Chemoprevention of Barley and Sage against acrylamide-Induced genotoxic, biochemical and histopathological alterations in rats

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Abstract
Acrylamide (ACR) has recently been found in fried and backed foods, suggesting widespread public exposure. ACR is an industrial chemical material that causes neurotoxicity in humans and was designated as a probable human carcinogen by IARC and USEPA. The aim of the present study was to evaluate the protective effects of barley and sage against ACR mutagenicity and biochemical and histopathological changes in rats. Forty mature male rats were divided into eight groups and were fed barley and/or sage-supplemented diet (5%) with or without ACR (50 mg/kg b.w). The biochemical results revealed that ACR increased Alanine amino transferase (ALT), Aspartate amino transferase (AST), triglycerides (TG), cholesterol and uric acid. Micronucleated polychromatic erythrocytes and chromosomal aberrations in somatic and germ cells were significantly increased in ACR-treated animals. Severe pathological lesions included testicular degeneration, oedema, spermatid giant cells formation and necrosis of spermatid cells were found in the testis of ACR-treated group. The kidney of this group showed degenerative changes. Cotreatment with barley and/or Sage and ACR resulted in a significant improvement in all the parameters tested. It could be concluded that these plants contain antioxidant compounds and may be useful when add as food additive to the food cooked on a higher temperature.

Key words: Acrylamide, Barley, Sage, chromosomal aberrations, kidney, testis.

Introduction
In the year 2002 the Swedish National Food Authority reported the presence of elevated levels of acrylamide (ACR) in certain types of food processed at high temperature. Since then ACR has been found in a lot of cooked and heat-processed foods in other countries, including Netherlands, Norway, Switzerland, United States, United Kingdom (Shelby, 2004), and Egypt. Human exposed to ACR through the consumption of heat-cooked food such as potato crisps, chips, cocktail snacks and gingerbread. Chronic ACR exposure has been associated with increased incidence of mesothelioma, cancer of central nervous system, thyroid gland and other endocrine glands, mammary glands and reproductive tracts in rats (Johnson et al., 1986). ACR is regarded as a potential mutagen based on experimental evidence that it can bind to DNA (Sega, 1990). ACR undergoes biotransformation by conjugation with glutathione (Miller, 1982) or reduction by microsomal cytochrome P-450 (Anzenbacher and Anzenbacherova, 2001). The major metabolite of ACR, glycidamide is an epoxide that may be more critical for carcinogenic and genotoxic properties in animals than the parent compound (Segerback et al., 1995).

ACR exposure in hamsters caused testicular atrophy with damage to spermatids and mature spermatozoa (Sakamoto et al., 1988). Reduced sperm motility and impaired fertility have also been reported in treated mice and rats
ACR caused chromosomal aberrations in mammalian cells in vitro and in vivo (Backer et al., 1989; Collins et al., 1992). Micronuclei were found in mouse bone-marrow cells and splenocytes following exposure to ACR (Collins et al., 1992). ACR induces pathological lesions in different body organs including variety of tumors in rats and mice (U.S.EPA, 1994; ACGIH, 1991). In human, inadequate evidence is available from human studies regarding the tumor formation by acrylamide.

Previous studies indicated that the leaves of sage (Salvia officinalis L.) are well known for their anti-oxidative properties (Chipault et al., 1956; Baricevic et al., 1996), used in the food processing industry but applicable also to the area of human health (Pearson et al., 1997). The plant is reported to have a wide range of biological activities, such as anti-bacterial, fungistatic, virustatic, astringent, eupeptic and anti-hydrotic effects (Dobrynin et al., 1976, Cherevaty et al., 1980). Experimental studies on sage extracts or sage essential oil showed hypotensive properties, central nervous system-depressant actions and anti-spasmodic activity (Newall et al., 1996). Moreover, the antimutagenic potential of sage extracts was demonstrated on Escherichia coli repair proficient strains (Baricevic et al.,1996; Filipic and Baricevic 1997 and1998).

