Antioxidant and histopathological effect of catechin and neem leaves extract in acrylamide toxicity of rats.

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SUMMARY

Recently, considerable alteration has been focused on dietary and medicinal photochemical that inhibit reverse or retard damage caused by oxidative and inflammatory processes. Catechin and neem leaves extract have both antioxidant and anti-inflammatory properties. In the present study we investigated wither catechin and neem extract can reduce the toxic effects of acrylamide. Acrylamide (2.8mM), catechin 1.5%, neem leave extract (200 mg/kg body wt), acrylamide + catechin and acrylamide + neem leave extract were given to rats. Catechin and neem leaves extract partially protects hemogram from anemia and leucopenia caused by Acrylamide treatment. In addition, these photochemical significantly modulated the lipid peroxidase (TBARS) associated with a reduction of GSH (reduced glutathione) and catalase enzyme observed in acrylamide groups. The microscopic examination of different organs in rats received acrylamide revealed a variable damage of tissues. The intensity of these changes were decline with a nearly degree in rats received catechin or neem leaves extract.

INTRODUCTION

Acrylamide (2-propenamide) is an industrial chemical used for the production of polymers 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 (LeQuense, 1980). Acrylamide monomer may form in certain foods cooked at high temperatures. Acrylamide is thought detected in food principally from the interaction of the amino acid asparagines with glucose or other carbohydrates Taubert et al., (2004). Acrylamide appears to be rapidly distributed to tissues (Marlowe, et al., 1986; and Sumner et al., 2001). Acrylamide (ACR) is an ß, ô-unsaturated carbonyl and is a well-recognized as neurotoxicant, reproductive toxicant and carcinogenic in animal LoPachin et al.,
Several studies with liver, kidneys, brain, and erythrocyte GST (glutathione-S-transferase) indicate that acrylamide causes oxidative stress (Sumner et al., 1997). P450 2E1 is possibly the cytochrome P450 enzyme involved in the metabolism of acrylamide. These may be an appropriate model for the investigation of the role of oxidative metabolism in the toxicity or carcinogenicity of these compounds Sumner, et al., and (1999). The activity of liver and brain antioxidant enzymes is rapidly lost following injection of acrylamide (50-200 mg / kg) Bergamini and Signorini, (1990).

Catechins (polyphenols) are a group of natural polyphenols found in green tea. Catechins have demonstrated significant antioxidant, anticarcinogenic, anti-inflammatory, thermogenic, antimutation, probiotic, and antimicrobial properties in vivo, and vitro studies (Kada et al., 1985; Muramatsu et al., 1986; Sagesaka et al., 1996; Fujiki et al., 1996, and Alschuler 1998). Catechin also restored the decreased nonenzymatic and enzymatic antioxidants of mitochondria Tabassum et al. (2007). Catechins have values comparable to that of α -tocopherol (vitamin E), but higher than ascorbate (vitamin C) which is a superior hydrogen donor (antioxidant) to polyphenols (Jovanavic et al., 1996 and Jovanavic et al., 1997).

Azadirachta indica (Neem leaves) and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, enhancing the antioxidant status, antimutagenic and anticarcinogenic properties Bhanwra, et al. (2000), Subapriya et al. (2003, 2004 and 2005). It has modulator effects on hepatic and blood oxidant-antioxidant status may play a key role in preventing cancer development at extrahepatic sites (Arivazhagan et al., 2004).

The purpose of this study was to elucidate the antioxidant activity and the hepato and cellular protective effect of catechin and neem leaves extract against acrylamide effects.

MATERIALS AND METHODS
MATERIALS:
- Acrylamide (99.9 purity) was purchased from Sigma chemical Co. It used in concentration 2.8 mM in drinking water for six weeks according to Barber et al., (2001).
- Catechins were extracted from green tea and add as the sole source of drinking water for rats at concentration of 1.5% according

- Neem leaves extract: ethanolic neem leaves extract was prepared and used at a dose of 200 mg/kg body wt. per os (Bhanwra, et al., 2000).

Experimental animals:

Thirty-six male albino rats weighing from 120 to 140 g were used in the present study. Rats were obtained from laboratory Animal House Ministry of Health, Helwan, Cairo. Animals were acclimatized in our laboratory condition for two weeks before being used. The rats were fed on balanced ration and free access water was allowed.

Experimental design:

The rats were divided into six groups each group contains six animals. Group (1) received basal diet and tap water throughout the experiment and served as the untreated control. Group (2) was administered acrylamide for six week. Group (3) was administered tea catechin extract throughout the experiment. Group (4) was administered neem leaves extract throughout the experiment. Group (5) was administered acrylamide for six week and tea catechin throughout the experiment. Group (6) was administered acrylamide for six week and neem leaves extract throughout the experiment.

