

A Trail of Using Green Tea for Competing Toxicity of Acrylamide on Liver Function

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Abstract: Acrylamide (AA) is neurotoxic to experimental animals and humans. Also, it has mutagenic and carcinogenic effects. This study was carried out to investigate the effects of green tea extract on liver function test in male rats received different doses of acrylamide. Animals were assigned at random to six groups: group 1 served as control, while groups 2, 3 were received 7, 14 mg/100g B.W /day of acrylamide, respectively in drinking water for 15 and 30 days. Group 4 received green tea 1.5% concentration and groups 5, 6 received 7, 14 mg/100 g B.W /day of acrylamide in a combination with green tea for 15 and 30 days. Aspartate aminotransferase (AST), and Alanine aminotransferase (ALT) and Alkaline phosphates (ALP), significantly increased whereas cholinesterase activity declined significantly in rats received AA in both concentrations and time in comparison with the control. However, the values of cholinesterase activity decreased when rats received green tea alone or in combination with AA.

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Key words: Acrylamide – Cholinesterase- Aspartate aminotransferase (AST) - Alanine aminotransferase (ALT) - alkaline phosphates (ALP) - Green tea.

1. Introduction

Acrylamide (AA) (2-propenamide) is an industrial chemical used for the production of polymers which are used as flocculants for purification of drinking and waste water, thickeners for agricultural sprays, gel chromatography and electrophoresis, soil stabilizers and in the paper and pulp industry. Acrylamide monomer may form in certain foods cooked at high temperatures¹. The highest concentrations of Acrylamide have been identified in potato and grain-based foods that are cooked at very high temperatures (e.g. frying, grilling or baking)². Acrylamide is thought to form in food principally from the interaction of amino acid asparagines with glucose or other carbohydrates³. Acrylamide has significant binding capacity to liver, kidney, brain and erythrocyte⁴. Acrylamide has been classified as a group 2A carcinogen by the international Agency for Research on Cancer⁵.

Several mechanisms have been suggested to explain Acrylamide induce its toxic effect. It is metabolized into glycidamide via the cytochrome P450 (CYP450) pathway to produce glycidamide (2E1), forming a DNA- reactive epoxide.⁶ Reactive with enzymes or receptors may induce changes in cellular functions and signal pathways, leading to carcinogenesis.⁷ changes in dopamine receptor affinity and alterations of thyroid stimulating hormones, prolactin, and testosterone levels. This observed in rats following AA treatment.⁸ Oxidative stress is considered as one of the important mechanisms of toxic effects of Acrylamide. AA causes oxidative damage through inducing the

generation of reactive oxygen species (ROS) which enhanced the production of lipid peroxidase reducing the antioxidant defense systems⁹.

Antioxidants are chemicals that reduce oxidative damage to cells and biochemical's. Some dietary antioxidants vitamins A, C, and E can be considered as protective agents against genotoxic action of Acrylamide¹⁰. Green tea getting so much attention from the science world because of the antioxidant Epigallocatechin-3 gallate (EGCG) that is the most abundant catechin preserved in green tea¹¹. Green tea is characterized by a high content of flavonoids. Flavonoids are large group of phenolic products of plant metabolism (strong antioxidant)¹².

The present study was carried out to investigate the effects of using green tea for coping toxicity of acrylamide on liver function.

2. Materials and Methods

Materials

Green tea

Green tea was provided by local market. It was prepared freshly and adds twice as the sole source of drinking water for rat at concentration 1.5%¹³.

Acrylamide (99.9% purity) was purchased from Sigma chemical Co. It used in two different concentrations 7 and 14 mg/100 g B.W.¹⁴.

Animals:

Sixty mature (2-4 months) male albino rats weighing from 120±10 g body weight were used in this study.

Animal ration:

Normal ration for rats given to control group. The rats were fed balanced rations and free access water was allowed.

Methods**Experimental design**

Six equal groups each of ten rats were subjected to dosing. The first group (G1) of rats kept as a control. The second (G2) and third groups (G3) of rats were received 7, 14 mg/100g B.W. /day of acrylamide, in drinking water for 15 and 30 days. While the fourth (G4) group rats received green tea 1.5% as sole source of water from day 1 to day 30, the fifth (G5) and sixth (G6) groups received with 7, 14 mg/100g B.W/day acrylamide respectively for 15 and 30 days and green tea 1.5% from the first day to day 30.