Barley (Hordeum vulgare L.) grains are relatively rich in dietary fibers such as glucan, arabinoxylans and cellulose. The consumption of glucan-rich diet results in several beneficial physiologic effects due to a relatively high concentration, soluble state and high molecular weight of this polysacride (Dongowski, et al., 2002). The extractability and viscosity of glucan are influenced by both the technologic conditions in the gastrointestinal tract (Robertson, et al., 1997, Johansen, et al., 1993). Generally, the previous studies concluded that dietary fiber-rich barley-containing diets have beneficial physiologic effects. The aim of the present study was to evaluate the protective effects of barley and sage against ACR-induced toxicity, mutagenicity biochemical and pathological changes in rats.

Materials and methods

Acrylamide

Acrylamide was purchased from MERCK-Schuchardt Chemical Co. (Hohenbrunn, Germany) with molecular formula NH2-C=O-CH2, with purity >99%.

Kits: Transamianse (ALT and AST) were purchased from Randex Laboratories (San Francisco, CA, USA). Cholesterol, triglycerides and uric acid were obtained from Biomerieux, Laboratory of Reagents and Products (Marcy Létoile, France).

Plants

Barley was purchased from local market at Cairo, Egypt. Whereas, Sage (Salvia officinalis) was supplied by the Department of crop production, Faculty of Agriculture, Ain-Shams University.

Animals and Treatments

Experimental Animals: Ten-week-old, adult male Sprague-Dawley rats weighting 180-200 g (purchased from Animal House Colony, Giza, Egypt) were maintained on standard lab diet (Protein: 16.04%; Fat: 3.63%; Fiber: 4.1%, and metabolic energy: 0.012 MJ) and water ad libitum at the Animal House Lab., National Research Center, Dokki, Cairo, Egypt. After an acclimation period of 1 week, animals were distributed into eight groups (5 rats /group) and housed in stainless steel cages housed in a temperature-controlled (23 ± 1 °C) and artificially illuminated (12 hr dark/light cycle) room free from any source of chemical contamination.

Experimental Design: After an acclimation period of one week, animals were divided into eight treatment groups (5 rats /group). Groups 1-4 were fed on barley diet only for three months whereas, groups 5-8 were fed on the standard lab diet for the same period. At the end of the 3rd month, animals within all groups were treated for five days as follow: group (1) control, group (2) treated orally with ACR (50 mg/kg b.w), group (3) fed on ad lib barley diet, group (4) fed on barley diet and treated orally with ACR, group (5) fed on standard lab diet...
supplemented with 5% dried sage leaves, group (6) fed on lab diet supplemented with dried sage leaves and treated orally with ACR, group (7) fed on barley diet supplemented with 5% dried sage leaves, and group (8) fed on barley diet supplemented with sage leaves and treated orally with ACR.

At the end of the experiment period, blood samples were collected from the retro-orbital venous plexus under diethyl ether anesthesia. Blood samples were left to clot and the sera were separated using cooling centrifugation and stored at −20°C until analysis. The activities of Alanine amino transferase (ALT) and Aspartate amino transferase (AST) were determined according to the method recommended by The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (1974). Cholesterol was determined in serum according to Charles and Richmond (1974), triglycerides (TG) was determined in serum according to Wahlefeld, (1974) and uric acid was determined in serum according to Haisman and Muller (1977). Then animals were injected intraperitonealy (i.p) with 0.1 ml of 0.5% of colchicine 3 h before sacrifice. The femora and testis were removed from all animals and prepared for the micronucleus and chromosomes studies as follow:

For micronucleus analysis: Slides were prepared according to Salamone et al. (1980) The bone marrow cells were mixed with one drop of fetal calf serum and smeared on clean glass slides. The slides were air dried and fixed in methyl alcohol for 5 min. then stained with 5% Giemsa and mounted with DPX. Two thousands PCEs/animal were recorded.

For chromosomal analysis: The bone marrow was prepared, stained and scored according to Preston et al. (1987). To study the effects of different treatments on the mitotic activity of the bone marrow, 100 cells per animal were counted and the number of dividing cells including prophases and metaphases were scored. The testis of the same animals were removed and slides were prepared according to the method adopted by Russo (2000). Seventy-five metaphase spreads were examined in spermatocytes.

