

Ameliorating Effect of White Tea Extract against Acrylamide Induced DNA Damage and Biochemical Changes in Male Rats

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Abstract

In this study, the protective effect of white tea (WT) aqueous extract against cytogenicity, DNA damage and some biochemical changes induced by acrylamide (ACR) in male albino rats was investigated. Six groups containing 5 rats each were used in this study. Group 1 served as a control. Groups 2 and 3 were given single daily oral doses of 10 and 20 mg /kg. b. wt. for 15 successive days. Group 4 permitted free access to solubilized WT (1.5%) as the sole drinking fluid. Groups 5 and 6 were given the same doses as group 2 and 3 in combination with solubilized WT. Rats received acrylamide in both tested concentrations significantly decreased the amount of DNA/gm of spleen and significantly increased in the percentage of MPCEs and PCE/NCE ratio as compared with the control group. Administration of acrylamide to rats significantly increased serum transaminases (AST and ALT), alkaline phosphatase (ALP), urea, creatinine and malondialdehyde in both doses (low and high), while it significantly decreased reduced glutathione. Administration of WT to intoxicated rats normalized all the tested parameters.

Conclusion: Dietary intake and occupational exposure to acrylamide (ACR) must be restricted besides raising the public awareness to ACR hazards. The white tea extract exhibits a protective effect against ACR damage.

Keywords: White tea, Acrylamide, DNA damage, Cytogenicity

Introduction

Acrylamide (ACR) is a reactive, small organic molecule with very high water solubility. These properties facilitate its rapid absorption and distribution through the body (**Mannaet al., 2006**). ACR represents an industrial chemical used in the manufacturing of polyacrylamides that are common in personal care and grooming products (e.g. lotions, cosmetics, deodorants), soil conditioning, wastewater

treatment, and paper and textile industries (**Friedman, 2003 and Exon, 2006**). In addition to such industrial and laboratory uses, high levels of ACR were detected in tobacco smoke (**Pruser and Flynn, 2011**).

Recently, it has been reported that acrylamide monomer may form in certain foods cooked at high temperatures, the highest concentrations of which have been identified in potato and grain-based (e.g., frying, grilling or baking) principally from the interaction of the amino acid asparagine with glucose or other carbohydrates (**Tareke et al., 2002**).

Acrylamide is readily absorbed into the circulation and thereafter distributed to various organs, reacts with cellular DNA, hemoglobin, nerve cells and enzymes (**Rayburn and Friedman, 2010**) and acts as animal carcinogen and germ cell mutagen (**Ghanayem et al., 2005a**) and as human neurotoxicant and suspected carcinogen (**Klaunig and Kamendulis, 2005 and Nuno et al., 2008**). Acrylamide is genotoxic through its metabolite glycidamide formed via epoxidation by CYP2E1 and leads to the formation of glycidamide-DNA and hemoglobin adducts (**Ghanayem et al., 2005b**).

In fact, herbal medicines derived from plant extracts are being increasingly utilized as adjunct treatment options for a wide variety of clinical disease. More attention has been paid to the protective effects of natural antioxidants against chemically induced toxicities (**Frei, 2003**). The health benefits of white & green tea have been extensively studied in the past few decades. Nowadays, tea is considered as a source of dietary constituents endowed with biological and pharmacological activities with potential benefits to human health. The increasing interest in the health properties of tea extract and its main catechin polyphenols have led to a significant rise in scientific investigation for prevention and therapy in several diseases (**Chung, et al 2009; Mandel et al, 2006 and Ostrowska and Skrzydlewska 2006**). Furthermore, many previous investigators reported that White and green tea extract (WT & GT) displays antioxidant and free radicals scavenger properties (**Koutelidakis et al ,2009 Chung, et al 2009 and Crespy, 2004**).

Therefore the present study aimed to elucidate has hepatotoxicity when administered orally to male rat separately or in combination with acrylamide as well as the hepatoprotective effect of WT against DNA damage which may result from ACR exposure.

