mg/100 g B.W./day AA for 15 and 30 days.

Creatinine. Table II indicated that the rats received AA elicited significant increase in creatinine on both doses (seven, 14 mg/100 g B.W./day) (G2 and G3) at 15 days of the mean values of 1.28 ± 0.12 ($p < 0.05$) and 1.44 ± 0.18 ($p < 0.01$), respectively, in comparison with (control) non-treated rats (G1) of the mean values of 0.86 ± 0.09. The two previous doses showed were a non-significant increase next to 30 days with the mean values of 1.25 ± 0.15 and 1.33 ± 0.18 compared with non-treated rats with the mean value 0.91 ± 0.09. Rats received green tea extract only (G4) showed no enzyme activities after 15 and 30 days. The mean values were 0.99 ± 0.10 and

Studied groups	Urea mg/dl 15 days	30 days
G1	34.10 ± 3.35	35.90 ± 3.80
G2	46.49 ± 3.60*	43.00 ± 4.81
G3	51.62 ± 4.07**	49.54 ± 3.06*
G4	30.46 ± 2.44	32.12 ± 3.21
G5	40.02 ± 2.17	38.28 ± 3.91
G6	45.33 ± 3.90	42.11 ± 4.41

Notes: $n = 5$. *$p < 0.05$; **$p < 0.01$

Table I. Effect of green tea extract on serum urea, in rats orally administrated AA (7 and 14 mg/100 g B.W./day for 15 and 30 days)

1.01 ± 0.11 compared to non-treated (control) rats (G1) (0.86 ± 0.09 and 0.91 ± 0.09), respectively, which indicated that the antioxidant green tea had activities of such enzymes but was fewer than that received AA. (G5) that had green tea extract (1.5 percent) with 7 mg/100 g B.W./day AA for 15 and 30 days, decreased the effect of AA on injuring cell with mean values 1.12 ± 0.13 and 1.07 ± 0.11, respectively. The rats in (G6) which had (1.5 percent) green tea extract with 14 mg/100 g B.W./day AA, respectively, for 15 and 30 days similarly decreased the effect of AA on injuring cell with the mean values of (1.21 ± 0.12 and 1.11 ± 0.13), respectively.

Serum uric. Table III displayed that there is a non-significant increase in serum uric with rats which had AA for both doses of (seven, 14 mg/100 g B.W./day) (G2 and G3) for 15 days with the mean value 2.07 ± 0.13 and 2.12 ± 0.16, respectively, compared to non-treated (control) rats (G1) with the mean values of 1.73 ± 0.14 and for 30 days, with the mean value of 2.2 ± 0.15 and 2.34 ± 0.12 in comparison with non-treated control (G1) with the mean values of 1.70 ± 0.13. Rats that received green tea extract only (G4) showed no significant effect on serum uric after 15 and 30 days with the mean value of 1.68 ± 0.11. This indicated that green tea (antioxidant) had no effect on increasing serum uric.

Serum protein

Total protein. Table IV showed in (G2 and G3) there is significant drop in serum Total protein on doses of (seven, 14 mg/100 g B.W./day) on the 15 days, with the mean values of 5.75 ± 0.15 ($p < 0.05$) and 5.06 ± 0.50 ($p < 0.05$), respectively, in comparison with the control non-treated rats (G1) with the mean values of 6.80 ± 0.36. At 30 days there is significant decrease in serum total protein on the dose 14 mg/100 g B.W./day with the mean 5.90 ± 0.36 ($p < 0.05$). Whereas there is none significant, reduction in total protein on dose

Table II.
Effect of green tea extract on serum createinin in rats orally administrated AA (7 and 14 mg/100 g b.wt./day for 15 day)

Studied groups	Creatinine mg/dl 15 days	30 days
G1	0.86 ± 0.09	0.91 ± 0.09
G2	1.28 ± 0.12*	1.25 ± 0.15
G3	1.44 ± 0.18**	1.33 ± 0.18
G4	0.99 ± 0.10	1.01 ± 0.11
G5	1.12 ± 0.13	1.07 ± 0.11
G6	1.21 ± 0.12	1.11 ± 0.13

Notes: $n = 5$. *$p < 0.05$; **$p < 0.01$

Table III.
Effect of green tea extract on serum uric in rats orally administrated AA (7 and 14 mg/100 g B.W./day for 15 days)

Studied groups	Uric mg/dl 15 days	30 days
G1	1.73 ± 0.14	1.70 ± 0.13
G2	2.07 ± 0.13	2.2 ± 0.15
G3	2.12 ± 0.16	2.34 ± 0.12
G4	1.68 ± 0.11	1.76 ± 0.13
G5	1.91 ± 0.09	1.99 ± 0.11
G6	1.98 ± 0.13	2.01 ± 0.12

Notes: $n = 5$. G5 &G6 that had 1.5 percent green tea extract with 7 & 14 mg/100 g B.W/day AA for 15 and 30 days, respectively, showed a none significant effect in reducing serum uric