Blood Sampling

Five blood samples were collected from rats from each group from eye plexus at 15 and 30 days in clean dry sterile and labeled centrifuge tubes. Separating serum was done by centrifugation at 3000 r.p.m for 10 minute.

Animal Ration analysis

Analytical measurements include; Determination of crude protein content by AOAC (1995)¹⁵ and crude fiber percentage according to Less, (1975)¹⁶ Determination of fat, Ash and moisture content by either extraction as described by Horwitz (1980)¹⁷ Aflatoxin content¹⁸ and determination of acid number in fat¹⁹.

Preparation of green tea extract²⁰.

15 g of green tea powder were soaked in 1 liter of boiling distilled water for 5 minutes and filtered to make 1.5 % green tea solution. Green tea extracted (GTE), was provided to rats as their sole source of drinking water.

Biochemical Analysis

Serum samples were subjected to quantities determination of Serum Aspartate amino transferase (AST) and Serum Alanine amino transferase (ALT), according to Retman, (1975),²¹ Serum Alkaline phosphates (ALP), according to Roy,(1970)²² Serum Cholinesterase activities according to Tietz, (1986).²³

Statistical Analysis was carried out by Snedecor Snedecor, (1995).²⁴

3. Results**Aspartate aminotransferase (AST)**

Statistical analysis of data represent in **Table 1**. Shows that AA elicited significant increase of Aspartate aminotransferase (AST) at the two doses (7, 14 mg/100g B.W. /day) G2 & G3 respectively for 15 days with the mean values of 48.54 ± 3.38 ($P < 0.01$) and 55.46 ± 3.61 ($P < 0.001$) respectively compared with non- treated (control) rats G1 with the mean values of 29.33 ± 2.82 . There was slightly significant increase at the two previous doses after 30 days with the mean values of 41.25 ± 2.90 ($P < 0.05$) & 48.00 ± 3.11 ($P < 0.01$) compared with non-treated rats with the mean value 30.67 ± 2.29 .

Rats received green tea extract only (antioxidant) group 4 had no effect on enzyme activities after 15 & 30 days. The mean values were 28.66 ± 1.41 & 29.67 ± 2.77 compared to non- treated (control) rats (29.33 ± 2.82 & 30.67 ± 2.29), respectively. This indicated that green tea (antioxidant) had activities of such enzymes but was less than that received Acrylamide. Group 5 which received green tea extract (1.5%) with 7 mg/100g B.W/day Acrylamide for 15 and 30 days showed good effect in decreasing the effect of Acrylamide on damaging cell with mean values 37.23 ± 2.96 & 33.76 ± 3.43 respectively. The rats in group 6 given green tea extract (1.5%) with 14 mg/100g B.W/day Acrylamide respectively for 15 and 30 days also showed good effect in decreasing the effect of Acrylamide on damaging cell with mean values of 42.67 ± 3.21 ($P < 0.05$) & 39.78 ± 3.01 ($P < 0.05$) respectively.

Table 1. Effect of green tea extract on Aspartate Aminotransferase (AST) in rats orally administrated Acrylamide (7 and 14 mg/100g B.W./days for 15&30 day) n=5

Studied groups	AST u / l	
	15 days	30 days
G1	29.33 ± 2.82	30.67 ± 2.29
G2	48.54 ± 3.38 ***	41.25 ± 2.90 *
G3	55.46 ± 3.61 ***	48.00 ± 3.11 **
G4	28.66 ± 1.41	29.67 ± 2.77
G5	37.23 ± 2.96	33.76 ± 3.43
G6	42.67 ± 3.21 *	39.78 ± 3.01 *

N=Number of rats; * Significant at $P < 0.05$; **Significant at $P < 0.01$; *** Significant at $P < 0.001$

Alanine aminotransferase (ALT)

Statistical analysis of data represent in **Table 2**. Shows that AA elicited significant increase of Alanine aminotransferase (ALT) at the two doses (7, 14 mg/100g B.W. /day) G2 & G3 respectively for 15 days with the mean values of 23.90 ± 3.34 ($P < 0.01$) and 41.10 ± 2.85 ($P < 0.001$) respectively compared with non- treated (control) G1 rats with the mean values of 17.71 ± 2.39 . There was slightly significant increase at the two previous doses after 30 days with

the mean values of 27.11 ± 2.17 ($P < 0.05$) & 30.06 ± 2.59 compared with non- treated rats with the mean value 18.00 ± 2.01 .