Materials and Methods

Materials

White tea

White tea (WT) was provided by local market. WT extract was prepared freshly and added twice a day as the sole source of drinking water for the rat at a concentration 1.5% as reported by **Mohamdin, et al, (2005**

Acrylamide

ACR compound purity is 99% and purchased from Sigma Chemical Company. It is a water-soluble vinyl monomer (**Shan *et al.*, 2006**).

ACR synonyms: 2- propenamide; acrylic acid amide; acrylic amide; acrylamide monomer; acrylamide; propenoic acid amide ; vinyl amide ; ethylene carboxamide

Molecular formula: C₃H₅NO

Chemical formula: CH₂CHCONH₂

Chemical structure: CH₂=CH-C-NH₂ (**Ghanayem, *et al.*, 2005b**)

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Animals and dosage

Thirty mature male albino rats with an average body weight ranging from 160-180 g were obtained from laboratory Animal House Ministry of Health, Helwan, Cairo. Animals were kept in metal cages during the whole experimental period under hygienic conditions, fed on commercial rat pellets and water was provided *ad libitum*, throughout the experiment.

Methods

Experimental design:

Six equal groups each of ten rats were subjected to dosing and divided as following:

The first group (G1): Rats were kept as a control.

The second (G2) and third groups (G3): Rats were received 10, 20 mg/kg. B.W. /day of acrylamide, respectively, in drinking water for 15 days.

The fourth (G4) group: rats were received white tea at 1.5% concentration as a sole source of water from day 1 to day 15.

The fifth (G5) and sixth (G6): groups were received 10 and 20 mg/kg. B.W./day acrylamide respectively for 15days combined with white tea at 1.5% concentration.

Preparation of white tea extract (**Maity *et al.*, 1998**)

Fifteen gram of white tea powder were soaked in 1 liter of boiling distilled water for 5 minutes and filtered to make 1.5 % white tea solution. white tea extracted (WTE) was provided to rats as their sole source of drinking water.

Samples:

- Spleen samples were taken from the sacrificed rats in all experimental groups. Samples were collected in clean dry plastic bags and kept at -20°C for determination of DNA.
- Bone marrow samples were collected from both femurs of rats of each groups at the end of experimental period and used for micronucleus analysis.

- Individual blood samples were obtained at the end of experiment from rats of each group, left to clot, sera were separated and kept at - 40°C for biochemical analysis. Then animals were sacrificed and autopsy performed immediately; liver tissue was removed and washed with saline solution, then minced and homogenized (10% w/v) in ice-cold normal saline. The homogenate was centrifuged at 10,000xg for 20 min at 4°C and the resultant supernatant was used for antioxidant assay (**Chitra *et al.*, 1999**).

1- DNA isolation:

The isolation of DNA from spleen of rats according to the following protocol based on the solubility of nucleoprotein in water and the high ionic strength of the solution, these used in the initial extraction.

The tissue at first homogenized in isotonic saline buffered with sodium citrate, pH 7, then protein is removed by treatment with a chloroform/ amyl alcohol mixture and the DNA precipitated with ethanol (**Schwader and Singer, 1950**). Estimation of isolated DNA was carried according to **Dische and Schwarz, (1973)**.

2- Cytogenetic analysis:

The following protocol established by **Salamon *et al.*, (1980)** from each group, bone marrow cells of five mature rats from each group were extracted with a pin into a clean dry glass slide and homogenized with two drops of fetal calf serum. Cells were smeared on the slide, air dried, fixed in absolute methanol and stained with Giemsa in phosphate buffer pH 6.8. The polychromatic erythrocytes (PCEs, 1000/animal) were screened for micronuclei and the changes in mitotic activity (**Hart and Engberg-Pederson, 1983 and Al-Bekairi *et al.*, 1991**) were assessed on basis of the ratio of polychromatic to normochromatic erythrocytes (PCE/NCE).