7 mg/100 g B.W./day with the mean values of 6.08 ± 0.34 compared with control non-treated (G1) 7.03 ± 0.31. The same results were observed for rats received green tea extract but the alteration in the serum protein was moderate. Rats, which had only, green tea extract (G4) showed no effect on total protein after 15 and 30 days with the mean values of 6.99 ± 0.45 and 6.90 ± 0.27 compared to non-treated (control) rats (G1) (6.80 ± 0.36 and 7.03 ± 0.31), respectively. (G5) which had (1.5 percent) green tea extract with 7 mg/100 g / B.W./day AA for 15 and 30 days, showed a non-significant effect in decreasing the effect of AA on injuring cell with mean values 6.11 ± 0.23 and 6.33 ± 0.15, respectively. Rats in (G6) given (1.5 percent) green tea extract with 14 mg/100 g B.W./day AA, respectively, for 15 and 30 days decreased the effect of AA on destroying cell significantly with the mean values 5.86 ± 0.31 ($p < 0.05$) and 6.17 ± 0.26 ($p < 0.05$), respectively.

Albumin g/dl. Table IV also showed that there is a significant drop in Albumin on the 15 days on both doses (seven, 14 mg/100 g B.W./day) (G2 and G3), with the mean values of 1.39 ± 0.09 ($p < 0.01$) and 1.26 ± 0.16 ($p < 0.01$), respectively, in comparison with control non-treated group (G1) with the mean values of 1.93 ± 0.14. At 30 days there is significant decrease in serum albumin on both dose seven, 14 mg/100 g B.W./day with the mean 1.60 ± 0.10 ($p < 0.05$) and 1.57 ± 0.13 ($p < 0.05$) in comparison with non-treated control (G1) 2.06 ± 0.17. The rats in (G4) had only green tea extract showed no effect after 15 days on Albumin with the mean values 1.91 ± 0.21 compared to (G1) non-treated rats with the mean value 1.93 ± 0.14 whereas after 30 days there is a significant reduction in albumin with the mean values 1.93 ± 0.19 ($p < 0.05$). (G5) which had (1.5 percent) green tea extract with 7 mg/100 g B.W./day AA during 5 and 30 days, showed a non-significant result in lessening the effect of AA on injuring cell with mean values 1.50 ± 0.17 and 1.76 ± 0.14, respectively. The rats in (G6) given (1.5 percent) green tea extract with 14 mg/100 g B.W./day AA, respectively, at 15 days revealed a significant effect in lessening the effect of AA on injuring cell with the mean values 1.46 ± 0.12 ($p < 0.05$) whereas there is no significant effect at 30 days with mean values 1.64 ± 0.13.

Globulin. Table IV reveals that AA has no effect on globulin at the two doses (seven, 14 mg/100 g B.W./day) in (G2 and G3) for 15 days with the mean values of 4.36 ± 0.14 and 3.80 ± 0.24, respectively, in comparison with (control) non-treated rats (G1) with the mean values of 4.87 ± 0.42. The same result was observed at 30 days, as there is no significant change in globulin on both dose seven, 14 mg/100 g B.W./day with the mean 4.48 ± 0.18 and 4.33 ± 0.21compared with non-treated control (G1) with the mean value 4.97 ± 0.32. Rats received green tea extract only (antioxidant). (G4) had no effect on Albumin after 15 and 30 days with the mean values 5.08 ± 0.23 and 4.97 ± 0.19 compared to non-treated (control) rats (G1) with the mean value 4.87 ± 0.42 and 4.97 ± 0.32, respectively, (G5) which had (1.5 percent) green tea extract with 7 mg/100 g B.W./day AA for 15 and

Studied groups	Total protein g/dl		Albumin g/dl		Globulin g/dl		A/G Ratio	
	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days
G1	6.80 ± 0.36	7.03 ± 0.31	1.93 ± 0.14	2.06 ± 0.17	4.87 ± 0.42	4.97 ± 0.32	0.40 ± 0.02	0.41 ± 0.02
G2	5.75 ± 0.15*	6.08 ± 0.34	1.39 ± 0.09**	1.60 ± 0.10*	4.36 ± 0.14	4.48 ± 0.18	0.32 ± 0.01**	0.36 ± 0.02
G3	5.06 ± 0.50*	5.90 ± 0.36*	1.26 ± 0.16**	1.57 ± 0.13*	3.80 ± 0.24	4.33 ± 0.21	0.33 ± 0.01**	0.36 ± 0.01*
G4	6.99 ± 0.45	6.90 ± 0.27	1.91 ± 0.21	1.93 ± 0.19*	5.08 ± 0.23	4.97 ± 0.19	0.37 ± 0.03	0.39 ± 0.02
G5	6.11 ± 0.23	6.33 ± 0.15	1.50 ± 0.17	1.76 ± 0.14	4.61 ± 0.16	4.57 ± 0.22	0.33 ± 0.03	0.38 ± 0.02
G6	5.86 ± 0.31*	6.17 ± 0.26*	1.46 ± 0.12*	1.64 ± 0.13	4.40 ± 0.21	4.53 ± 0.17	0.34 ± 0.02	0.36 ± 0.03