Histopathological examination: - Samples of testis and kidneys were collected from each animal within each treatment immediately after scarification and fixed in 10% neutral buffered formalin. Tissue specimens were processed routinely for paraffin sections of 4-5 micron thickness, stained with Hematoxylin and Eosin (H&E) according to Bancroft et al. (1996). Whereas, Crystal violet-eosin stain was used to determine the normal and abnormal spermatozoa in the testis (Deeb, 1976).

Statistical analysis: All data for biochemical analysis were statistically analyzed using the General Linear Model Procedure of the Statistical Analysis System (SAS, 1982). The significance of the differences among treatment groups was determined by Waller-Duncan k-ratio (Waller and Duncan, 1969). All statements of significance were based on probability of $P < 0.05$. For micronucleus test and chromosomal aberrations study, the comparison of reliability analysis (F. test) was applied (Snedecor and Cochran, 1961).

Results Results of the biochemical analysis (Table 1) revealed that treatment with ACR caused a significant increase in all tested parameters. Sage alone resulted in a significant decrease in all tested parameters except AST which was comparable to the controls. Rats fed barley diet only showed a significant decrease in TG, Cholesterol and uric acid levels in serum, whereas, ALT and AST were comparable to the control. Both sage and barley were comparable to the control regarding AST, whereas the other biochemical parameters were found to decrease than the control values. Addition of sage to the diet of animals treated with ACR resulted in a significant improvement in all the parameters tested. This treatment was effective in decreasing the elevated levels of all the biochemical parameters although they were still differ significantly than the control. Animals fed barley diet and received ACR were comparable to the
control regarding ALT while the other parameters were still significantly higher than the control. Animals treated with ACR and received barley plus sage showed a normal AST, TG and cholesterol levels whereas, a significant decrease and a significant increase was found for ALT and uric acid respectively.

**Micronucleus study**

Data presented in table (2) and fig. (1a) show the effects of different treatments on polychromatic erythrocytes (PCEs) in bone marrow of rats. Treatment with ACR resulted in a significant increase (P< 0.01) in the micronucleated polychromatic erythrocyte (MnPCEs) whereas; no significant differences were noticed in the MnPCEs between the control group and groups treated with barley or sage. Animals fed barley for three months showed an inhibition of MnPCEs reached 69.3%. Meanwhile, sage alone showed a significant (P<0.01) inhibition in MN-PCEs (35.42%).

**Somatic cells:**

In the present study, the chromosomal examination showed that structural aberrations include chromatid and chromosomal gaps (Fig. 1c), deletions and end to end associations (Fig. 1d), chromatid and chromosomal breaks (Fig. 1e), and fragments (Fig. 1f). Numerical aberrations were studied represented by polyploidy (Table 3) (Fig. 1g).

Statistical analysis showed a significant increase (P< 0.001) in total structural chromosomal aberrations with or without gaps and total aberrations in ACR-treated group compared with the control group. The total structural aberrations and total aberrations recorded a significant increase (P<0.01) in ACR-sage treated group compared with sage group. These differences in total structural aberrations were also significant (P<0.05) compared with those of animals fed barley alone or barley plus sage (Table 3).

The main frequent types of structural aberrations in the animals treated with ACR plus barley was chromosomal breaks which found to be significant (P< 0.05) whereas, the other types of aberrations were nonsignificant. Animals treated with ACR plus sage showed a significant increase in case of gaps and the other types of aberrations (p<0.01 and 0.05 respectively). Animals pretreated with barely or fed barley supplemented with 5% sage, or those fed control diet supplemented by 5% sage showed an inhibition in chromosomal aberrations in bone marrow (59.6%, 63.1%, and 37% respectively. Animals fed barley supplemented with 5% sage showed insignificant changes in all other type of aberrations.

**Spermatocytes:**

Concerning the spermatocytes abnormalities, data presented in table (4) and fig (2) clearly indicated that treatment with ACR resulted in a significant increase (P<0.01) in total structural aberrations. The main types of abnormalities observed were chain (fig. 2b), autosomal univalent (fig. 2c), X-Y univalent and polyploidy (fig. 2d). Animals treated with sage, barley or barley plus sage showed nonsignificant increase compared with the control group. Treatment with barley, sage or barley plus sage succeeded to decrease significantly the abnormalities of spermatocytes (71.4, 46 and 85.7% respectively) although some abnormalities such as x-y univalents, chain, autosomal univalents and total structural aberrations were still significant in the animals received the combined treatment (P< 0.05).