Sampling

1- Blood samples

Six blood samples were collected from rat orbital sinus at end of experiment (six weeks), in two clean, dry, sterile and labeled centrifuge tubes.

A- The 1st tube contained the anticoagulant (heparin) was used for the determination of hemogram. Hemoglobin, red blood cell count, total and differential leukocytic counts according to the method described by Schalm et al., (1975).

B- The 2nd tube was used for separating serum by centrifugations at 3000 r.p.m for 10 minutes. Serum samples were subjected to estimations of vitamin E, A, C and β-carotene according to Henry, et al. (1974).

2- Minced liver tissue

Liver was removed and washed with saline solution, then minced and homogenized (10% w/v) in ice-cold normal saline. The homogenate was centrifuged at 10,000 x g for 20 min at 4°C and the resultant supernatant was used for biochemical assay Chitra, et al., (1999)

The protein contents of liver tissue were estimated by the method of Bradford (1976). The biochemical assays of catalase, lipid peroxidation (TBARS) and reduced glutathione (GSH) were determined as mentioned by Aebi, (1974); Ok-
hawa, et al. (1979) and Ellman (1959), respectively. The activity of catalase was expressed as IU per mg protein.

Histopathological examination:

The neurotoxicity of Acrylamide and its effect on reproductive organs have been extensively studied in various animal models, including rats, mice, monkeys, dogs and cats by numerous dosing regimens and durations of dosing (Carrington et al., 1991; HSDB, 1994 and Exon, 2006). So, the present study will discover and deal with the effect of Acrylamide on the other organs. The rats slaughtered after at the end of experiment and fresh tissue specimens were collected from liver, kidney, heart, stomach, spleen, intestine and lung. The tissue specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin and examined by light microscopy (Bancroft et al, 1996).

The results were subsequently analyzed by following the statistical methods as established by Snedecor (1969).

RESULTS AND DISCUSSION

Acrylamide has been classified as a Group 2A carcinogen by the International Agency for Research on Cancer (IARC, 1994) and a Category 2 carcinogen and Category 2 mutagen by the European Union, this finding has caused worldwide concern (World Health Organization (WHO), 2002; European Commission, 2002).

In concern to the effect of acrylamide on the erythrogram, acrylamide produced a significant decrease in red and white blood cell counts, haemoglobin concentration, and haematocrit values in rats received Acrylamide (table 1). Similar finding were recorded by (Burek et al., 1980) who reported that rats were administrated acrylamide at a dose 0.05, 1.5 or 20 mg/kg/day had a decrease in red blood cells count, heamoglobin and packed cell volume. Also observed by Abramsson et al., (2005) at a dose of 50 mg/kg/b.wt of acrylamide for 3 days. Our results were disagree with Albassam et al. (1987) who mentioned that there is an increase in concentration of haematocrite in rainbow trots exposed to acrylamide. Regarding to the effect of acrylamide on the leuckocytic count, the present study showed a significant decrease in leuckocytic count, which is correlated by Doerge et al., (2005) who reported the levels of leukocytes count were significant decreased post dose of 50 mg/kg.

The results of this study indicate that catachins extract and neem leaves extract partially protects hemogram from the changes caused by Acrylamide treatment. These
results agree with Chow et al., (2004) and Mbah et al. (2007). Catachins showed a significant increase in leukocyte count and maintained it more effectively and also enhancing vitamin C which is important in preventing anemia (Jacob, 1977, Zhu, et al., 1999) and Trevisanato and Kim 2000). Neem leaves extract was observed to stimulate hematological systems as evidenced by the increase in total count of RBC, WBC and platelets as well as, hemoglobin percent Ghosh et al. (2006), and Haque et al. (2006). In addition, neem leaves extract has an immunostimulant effect through enhancing the activities of cell mediated immuno response (Sadekar et al., 1998).

DNA damage was assessed in liver and lung by administered acrylamide Ghanayem et al (2005). 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, 2).

Acrylamide could be generating ROS, (Reactive Oxygen Species) which enhanced lipid peroxidase production. Cellular fatty acids are readily oxidized by ROS to produce lipid peroxyl radicals and lipid hydroperoxides (Rice-Evans and Burdon, 1993). Lipid peroxyl 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 2). ACR is an α, β-unsaturated aldehyde with electrophilic reactivity at the carbonyl carbon atom. As a soft electrophile, ACR could conceivably adduct amines, imidazoles and sulphydryl groups on proteins via the Michael carbonyl condensation reaction (Friedman, 1973; Kemp and Vellaccio, 1980, Becalski et al 2003). However, the reactivity of free thiols is greater than that of other soft nucleophilic centers and, consequently, the preferential in vivo target of ACR is sulphydryl groups on protein cysteine residues and glutathione (Calleman, 1996). 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).