Notes: $n = 5$. *$p < 0.05$; **$p < 0.01$

Table IV. Effect of green tea extract on serum total protein, albumin (A), globulin (G) and A/G ratio in rats administrated orally AA (7 and 14 mg/100 g b.wt./ day for 15 days)

30 days, revealed a non-significant effect in lessening the effect of AA with mean values 4.61 ± 0.16 and 4.57 ± 0.22, respectively. The rats in (G6) given (1.5 percent) green tea extract with 14 mg/100 g B.W./day AA, respectively, at 15 and 30 days revealed no effect in lessening the effect of AA with the mean values 4.40 ± 0.21 and 4.53 ± 0.17

Albumin/globulin ratio (A/G ratio). Table IV also shows that AA has significant effect on (A/G ratio) at doses (seven, 14 mg/100 g B.W./day) (G2 and G3) during 15 days with the mean values 0.32 ± 0.01 ($p < 0.01$) and 0.33 ± 0.01 ($p < 0.01$), respectively, in comparison with (control) non-treated rats (G1) of the mean values 0.40 ± 0.02. No significant effect was observed with G2 at 30 days with the mean 0.36 ± 0.02. Whereas a significant result was observed with G3 at 30 days with the mean value 0.36 ± 0.01, ($p < 0.05$) compared with control (G1) non-treated with the mean value 0.41 ± 0.02. There is no effect on (A/G ratio) with rats in (G4) which received green tea extract only after 15 and 30 days with the mean values 0.37 ± 0.03 and 0.39 ± 0.02. There is a non-significant result in decreasing the effect of AA with (G5) which had (1.5 percent) green tea extract with 7 mg/100 g B.W./day AA during 15 and 30 days, with mean values 0.33 ± 0.03 and 0.38 ± 0.02, respectively. The rats in (G6) which had given (1.5 percent) green tea extract with 14 mg/100 g B.W./day AA, respectively, during 15 and 30 days revealed a non-significant effect in lessening the effect of AA with the mean values 0.34 ± 0.02 and 0.36 ± 0.03, respectively.

Discussion

AA could be made in some foods by heating, principally from the precursor asparagines and it was found to be carcinogenic in animal trials. AA have strong binding power to liver, kidneys, brain and erythrocyte GST (Sumner *et al.*, 1997). According to (IARC, 1994) AA stimulates gene mutation in mouse germ cells and chromosomal abnormalities in mice and rats germ cells and constitute covalent adducts with protamines in murine cells.

Biochemical changes

Effect of AA on kidney function. Kidneys have metabolic, humeral and excretory function. Evaluation of serum chemistries, including blood urea nitrogen, creational and uric acid is required to confirm renal dysfunction (Susan and May, 1998). From results in Tables I and II concerning the effect of AA on serum urea and creatinine, a significant increase were noticed in both urea and creatinine. These results were confirmed by Alturfan *et al.* (2008) and Mucci *et al.* (2004) who reported the relationship between cancer of the bladder and kidney with AA intake. Aside from the clear benefits, green tea extract has a significant decrease of serum levels of creatinine and urea. This resulted agreed with Yokozawa *et al.* (2003) who stated that green tea reduce serum creatinine, serum urea nitrogen and reduce production of uremic toxins. The same table shows that the co-administration of green tea extract give a protective action against the moderate increase in kidney function induced by AA. Green tea has chemo preventive action on renal epithelial cells treated with renal chemical carcinogen (Takahashi *et al.*, 2004).

Effect of AA on protein and its fraction. Concerning the effect of AA in both doses on serum total protein, Table IV showed detected that AA caused a significant decrease in serum total protein, total albumin and total globulin, while A/G ratio increased.

Our existent data were supported by Barber and LoPachin (2004) and Yousef and El-Demerdash (2006) who demonstrated that AA could form adduct by nucleophilic remains on protein and thus change consequent function and structure. Moreover, AA decrease in thiol group, which required for activity of many biological important proteins. It has been demonstrated by Nordin-Andersson *et al.* (2003) that AA induced 20 percent decrease in protein synthesis rate at 0.17 mmol/L after 72 h of exposure.

Administration of green tea extract together with AA prevented the toxic effect induced by AA. These result was confirmed by Zhu *et al.* (1999) that green tea act as an immunodulators in immune dysfunction caused by carcinogen treatment. Also Balasubramanian *et al.* (2005) found that the green tea catachens increase serum protein levels.

Conclusion

Green tea (antioxidant) reduced the genotoxic severity of AA effects and the alteration in blood and serum kidney parameters produced by AA in rats. Green tea also improved anti-oxidative capabilities in kidney and defends the cells membrane of kidney against AA action.

Acknowledgment

The authors would like to note that they are working on testing the effect of green tea as antioxidant on reducing the toxicity effect of AA on different body organs. The methodology used in this paper is therefore similar to the methodology used in the paper "A Trail of Using Green Tea for Competing Toxicity of AA on Liver Function" published by the *Journal of American Science* in 2011 and found at www.kau.edu.sa/Files/0053658/Researches/61046_31946.pdf. The two papers are however, entirely different studies on different body organs with significantly different results.

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