Results in table (4) indicated the numerical aberrations recorded. These results showed a significant increase in ACR group compared with the control (P< 0.01). Meanwhile, the pretreatment with barley or sage plus barley inhibited the total numerical aberrations.

**Histopathological results:**

The histopathological examination of the animal treated with ACR alone revealed severe pathological lesions in the testicular tissues. A large number of residual bodies in the lumen of the seminiferous tubules were noticed (Fig. 3a). Some of the seminiferous tubules were completely devoid of mature sperms while others showed few number as demonstrated by positive reaction of the mature sperms to crystal violet stain (Fig.3b). Necrotic spermatocytes with chromatolysis were also noticed (Fig3, c,d). The most common
testicular lesion in the animals in this group was the presence of varied number of spermatid giant cells. Some of the seminiferous tubules appeared with single giant cell with foamy cytoplasm (Fig. 4a) while the others were impacted with 3-6 spermatid giant cells (Figs. 4b,c). The nuclei of these cells were varied in number and ranged from 2-5 nuclei (Figs. 4d,e). Interstitial oedema was also observed in the testis with spermatid giant cells (Fig. 4f).

The animal fed barley and treated with ACR showed noticeable decrease of the testicular lesions. The seminiferous tubules appeared impacted with mature spermatocytes, which confirmed by positive reaction to crystal violet (Fig. 5a,b). On the other hand, animals fed sage supplemented diet and treated with ACR appeared with low number of mature spermatocytes but without obvious pathological lesions (Fig. 5c,d).

The kidneys of the animals in ACR-treated group showed mild degenerative changes with shrunken glomerular capillary tufts (Fig. 6a). Animals fed barley and/or sage showed no obvious pathological lesions in the testicular and kidney tissues. The kidneys of both groups and received ACR were apparently normal (Fig 6b).

Table (1). Effect of ACR with or without barley or sage on serum biochemical parameters in rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ACR</th>
<th>Barley</th>
<th>ACR + Barley</th>
<th>Sage</th>
<th>ACR + Sage</th>
<th>Barley + Sage</th>
<th>ACR + Barley + Sage</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L)</td>
<td>36.52a ± 0.85</td>
<td>46.99c ± 1.53</td>
<td>36.84a ± 0.68</td>
<td>36.33a</td>
<td>33.65b</td>
<td>34.03b</td>
<td>34.28b</td>
<td>31.24d ± 0.95</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>42.17a ± 0.79</td>
<td>56.50c ± 1.18</td>
<td>43.33b ± 0.88</td>
<td>44.67b</td>
<td>41.08a</td>
<td>45.33b</td>
<td>41.62a</td>
<td>41.57a ± 0.79</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>55.00a ± 1.41</td>
<td>71.50c ± 1.41</td>
<td>43.17b ± 1.08</td>
<td>58.67ad</td>
<td>42.83b</td>
<td>62.17d</td>
<td>39.67b</td>
<td>56.17a ± 1.30</td>
</tr>
<tr>
<td>Cholesterol (mg/l)</td>
<td>68.00a ± 1.41</td>
<td>91.67c ± 1.82</td>
<td>56.00b ± 1.15</td>
<td>69.83ad</td>
<td>57.50b</td>
<td>71.83d</td>
<td>54.17b</td>
<td>67.00a ± 0.97</td>
</tr>
<tr>
<td>Uric Acid (mg/dl)</td>
<td>0.47a ± 0.02</td>
<td>0.94c ± 0.02</td>
<td>0.36b ± 0.01</td>
<td>0.64d</td>
<td>0.38b</td>
<td>0.66d</td>
<td>0.34b</td>
<td>0.61d ± 0.02</td>
</tr>
</tbody>
</table>

Within each raw, means superscript with the same letter are not significantly different (P< 0.05)

Table (2: The mean value of micronuclei detected in polychromatic erythrocytes of bone marrow cells induced ACR and the effect of antioxidants in rats