Catechin provides the first defense against oxygen toxicity by catalyzing the dismutation of superoxide anion to hydrogen peroxide and decomposition of hydrogen
peroxide to water and molecular oxygen (Corrocher et al., 1986). The activity of liver antioxidant enzymes is rapidly lost following injection of acrylamide (50-200 mg/kg) Bergamini and Signorini (1990).

Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human and animal's diseases (Stajner et al., 1998 and Malencic et al., 2000). There are many reports that support the use of antioxidant supplementation in reducing the level of oxidative stress and in slowing or preventing, the development of complications associated with diseases (Rose et al., 1982).

Administration of catechin and neem leaves extracts significantly lowered lipid peroxidation and enhanced the hepatic levels of glutathione (GSH) and catalase enzymes. High doses of catechin (3 g/d for 30 days or 1.5 g/d for 0 days) increased blood levels of vitamin E and the activities of the antioxidant enzymes catalase and glutathione peroxidase in patients with chronic hepatitis (Pár et al., 1985 and Kaviarasna et al., 2007).

We speculate that catechin and neem leaves significantly alter cancer development at extrahepatic sites by influencing hepatic biotransformation enzymes and antioxidants (Skrzymlewska et al., 2002; Paulsson et al., 2003 and Subapriya et al., 2004). The results of the present study demonstrate that catechin and neem leaves extract exerts protective effects against acrylamide induced oxidative stress by augmenting host antioxidant defense mechanisms.

catechins and neem leaves extract 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, peroxyl and lipid radicals and superoxide anions), glutathione metabolism and cytochrome P450 2E1 activity (Wolin, 2000; Sithisarn et al., 2006 and Raza and John, 2007).

Our finding of low plasma retinol, α-tocopherol, ascorbic acid and β-carotene in acrylamide-toxicated rats compared to control group (table 3) was shown in pervious studies by Clarke et al. (1984) and Vatassery et al. (1986).

Acrylamide causes oxidative stress by inducing the generation of reactive oxygen species, reducing the antioxidant defense systems of cells via depleting non enzymatic antioxidant system (vitamins and glutathione) and/or increasing susceptibility of cells to oxidative attack by altering the membrane integrity and fatty acid composition.
Feeding a mixture of tea catechins (10 g/kg diet, amounting to 140 mg CAT/kg diet) to catechins exert a significant protection to vitamin E, C, A and β-carotene (Lotito and Fraga, 2000). Catechins have been shown to reduce the oxidative consumption of vitamin E in vitro (Pedrielli and Skibsted, 2002). It is can regenerate the antioxidant (Frank et al., 2003). Feeding tea catechins to rats resulted in a significant increase in vitamin E concentrations in plasma and erythrocytes (Nanjo et al., 1993).

Dietary catechins and neem leaves extract prevent the decrease of non-enzymatic antioxidants and enhancing the antioxidant status (Kasaoka et al., 2002; Frank et al. 2003 and Subapriya et al., 2003).

The main histopathological studies in rats received Acrylamide alone (G-2) or in-combination with catechin (G-5) or neem leaves extract (G-6) revealed similar pathological alterations. The intensity of change were more obvious in (G-2) and the severity were decline with a nearly degree in (G-5) and (G-6).

The microscopical examination of different organs of rats (G-2) demonstrated variable tissue damage. The liver showed diffuse vacuolar degeneration of hepatocytes of nearly all animals. Congestion of central veins and hepatic sinusoids as well as blood vessels of portal area were observed. Hemorrhage was pronounced in the hepatic parenchyma especially in the subcapsular area (Figs., 1 & 2).

The kidney revealed an interstitial hemorrhage and oedema. Vascular degeneration of the convoluted tubular epithelium as well as glomerular degeneration with albuminous material in the capsular space was seen. Hyaline cast within tubular lumen was also observed (Fig., 3).

The lung showed focal area and red hepatization with peribronchial aggregation of inflammatory cells (Fig, 4). Some alveoli contain homogenous esinophilic exudates. Emphysema, congestion as well as hemosidrosis were also seen (Fig., 5).