3-Serum biochemical studies:

The biochemical assays of serum alkaline phosphatase (ALP) activity according to **Tietz, (1996)**, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (**Reitman and Frankel 1957**), serum urea level according to **Wybenga *et al.* (1971)**, creatinine according to **Faulkner and King (1976)**. Catalase activity; lipid peroxidation as malonaldehyde (MDA) and reduced glutathione (GSH) in homogenate liver tissues were determined according to **Aebi (1974); Okhawa *et al.*, (1979) and Ellman (1959), respectively**.

The obtained data were statistically analyzed using t-test after **Petrie and Watson (1999)**.

4- Statistical analysis:

Data were expressed as mean \pm S.E. Significant different using student "t" test .(Snedecor,1982) .

Results and Discussion

Acrylamide (ACR) 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 (**Calleman *et al*, 1990 and Sumner *et al*, 1997**).

The results recorded in **Table 1 and 2** showed that acrylamide at both doses (low and high) induced DNA damage and significant increased the frequency of micronuclei formation in rat bone marrow cells.

DNA analysis revealed that acrylamide induced a significant decreased in the amount of DNA / gm of spleen in the group of rats given ACR (G2 and G3) compared with control group. These finding agreed with the results of (**Ghanayem *et al* 2005a**) who reported that ACR enhance the genotoxicity and inhibit DNA repair process (**Rice –Evans and Burdon, 1993 and Khlood *et al* ,2011**) indicate that ACR blocks DNA repair by distributing protein interaction involved DNA damage recognition. Also, ACR leads to DNA strand breaks and dominant lethal mutations (**Tyla *et al.*, 2000 and Ao *et al.*, 2008**).

The group of rats administered white tea (WT) showed significant increased in the amount of DNA / gm of spleen as compared with control –ve and control +ve groups.

White & Green tea polyphenols are believed to have the potential as neuropreventive agents for the treatment of neurodegenerative diseases and DNA damage via antioxidant properties (**Kumar *et al* 2012, Nie *et al*, 2002 and Elahe *et al*, 2015**).

The in vivo micronucleus assay is one of the most assays to reflect chromosomal aberration and DNA damage (**Celik *et al*, 2005**) .Micronucleus test revealed significant increased in the percentage of micronucleated polychromatic erythrocytes (MPCEs) in ARC treated group with an increase in polychromatic erythrocytes / Normochromatic erythrocytes (PCE/NCE) ratio. These finding are in agreement with **Elahe *et al*, (2015)** who recorded that the administration of ACR lead to a significant increased in the frequency of micronuclei in rats .

Rats administered WT in combination with ACR in both doses, reduce the micronucleus frequencies in polychromatic erythrocytes (MPCEs) and PCE/NCE ratio of bone marrow (**Table 2**). Our results agree with those of **Vuillaume (1987)**;

Azram *et al* (2004) and Chung *et al* (2009).

Data in **Table 3** shows that ACR elicited significant increase of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and alkaline phosphatase (ALP) at the two doses (10 and 20 mg/kg. B.W. /day) G2 and G3 respectively for 15 days compared with non- treated (control) rats

Rats received white tea extract only (antioxidant) group 4 had no effect on enzyme activities after 15 days compared with control group. This indicated that white tea (antioxidant) had activities of such enzymes but was less than that received acrylamide. Group 5 and 6 which received white tea extract (1.5%) with 10 mg/kg. b.wt/day acrylamide for 15 days showed good effect in decreasing the effect of acrylamide on damaging cell.

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 (**Susan and May 1998**). 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 15 days. These results agree with those of **Yousef and El-Demerdash, (2006)** and with the study of **Higdon JV, and Frei B (2003)** which revealed that white & green tea polyphenols, particularly EGCG(-)epigallocatechingallate are naturally occurring strong antioxidants. Tea catechin 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 co-administration of acrylamide together with green tea reduces the effect of acrylamide on the hepatological markers in serum AST, ALT and ALP. **Hasegawa, et al (1995)**, . **Imai and Nakachi (1995)** and **Sai et al (1998)**. Green tea enhanced antioxidative abilities in liver and protects liver cells and liver cell membrane against acrylamide action. **Ostrwsk (2004)** showed that white & green tea reduce the morphological and biochemical alterations induced by hepatic toxicity agents.