<table>
<thead>
<tr>
<th></th>
<th>Mononuclear</th>
<th>Binuclei</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>1.0 ± 0.41</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>ACR (50mg/kg b.w)</td>
<td>13.29 ± 0.29***</td>
<td>1.0 ± 0.41</td>
<td></td>
</tr>
<tr>
<td>barley</td>
<td>1.75 ± 0.85</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>ACR + barley</td>
<td>4.5 ± 0.86</td>
<td>0.25 ± 0.25</td>
<td>69.3%</td>
</tr>
<tr>
<td>Sage</td>
<td>2.5 ± 1.04</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>ACR + Sage</td>
<td>9.25 ± 1.11**</td>
<td>0.5 ± 0.29</td>
<td>35.42%</td>
</tr>
<tr>
<td>Sage + barley</td>
<td>1.5 ± 0.65</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>ACR + Sage + barley</td>
<td>3.25 ± 0.25</td>
<td>-----</td>
<td>86.5 %</td>
</tr>
</tbody>
</table>

= P < 0.05 ** = P < 0.01
Table (3): Mean value of different chromosomal aberrations induced by ACR and the effect of antioxidants in bone marrow of male rats (M ± SE)

<table>
<thead>
<tr>
<th>Types of aberrations</th>
<th>Treatments</th>
<th>control</th>
<th>ACR</th>
<th>barley</th>
<th>ACR+barley</th>
<th>Sage</th>
<th>ACR+Sage</th>
<th>ACR+Sage+barley</th>
</tr>
</thead>
</table>
| Total aberrations    |                    | 1.0 ± 0.41 | 40.3 ± 1.8**) | 2.75 ± 0.47 | 18.0 ± 0.71) | 4.0 ± 0.91 | 27 ± 0.41**) | 3.25 ± 0.63 | 17.0 ± 4.4*
| Total struct. aberrations |            | 0.75 ± 0.25 | 32.75 ± 1.31**) | 2.2 ± 0.48 | 12.25 ± 2.25**) | 3.75 ± 0.25 | 23.25 ± 1.75**) | 2.5 ± 0.65 | 13.75 ± 2.1
| Total struct. Ex. gap |                   | 0.5 ± 0.29 | 25.25 ± 1.31)** | 1.5 ± 0.29 | 10 ± 2.01) | 2.25 ± 0.85 | 19 ± 1.47**) | 1.5 ± 0.87 | 10.5 ± 1.9
| Gap                  |                   | 0.25 ± 0.25 | 7.5 ± 0.64**) | 0.75 ± 0.25 | 2.5 ± 1.04 | 1.0 ± 0.41 | 4.25 ± 0.63 | 1.0 ± 0.41 | 3.75 ± 1.25
| Chromos. gaps        |                   | ——— | 4.25 ± 0.75**) | ——— | 2.5 ± 0.96 | 0.5 ± 0.29 | 2.75 ± 0.25 | 0.25 ± 0.25 | 1.75 ± 0.63
| Breaks               |                   | 0.25 ± 0.25 | 5.5 ± 0.65**) | 0.5 ± 0.29 | 2.75 ± 0.48 | 0.75 ± 0.29 | 3.75 ± 0.93 | 0.75 ± 0.25 | 2.25 ± 0.75
| Chromos. breaks      |                   | ——— | 4.5 ± 0.29**) | 0.25 ± 0.25 | 2.25 ± 0.49 | 0.5 ± 0.29 | 3.5 ± 0.64 | 0.25 ± 0.25 | 1.5 ± 0.65
| Deletions            |                   | ——— | 4.0 ± 0.41**) | ——— | 1.5 ± 0.65 | 0.5 ± 0.29 | 3.75 ± 0.85 | 0.25 ± 0.25 | 2.25 ± 0.85
| Fragments            |                   | ——— | 4.0 ± 0.41**) | ——— | 1.5 ± 0.65 | 0.5 ± 0.29 | 3.75 ± 0.85 | 0.25 ± 0.25 | 2.25 ± 0.85
| E.E                  |                   | 0.25 ± 0.25 | 7.5 ± 0.29**) | 0.5 ± 0.29 | 3.0 ± 0.41* | 0.75 ± 0.25 | 3.75 ± 0.85* | 0.75 ± 0.25 | 3.25 ± 0.48

E.E= end to end associations Total struct. Ex. gap = Total structural excluding gaps
* = P < 0.05 ** = P < 0.01