The heart revealed hemorrhages between the myocardial muscle fibers accompanied with myocardial degeneration and loss of accompanied striation (Figs, 6 & 7). Spleen showed moderate hemorrhages associated with haemosidrin laden macrophages (Fig., 8). Stomach revealed sever vacuolar degeneration of the epithelial lining stomach mucosa, in addition to mononuclear cells infiltration in the tunica muscularosa (Fig., 9). Intestine showed
necrosis of the lining epithelium of some villi as well as scattered areas of hemorrhage. No pronounced histopathological change could be detected in the different organs of rats supplement with catechin extract (G-3) or neem leaves extract (G-4) alone. The histopathological alterations observed in Acrylamide group are likely related to affinity of Acrylamide binding to sulfhydryl groups on protein and so, inactivate the enzyme involved in DNA repair and other critical functions (Nordin and Andersson et al., 2003 and Exone, 2006).

These results were parallel with those observed by (Hashimoto et al., 1981, Hashimoto and Sakamoto, 1982) who noted degeneration changes in renal convoluted tubular epithelium and glomerulus in addition to degeneration and necrosis of hepatic parenchyma in monkeys received large dose of Acrylamide. Also, our results agree with Mc Collister et al (1964) who observed degeneration in convoluted tubular epithelium and hepatic cells as well as congestion and hepatic sinusoids.

Catechin and neem leaves extract administration led to modulation of all histological injury observed by Acrylamide toxicity.

The protective effect of catechin and neem leaves extract may be due to ability for regulation the immune mediated through the suppressive effect on the production of important inflammatory mediators. Also, may be due to the capacity of catehins to scavenge free radicals and protect against oxidant stress induced by acryamide. These results and discussion were similar to those observed by (El-Beshbishy, 2005. Wang et al., 2006 and Koul et al., 2006).

On conclusion, catechin and neem leaves extract have a nearly similar protective manner against Acrylamide toxicity by anti-inflammatory and antioxidant role.
Table (1): Mean (±SE) blood hematological values in rats fed acrylamide, tea catechin or neem extract singly and in combination (n=6).

<table>
<thead>
<tr>
<th></th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>14.01</td>
<td>10.98***</td>
<td>12.54</td>
<td>13.79</td>
<td>11.83**</td>
<td>12.85</td>
</tr>
<tr>
<td>±0.56</td>
<td>±0.55</td>
<td>±0.86</td>
<td>±0.72</td>
<td>±0.44</td>
<td>±0.75</td>
<td></td>
</tr>
<tr>
<td>RBCs X10⁶</td>
<td>7.53</td>
<td>6.04***</td>
<td>6.51</td>
<td>6.99</td>
<td>6.23**</td>
<td>6.35*</td>
</tr>
<tr>
<td>±0.3</td>
<td>±0.24</td>
<td>±0.46</td>
<td>±0.46</td>
<td>±0.34</td>
<td>±0.45</td>
<td></td>
</tr>
<tr>
<td>PCV%</td>
<td>43.9</td>
<td>34.2***</td>
<td>37.9*</td>
<td>39.8</td>
<td>38.4*</td>
<td>41.7</td>
</tr>
<tr>
<td>±1.51</td>
<td>±1.51</td>
<td>±1.76</td>
<td>±1.76</td>
<td>±1.10</td>
<td>±1.24</td>
<td></td>
</tr>
<tr>
<td>WBCs X10³</td>
<td>8.80</td>
<td>7.23**</td>
<td>8.57</td>
<td>9.4</td>
<td>7.79*</td>
<td>8.2</td>
</tr>
<tr>
<td>±0.33</td>
<td>±0.37</td>
<td>±0.26</td>
<td>±0.3</td>
<td>±0.26</td>
<td>±0.21</td>
<td></td>
</tr>
<tr>
<td>Lymph</td>
<td>42.1</td>
<td>46.6</td>
<td>40.0</td>
<td>39.8</td>
<td>44.3</td>
<td>43.5</td>
</tr>
<tr>
<td>±2.71</td>
<td>±1.06</td>
<td>±1.41</td>
<td>±1.3</td>
<td>±2.17</td>
<td>±1.24</td>
<td></td>
</tr>
<tr>
<td>MID</td>
<td>31</td>
<td>25.4***</td>
<td>28.7</td>
<td>33.1</td>
<td>28.4</td>
<td>30.9</td>
</tr>
<tr>
<td>±1.11</td>
<td>±1.21</td>
<td>±0.76</td>
<td>±1.2</td>
<td>±1.35</td>
<td>±1.01</td>
<td></td>
</tr>
<tr>
<td>Gran</td>
<td>26.9</td>
<td>28.0</td>
<td>31.3</td>
<td>27.1</td>
<td>27.3</td>
<td>25.6</td>
</tr>
<tr>
<td>±1.83</td>
<td>±2.17</td>
<td>±1.57</td>
<td>±1.41</td>
<td>±1.82</td>
<td>±1.63</td>
<td></td>
</tr>
</tbody>
</table>

Table (2): Mean values (±SE) of liver Malondialdehyde, GHS and Catalase values in rats fed acrylamide, tea catechin or neem extract singly and in combination (n=6).