Toxic effects of acrylamide in our study on liver tissue involve a significant increase in malonaldehyde (indication for lipid peroxidase) associated with a reduction of GSH and catalase enzyme levels (**Table 4**).

Acrylamide could be generating ROS, (Reactive Oxygen Species) which enhanced lipid peroxidase production. Cellular fatty acids are readily oxidized by ROS to produce lipid peroxy radicals and lipid hydroperoxides (**Rice-Evans and Burdon, 1993**). Lipid peroxy radicals can subsequently propagate into malondialdehyde (MDA) and these result attributed the significant increased in acrylamide treated rats (**Dobrzynska, et al., 2004**). MDA levels in liver may also be

used to investigate the oxidative damage of protein and lipoproteins, which is a possible pathogenic mechanism for liver injury (**Kojic, et al., 1989**).

A reduction of liver cellular GSH level by acrylamide treatment was observed in our study (**table 4**). ACR could conceivably adduct amines, imidazoles and sulfhydryl groups on proteins via the Michael carbonyl condensation reaction (**Kempand Vellaccio, 1980 and Becalski et al 2003**). This may result in a depletion of cellular GSH stores and result in a change in the redox status of the cell (**Park, et al., 2002**).

Administration of WT significantly lowered lipid peroxidation and enhanced the hepatic levels of glutathione (GSH) and catalase enzymes. In agreement with **Kaviarasan et al., (2007)** who reported that the administration of catechin increased blood levels of vitamin E and activities of the antioxidant enzymes catalase and glutathione peroxidase in patients with chronic hepatitis .

Sithisarn et al. (2006) and Raza and John (2007) reported that catechin prevent molecular degradation in oxidative stress conditions by directly altering the subcellular ROS production (prevent oxygen radical formation and to scavenge free radicals such as hydroxyl, peroxy and lipid radicals and superoxide anions), glutathione metabolism and cytochrome P450 2E1 activity.

ACR doses, there were significant decreases in serum urea levels and creatinine. The decreased urea levels may be a result of impaired urea synthesis due to hepatic insufficiency because of liver damage (**Agency USEB, 2011**). The results agree with those recorded by **Hammad et al. (2013)** who used different doses of ACR (10, 30, 60, 90 mg/kg) for 6 weeks, and **El-Mottaleb and Rashed (2008)**, who treated the specimens with 1/20 and 1/10 of LD50 of ACR for 28 days. The results from present study disagrees with **Alturfan et al. (2011)**; they reported that the BUN and creatinine levels significantly increased in the ACR treated group (40 mg/kg/day intraperitoneally) compared to those of the control group.

Creatinine formation begins with the transamidation from arginine to glycine to form glycoamine or guanidoacetic acid (GAA). This reaction occurs primarily in the kidneys, in the mucosa of the small intestine, and the pancreas. The GAA is transported to the liver where it is methylated by S-adenosyl methionine (SAM) to form creatine. Creatine enters circulation and 90% is taken up and stored by muscle tissue. In a reaction catalyzed by creatine phosphokinase (CPK), most of this muscle creatine is phosphorylated to creatine phosphate. The results of the present work are agree with the findings presented by **Shler et al (2015) and Rawi et al. (2012)** who reported that there were significant changes between immature female rats treated with 15 mg/kg of ACR for 28 days and the control group.

In conclusion

dietary intake and occupational exposure to acrylamide (ACR) must be restricted besides raising the public awareness to ACR hazards. The white tea extract exhibits a protective effect against ACR damage.

Table (1): Effect of treated White tea on the amount of DNA mg/gm of spleen in Acrylamide intoxicated rats.