Table (4): Mean values of different aberrations induced by acrylamide and the effect of antioxidants in rat spermatocytes (M ± SE)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Types of aberrations</th>
<th>Chain</th>
<th>Auto</th>
<th>X-Y</th>
<th>Total</th>
<th>Ploidy</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td></td>
<td>0.0± 0.0</td>
<td>0.25</td>
<td>0.75</td>
<td>1.25</td>
<td>0.25 ± 0.25</td>
</tr>
<tr>
<td>ACR</td>
<td></td>
<td>3.75 ± 0.63**)</td>
<td>4.0 ± 0.42**)</td>
<td>5.0 ± 0.71**)</td>
<td>13.0 ± 0.71**</td>
<td>2.75 ± 0.48**)</td>
</tr>
<tr>
<td>barley</td>
<td></td>
<td>0.25 ± 0.25</td>
<td>0.75</td>
<td>1.0</td>
<td>2.75</td>
<td>0.75 ± 0.48</td>
</tr>
<tr>
<td>ACR+barley</td>
<td></td>
<td>1.25 ± 0.48*</td>
<td>1.5</td>
<td>2.5</td>
<td>5.25</td>
<td>1.25 ± 0.69</td>
</tr>
<tr>
<td>Sage</td>
<td></td>
<td>0.5 ± 0.29</td>
<td>0.75</td>
<td>1.25</td>
<td>2.25</td>
<td>0.75 ± 0.25</td>
</tr>
<tr>
<td>ACR+Sage</td>
<td></td>
<td>2.25 ± 0.48*</td>
<td>3.0</td>
<td>3.5</td>
<td>9.0</td>
<td>1.75 ± 0.25</td>
</tr>
<tr>
<td>Sage+barley</td>
<td></td>
<td>0.25 ± 0.25</td>
<td>0.75</td>
<td>0.75</td>
<td>1.5</td>
<td>1.0 ± 0.41</td>
</tr>
<tr>
<td>ACR+sage+barley</td>
<td></td>
<td>0.75 ± 0.48</td>
<td>1.0</td>
<td>2.25</td>
<td>4.25</td>
<td>1.75 ± 0.63</td>
</tr>
</tbody>
</table>

Auto = autosome univalents X-Y = x-y univalents
* = P < 0.05 ** = P < 0.01
Fig. (1):

a- Photomicrograph of a rat bone marrow showing normal PCEs and contains micronucleus MnPCEs.
b- Normal rat metaphase spread
c- Metaphase spread of a treated rat with a gap (G) and chromosomal gap (ch.G)
d- Metaphase spread of a treated rat with a deletion (D) and end to end associations (E.E)
e- Metaphase spread of a treated rat with a chromosomal break (ch.B)
f- Metaphase spread of a treated rat with a fragment (F)
Fig. (2):  
**a**- A normal rat spermatocyte  
**b**- Treated rat spermatocyte showing a chain  
**c**- Treated rat metaphase spread showing autosomal univalents  
**d**- Treated rat metaphase spread showing X-Y univalents and polyploidy

Fig. (3): Testis of albino rat orally exposed to acrylamide showing, (a) large number of residual bodies in the lumen of seminiferous tubules (H&E x 400). (b) Remnants of mature spermatozoa in the lumen (Crystal violet x 1000). (c) Different stages of necrosis in spermatocytes with pyknotic (p) and karyolytic (k) nuclei together with disturbed cytoplasm and nuclei of Leydig cells (H&E x 1000). (d) Prominent chromatolysis in the nuclei of spermatocytes (H&E x 1000)
Fig. (4): Testis of rat treated orally with ACR showing (a) Spermatid giant cell with foamy cytoplasm and karyolytic nuclei (H&E x 1000). (b) Few number of giant cells with two or three nuclei (H&E x 1000). (c) Multiple spermatid giant cells in the lumen of seminiferous tubules (H&E x 1000). (d) Multinucleated spermatid giant cell with five nuclei (H&E x 1000). (e) Spermatid giant cell with three nuclei (H&E x 1000). (f) Seminiferous tubules containing spermatid giant cells with marked interstitial oedema in between the shrunken seminiferous tubules (H&E x 200).
Fig. (5): Testis of rat fed barley or sage-supplemented diet and treated orally with ACR (a) ACR plus barley group showing some seminiferous tubules append normal, others appeared affected and contained disturbed spermatogenic activity (H&E x 100). (b) ACR plus barley showing large number of normal spermatozoa (Crystal violet stain x 1000). (c) ACR plus sage showing few number of spermatozoa in the lumen and abnormal leydig cells (H&E x 400). (d) ACR plus sage showing few number of normal spermatozoa in the lumen (Crystal violet stain x 1000).