Rats received green tea extract alone (antioxidant) group 4 had no effect on enzyme activities after 15 & 30 days. The mean values were 16.23 ± 2.01 & 18.50 ± 2.59 U/L compared to non-treated (control) rats (17.71 ± 2.39 & 18.00 ± 2.01 U/L), respectively. This indicated that green tea (antioxidant) had activities of such enzymes but was less than that received Acrylamide. Group 5 rats which received green tea extract (1.5%) with 7 mg/100g B.W/day Acrylamide for 15 and 30 days showed good effect in decreasing the effect of Acrylamide on damaging cell with mean values 27.20 ± 3.20 & 23.48 ± 1.93 respectively. The rats of group 6 given green tea extract (1.5%) with 14 mg/100g B.W/day Acrylamide respectively for 15 and 30 days also showed good effect in decreasing the effect of Acrylamide on damaging cell with mean values of 32.66 ± 4.11 ($P < 0.05$) & 26.01 ± 2.22 ($P < 0.05$) respectively.

Table 2. Effect of green tea extract on Alanine Aminotransferase (ALT) in rats orally administrated Acrylamide (7 and 14 mg/100g B.W./days for 15&30 day) n=5

Studied groups	ALT U/L	
	15 days	30 days
G1	17.71 ± 2.39	18.00 ± 2.01
G2	23.90 ± 3.34 **	27.11 ± 2.17 *
G3	41.10 ± 2.85 ***	30.06 ± 2.59 **
G4	16.23 ± 2.01	18.50 ± 2.59
G5	27.20 ± 3.20	23.48 ± 1.93
G6	32.66 ± 4.11 *	26.01 ± 2.22 *

N=Number of rats; * Significant at $P < 0.05$; **Significant at $P < 0.01$; *** Significant at $P < 0.001$

Alkaline phosphatase (ALP)

Statistical analysis of data represent in **Table 3**, shows that AA elicited significant increase of alkaline phosphatase (ALP) at the two doses (7, 14 mg/100g B.W. /day) (group2 & group3) for 15 days with the mean values of 178.96 ± 4.10 U/L ($P < 0.05$) and 181.22 ± 3.93 ($P < 0.001$) respectively compared with non- treated (control) rats (group 1) with the mean values of 160.56 ± 4.07 . There was significant increase at the two previous doses after 30 days with the mean values of 185.33 ± 4.09 ($P < 0.05$) & 198.86 ± 4.81 ($P < 0.01$) compared with non- treated rats with the mean value 168.31 ± 4.11 .

Rats received green tea extract only (antioxidant) G 4 had no effect on enzyme activities after 15 & 30 days. The mean values were 158.73 ± 3.24 & 159.89 ± 4.33 compared to non-treated (control) rats (160.56 ± 4.07 & 168.31 ± 4.11), respectively. This indicated that green tea had

activities of such enzymes but was less than that received Acrylamide. Group five rats which received green tea extract (1.5%) with 7 mg/100g B.W/day acrylamide for 15 and 30 days showed good effect in decreasing the effect of acrylamide on damaging cell with mean values 171.11 ± 3.91 & 169.94 ± 4.39 respectively. The rats of group six given green tea extract (1.5%) with 14 mg/100g B.W/day Acrylamide respectively for 15 and 30 days also showed good effect in decreasing the effect of Acrylamide on damaging cell with mean values of 176.12 ± 4.08 & 179.73 ± 3.49 respectively.