<table>
<thead>
<tr>
<th>groups</th>
<th>Malondialdehyde (mM/100g)</th>
<th>GHS (µmol/mg protein)</th>
<th>Catalase (IU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>1.93±0.41</td>
<td>39.61±2.01</td>
<td>6.38±0.047</td>
</tr>
<tr>
<td>G2</td>
<td>6.17±0.52***</td>
<td>23.17±1.31***</td>
<td>4.19±0.38***</td>
</tr>
<tr>
<td>G3</td>
<td>1.39±0.32</td>
<td>38.03±1.99</td>
<td>7.58±0.75</td>
</tr>
<tr>
<td>G4</td>
<td>1.40±0.43</td>
<td>40.11±1.67</td>
<td>7.43±0.69</td>
</tr>
<tr>
<td>G5</td>
<td>3.58±0.46*</td>
<td>32.21±1.79</td>
<td>5.18±0.41</td>
</tr>
<tr>
<td>G6</td>
<td>4.11±0.47**</td>
<td>30.33±1.84**</td>
<td>5.00±0.36*</td>
</tr>
</tbody>
</table>

Values represent the mean ±SE

*P<0.05    **P<0.01    ***P<0.001   "students "t" test"
Table (3): Mean (±SE) serum vitamin C, E, A and β-carotene values in rats fed acrylamide, tea catechin or neem extract singly and in combination (n=6).

<table>
<thead>
<tr>
<th>groups</th>
<th>Vitamin C µg/dl</th>
<th>Vitamin E µg/dl</th>
<th>Vitamin A µg/dl</th>
<th>β-carotene µg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>0.68±0.36</td>
<td>556.5±13.1</td>
<td>46.47±2.35</td>
<td>24.10±1.81</td>
</tr>
<tr>
<td>G2</td>
<td>0.33*±0.34</td>
<td>414.00*±10.13</td>
<td>31.03**±3.98</td>
<td>13.98**±1.94</td>
</tr>
<tr>
<td>G3</td>
<td>0.61±0.27</td>
<td>680.11*±21.17</td>
<td>50.59±1.71</td>
<td>25.76±1.36</td>
</tr>
<tr>
<td>G4</td>
<td>0.72±0.41</td>
<td>618.73±37.75</td>
<td>56.62*±1.76</td>
<td>29.15±1.61</td>
</tr>
<tr>
<td>G5</td>
<td>0.41±0.34</td>
<td>495.34±25.17</td>
<td>41.13±2.1</td>
<td>17.17*±1.71</td>
</tr>
<tr>
<td>G6</td>
<td>0.50±0.36</td>
<td>439.57*±15.93</td>
<td>43.06±1.66</td>
<td>18.11*±1.94</td>
</tr>
</tbody>
</table>

Values represent the mean ±SE
*P<0.05    **P<0.01    "students"t"test"
Fig. (1, 2): Liver of gp (2) and (5) showing congestion, interstitial hemorrhage and vacuolar degeneration of hepatocytes (H&E X200)
Fig. (3): Kidney of gp (2) showing interstitial hemorrhage as well as hyaline cast within tubular lumens (H&E X200)

Fig. (4): Lung of gp (2) showing red hepatization with peribronchial aggregation of inflammatory cells (H&E X400)
Fig. (5): Lung of gp (6) showing red hepatization as well as emphysema and congestion (H&E X200)
Fig (6, 7): Heart of gp (2) and (5) showing hemorrhage of the myocardial muscle fibers accompanied with myocardial degeneration and loss of their striation (H&E – fig 6- X200 & fig 7- X400)
Fig (8): Spleen of gp (2) showing moderate hemorrhage associated with hemosidrin laden macrophages (H&E X200)

Fig (9): Stomach of gp (2) showing vacuolar degeneration of the epithelial lining stomach mucosa. (H&E X400).
REFERENCES


lipid peroxide status in patients with chronic hepatitis."


Rice-Evans, C. and Burdon, R. (1993): "Free radical-lipid interactions and their pathologi-