Fig. (6): (a) Kidney of rat treated orally with ACR showing mild degenerative changes with shrunken glomerular capillary tufts and distented Bowman's capsules. Some proximal and distal convoluted tubules appeared highly affected. Debris of their nuclei could be detected (karyolysis) (H&E x 400). (b) Treated with ACR plus barley and /or sage showing apparently normal tissue (H&E x 200).
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Discussion

ACR has recently been found in fried and baked foods, suggesting widespread public exposure (Erdreich and Friedman, 2004). The toxicity of ACR on human and experimental animals were well documented in a series of reports since the Swedish Food Administration alarm in 2002 (Konings et al., 2003, LoPachin, 2004, Mucci et al., 2004, Ruden, 2004, Van Ledingham et al., 2004). ACR has been shown to be carcinogenic in animals, and has been classified by the WHO/IARC among others as “probably carcinogenic for humans” this prompted us to investigate the protective effects of barley and sage against the ACR-induced toxicity and genotoxicity in rats. The selective dose of ACR was literaturaly based (Lehning et al., 2002). The LD50 values in rats were in the range 107-251 mg/kg b.w (Shelby, 2004). In this study, the animals treated orally with ACR (50 mg/kg b.w) for five days. The cumulative dose was comparable to the LD50 value reported in the literature. Our results indicated that ACR is a potent toxic material to the liver as indicated by the elevated levels of the biochemical parameters tested. Although, the only statistically and toxicologically significant decrease in serum-cholinestrase activity and increased serum alkaline phosphatase activity, there is some evidences that ACR induces an increase in the relative weight of the liver indicating that the liver tissues may be a target for the toxicity of ACR (Shelby, 2004).

The current study indicated that ACR induced micronuclei in polychromatic erythrocytes in bone marrow cells of rats. Similar to these results, Adler et al. (1988), Chihak and Vontorkova (1990) and Dobrzynska and Gajewski (2000) reported that ACR increased the micronucleus frequency in bone marrow cells in mice treated with 25 mg/kg b.w (two doses) or 50 mg/kg b.w (single dose). Significant increase in micronucleus frequency was also reported in reticulocytes and splenic lymphocytes from mice i.p. injected with ACR at doses of 50 mg/kg b.w or more (Backer et al. 1989; Kligerman et al. 1991; Russo et al. 1994).

The present study revealed that ACR increases significantly all types of chromosomal aberrations in somatic cells of rats. In the study of Marchetti et al. (1996), using cytogenetic analysis of first cleavage zygotes, almost all zygotes had at least one structural aberration after paternal treatment with ACR. Moreover, Chihak and Vontorkova (1988) found that a single i.p. injection of 100 mg/kg b.w ACR monomer elevated the frequency of chromosomal aberrations and micronucleated polychromatic erythrocytes in the bone marrow of male mice.

Our data revealed that ACR induced chromosomal aberrations in germ cells. In this regard, some literature data suggested that ACR mainly interact with protamines (Sega et al., 1989) whereas, other data suggested a direct interaction between ACR and DNA (Segerback et al., 1995). ACR is regarded as a potential mutagen based on experimental evidence that it can bind to DNA. Dearfield et al. (1988) suggested that ACR did not produce gene mutation. While ACR does not produce gene mutation in bacteria, but the epoxide metabolite glycidamid does in the absence of metabolic activation. This corroborates fairly with our findings which proved the role of ACR in producing the greatest impact of paternal ACR on abnormalities. We also suggest that chromatin adducts are likely to be converted into cytogenetic abnormalities when they are exposed to DNA repair.