Table 3: Effect of green tea extract on Alkaline phosphates (ALP) in rats orally administrated Acrylamide (7 and 14 mg/100g B.W./day for 15&30 days) n=5

Studied groups	ALP U/L	
	15 days	30 days
G1	160.56 ± 4.07	168.31 ± 4.11
G2	178.96 ± 4.10 *	185.33 ± 4.09 *
G3	181.22 ± 3.93 **	198.86 ± 4.81 **
G4	158.73 ± 3.24	159.89 ± 4.33
G5	171.11 ± 3.91	169.94 ± 4.39
G6	176.12 ± 4.08	179.73 ± 3.49

N=Number of rats; * Significant at $P < 0.05$; **Significant at $P < 0.01$; *** Significant at $P < 0.001$

Cholinesterase activity

Statistical analysis of data represent in **Table 4**, showed that Cholinesterase activity declined significantly in rats received acrylamide in both concentrations and time (7, 14 mg/100g B.W. /day for 15&30 days) G2 & G3 with the mean values 492.84 ± 28.17 ($P < 0.01$), 340.65 ± 21.33 ($P < 0.001$), 540.69 ± 24.97 ($P < 0.05$) and 498.31 ± 26.82 , ($P < 0.01$) respectively in comparison with the non treated control rat G1 (631.20 ± 26.01 & 622.55 ± 23.76). However, the values of cholinesterase activity showed continuous decrease when rats treated with green tea alone G4 with the mean values 581.11 ± 22.60 & 518.65 ± 29.13 ($P < 0.05$) or in combination with acrylamide with the mean values 408.67 ± 22.49 ($P < 0.001$), 500.38 ± 22.56 ($P < 0.01$), 321.25 ± 21.91 ($P < 0.01$), and 453.98 ± 27.43 ($P < 0.001$) G5 & G6 respectively.

Rats received green tea extract (antioxidant) only, (group 4), had no effect on enzyme activities after 15 days with the mean values of 581.11 ± 22.60 & but had a significant effect after 30 days by 518.65 ± 29.13 ($P < 0.05$) compared to non- treated (control) rats (631.20 ± 26.01 & 622.55 ± 23.76), respectively. Group 5 which received green tea extract (1.5%) with 7 mg/100g B.W/day Acrylamide for 15 and 30 days showed a highly significant effect in decreasing the effect of Acrylamide on damaging cell with mean 408.67 ± 22.49 ($P < 0.001$) & $500.38 \pm$

22.56 (**P<0.01**) respectively. The rats of group 6 given green tea extract (1.5%) with 14 mg/100g B.W/day Acrylamide respectively for 15 and 30 days also showed highly significant effect in decreasing the effect of Acrylamide on damaging cell with mean values of 321.25±21.91 (**P<0.01**) & 453.98±27.43 (**P<0.001**) respectively.

Table 4. Effect of green tea extract on cholinesterase activity in rats orally administrated Acrylamide (7 and 14 mg/100g B.W. /day for 15&30 day) n=5

Studied groups	Cholinesterase U / L	
	15 days	30 days
G1	631.20±26.01	622.55± 23.76
G2	492.84±28.17 **	540.69±24.97 *
G3	340.65±21.33 ***	498.31±26.82 **
G4	581.11±22.60	518.65±29.13 *
G5	408.67±22.49 ***	500.38± 22.56 **
G6	321.25±21.91 **	453.98±27.43 ***

N=Number of rats; * Significant at P < 0.05; **Significant at P < 0.01; *** Significant at P < 0.00

4. Discussion

Acrylamide (AA) can be formed in certain foods by heating, predominantly from the precursor asparagines. It is a carcinogen in animal experiments. Acrylamide was tested for carcinogenicity in one experiment in rats by oral administration. Acrylamide have significant binding capacity to liver^{4; 6; 25; 26; 27; 28; 29}

The liver performs numerous functions that include but not limited to lipid, carbohydrate and protein metabolism. The liver also has immune logic activity, contributes to digestion, and is essential for detoxification of many endogenous and exogenous compounds.³⁰ In regard to the effect of used acrylamide on serum liver enzyme activity; our data revealed that treated groups displayed significant increase in (AST, ALT and ALP) at the two doses and slightly increase after 30 days. These results agree with Yousef. and El-Demerdash, (2006)³¹. Liver revealed congestion sinusoids with fatty degeneration and necrosis after administration a lethal dose of acrylamide to monkey was reported by Mccollister, (1964)³² AA had a significant capacity to liver Glutathione (reduce GSH)³³. In addition, it induced cellular transformation and increase in the degree of membrane permeability of liver³⁴. The disruption of these membranes may cause the translocation of liver enzyme ALT and AST into the blood, as observed in this study.