Groups	Amount of DNA mg/ gm spleen
G1 (C-ve)	0.164± 0.0089
G2: ACR (10mg/kg.b.wt)	0.47± 0.07***
G3 ACR(20mg/kg.b.wt)	0.08± 0.03***
G4 WT (1.5%)	2.27± 0.11***
G5 (WT+ ACR (1.5% &10mg/kg.b.wt).)	2.92± 0.024***
G6 (WT+ ACR (1.5% &20mg/kg.b.wt))	1.01± 0.00005***

Values represents the mean ± S.E.

***P < 0.001

Table(2): Effect of treated White tea on the incidences of MPCE on the relation of PCE / NCE in acrylamide intoxicated rats.

Group	PCE	MPCE/1000 PCE± S.E.	NCE screened	PCE/NCE ratio
G1(control)	5000	5.6± 0.32	2183	2.029±0.03
G2(ACR 10mg)	5000	14.8±1.89	1120	4.465±0.029*
G3(ACR 20mg)	5000	16.92±3.79***	952	5.252±0.21**
G4(WT 1.5%)	5000	6.96±1.24	2091	2.391±0.189
G5(ACR 10mg+WT 1.5%)	5000	9.6±1.62**	1371	3.647±0.28*
G6(ACR 20mg+WT 1.5%)	5000	14.56±2.45***	1144	4.371±0.283*

Values represents the mean ± S.E.

* P < 0.0** P < 0.01***P < 0.001

Table.3: Effect of White tea extract on liver enzymatic activity (AST,ALP&ALP) in rats orally administrated Acrylamide

Group	AST U/L	ALT U/L	ALP U/L
G1(control)	29.33±2.82	17.71± 2.39	160.56± 4.07
G2(ACR 10mg)	48.54±3.38 ***	23.90 ± 3.34 **	178.96±4.10 *
G3(ACR 20mg)	55.46± 3.61 ***	41.10±2.85***	181.22±3.93 **
G4(WT 1.5%)	28.66 ±1.41	16.23± 2.01	158.73±3.24
G5(ACR 10mg+ WT 1.5%)	37.23 ± 2.96	27.20 ± 3.20	171.11±3.91
G6(ACR 20mg+ WT 1.5%)	42.67 ± 3.21 *	32.66 ± 4.11 *	176.12±4.08

Values represents the mean ± S.E.

* P < 0.0 ** P < 0.01 ***P < 0.001

Table (4): Mean values (±SE) of liver Malondialdehyde, GHS and Catalase values in rats fed acrylamide and White tea (n=5)

Group	Malondialdehyde (mM/100g)	GHS (µmol/mg protein)
G1(control)	1.22±0.51	39.61±2.01
G2(ACR 10mg)	4.17±0.52***	30.33±1.81**
G3(ACR 20mg)	6.28±0.81***	24.17±1.297***
G4(WT 1.5%)	0.91±0.31	38.63±1.09
G5(ACR 10mg+ WT 1.5%)	2.71± 0.29*	36.11±1.97
G6(ACR 20mg+ WT 1.5%)	3.81±0.48*	32.21±1.79*

Values represents the mean ± S.E. * P < 0.0 ** P < 0.01 ***P < 0.001

Table.5: Effect of White tea extract on urea creatinine in rats orally administrated Acrylamide (n=5)

Group	Urea mg/dl	Creatinine mg/dl
G1(control)	34.1±3.35	0.86±0.07
G2(ACR 10mg)	46.47±3.52*	1.33±0.11*
G3(ACR 20mg)	51.62±4.07**	1.47±0.190**
G4(WT 1.5%)	30.41±2.41	0.93±0.09
G5(ACR 10mg+ WT 1.5%)	40.01±2.29	1.11±0.97
G6(ACR 20mg+ WT 1.5%)	45.31±3.88	1.21±0.12*

Values represents the mean ± S.E. * P < 0.0 ** P < 0.01 ***P < 0.001

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