Green tea (*Camellia sinensis*) contains high level of polyphenols including catechin, epicatechin, gallic acid, epigallocatechin, epicatechin gallate, and gallic acid gallate^{32; 33}. Polyphenols from green tea are efficient free radical and singlet oxygen scavengers^{34; 35} and green tea extract (GTE) inhibits

lipid peroxidation in vitro systems, in experimental animals, and in humans^{32, 33, 34;34; 35}.

Rats received green tea extract alone (antioxidant) in group 4 had no effect on enzyme activities. While the rats in Group 5 which received green tea extract (1.5%) with 7 mg/100g B.W/day Acrylamide for 15 and 30 days, showed a highly significant effect in decreasing the effect of Acrylamide on damaging cell. This result agreed with the study of Higdon JV, and Frei B (2003)³⁶ which revealed that green tea polyphenols, particularly EGCG(-)epigallocatechin gallate are naturally occurring strong antioxidants. Tea catechins and polyphenols are effective scavengers of reactive oxygen species in vitro and may also function indirectly as antioxidants through their effects on transcription factors and enzyme activities. The fact that catechins are rapidly and extensively metabolized emphasizes the importance of demonstrating their antioxidant activity in vivo. In humans, modest transient increases in plasma antioxidant capacity have been demonstrated following the consumption of tea and green tea catechins. The effects of tea and green tea catechins on biomarkers of oxidative stress, especially oxidative DNA damage, appear very promising in animal models, but data on biomarkers of in vivo oxidative stress in humans are limited.

A recent study showed that the addition of EGCG and other green tea constituents to tissue culture medium generated high levels of hydrogen peroxide (H₂O₂) and it was postulated that this might represent an artefact of cell culture³⁷. Another study showed similar results, although discriminating between different EGCG concentrations, while the presence of cells decreased H₂O₂ concentrations³⁸.

The co-administration of acrylamide together with green tea reduces the effect of acrylamide on the hepatological markers in serum AST&ALT and ALP^{39; 40; 41}. Green tea enhanced antioxidative abilities in liver and protects liver cells and liver cell membrane against acrylamide action. Bu-Abbas, et al (1945) and Ostrwsk (2004)^{35 and 42} showed that green tea reduce the morphological and biochemical alterations induced by hepatic toxicity agents.

Effect of Acrylamide on Cholinesterase and enzyme found in blood serum is synthesis by liver, hydrolyzes acetylcholine. Acetylcholine is a compound synthesized at the nerve endings, which acts in transmitting impulses from nerve to muscles fiber⁴³. Acrylamide is well-known neurotoxic compound that produces central and peripheral distal axonopathy⁴⁴. In the present study, there is a significant decrease in serum cholinesterase concentrations in group 2 and 3 after 15 and 30 days post high dose feeding Acrylamide as compared with

control (Table 4). Regarding the effect of acrylamide on Cholinesterase activity, our results agreed with that recorded by Tietz, (1996); Khanna, (1992) and Shell, (1992)^{43; 45; 46}. Cholinesterase levels inhibition are indicator for possible neurotoxic effect of organic compound (acrylamide)⁴⁷.

Also decrease in concentration of cholinesterase well appear by⁴⁸ they investigate the neurological defect (skeletal muscles weakness) associated with acrylamide intoxication are mediated by impaired neuro – transmission at central and peripheral synapses (decrease in Acetylcholine). Administered of green tea extract alone or in combination with AA significantly inhibited cholinesterase levels on the same table. Okello *et al.*, 2004⁴⁹ finding suggest that tea infusion contain biologically active principally, perhaps acting synergistically that may be due to the inhibition of acetyl cholinesterase and butyric cholinesterase

Conclusion

Supplementation of rats with antioxidant (green tea) reduces the effect of AA on the hepatogical markers in serum AST & ALT and ALP. Also green tea enhanced antioxidative abilities in liver and protects liver cells membrane against AA action.

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