According to Dearfield et al. (1988), ACR produced several genetic effects in germ cells such as chromosomal aberrations, micronuclei, sister chromatid exchange (SCE), polyploidy, aneuploidy and other mitotic disturbances. They also concluded that ACR impairs fertility in male rats, most likely through a direct toxic effect. In another study on germ cells, serial recovery of sperm from the caudate epididymides that were at the early spermatocyte (pre-meiotic/meiotic) stage to
the spermatozoa (post-meiotic) stage at the time of treatment showed no increase in unscheduled DNA synthesis (UDS); these stages correspond to those associated with dominant lethality (Sega et al. 1990). In contrast, DNA strand breaks were increased in these sensitive stages. DNA strand breakage in sperm in earlier stages of development at the time of treatment decreased with time, likely due to DNA repair, prior to become functional spermatozoa. Slight increase in DNA strand breaks in isolated human testicular cells have been reported. (Bjorge et al. 1996).

Adler et al. (1988) and Backer et al. (1989), reported that chromosomal aberrations and sister chromatid exchanges were not found in spermatogonia or early spermatocytes in contrast to positive results in late spermatids and spermatozoa. Further studies of DNA, total sperm head, and sperm protamine alkylations (Sega et al. 1989) suggested that the stage specificity can be explained by preferential binding to cysteine sulfhydryl groups in sperm protamine. In mid-to late spermatid stages, chromosomal histones are replaced by protamines that are relatively rich inarginine and cysteine. Alkylation of free sulfhydryl groups of cysteine in the “immature” protamine of late spermatids and early spermatozoa might prevent normal chromatin condensation leading to stress in the chromatin structure and strand breakage.

The clastogenicity results observed in the present study were confirmed by the histological examination of the testis.

The antioxidant and protective effects of barley and sage have been documented previously (Kanauchi et al., 1998; Bandoniene et al., 2001; Dongowski et al., 2002; Perry et al., 2003). In the present study, addition of either barley or sage to ACR resulted in a significant improvement in the serum clinical chemistry. This may be due to the higher content of antioxidant substances in these plants. Several reports indicated that blood cholesterol and lipoprotein concentrations can be reduced in human (Braaten et al., 1994) and animal (Kalra and Jood, 2001) by a-glucan from barley. We also observed that feeding on barley diet reduced aberrations produced by ACR. Similar results were noticed by Deguchi et al. (2000) who revealed that the novel purple pigment hordeumin, an anthocyanin-tannin pigment in barley, decreased a reverse mutation from Trp-P-1, Trp-P-2, IQ, and B_iP. Moreover, hordeumin also decreased the reverse mutation from dimethyl sulfoxide extracts of grilled beef in Salmonella typhimurium TA98.

The present study indicated that sage-supplemented diet decreased ACR mutagenicity. Similar results were observed by Karakaya and Kavas (1999) who noticed a decrease in sodium azide mutagenicity in S. typhimurium TA 100 by sage. According to Farhat et al. (2001) the antioxidant activity of sage may be due to 1,8-cineole, the main constituent, although, other components included ketones such as camphor and alpha, beta-thujone, terpenes such as limonene and alpha, beta-pinene, and alcohols such as borneol and linalool.

From pathological point of view, this study revealed testicular lesions in rats treated with ACR alone. These lesions were characterized by the presence of residual bodies in the seminiferous tubules, decreased number of mature sperms and the presence of spermatid giant cells together with necrosis of spermatocytes. The necrotic spermatocytes appeared with pyknotic and karyolitic nuclei. Debris of most spermatogenic cells revealed disturbed architecture of the seminiferous tubules which appeared devoid of sertoli cells.

The histological abnormalities in the testis reported by U.S EPA (2002) in animals treated with 150 mg/kg ACR appeared in spermatids particularly round spermatids (Golgi and cap phase) one day after treatment. Nuclear vacuolization and swelling were the most common lesions in the spermatids. Degeneration of spermatocytes and spermatogonia was also noted. By the second day after treatment, spermatid degeneration was most prominent. On day 3, multi-nucleated giant cells were frequent. By day 7-10, clearing of the histologic abnormalities was evident. These pervious results supported our findings regarding the degenerative lesions in the testicular tissues and clearly indicated that
ACR had toxic effects on male reproductive organs and also has a direct effect on Leydig cells. In this concern, Kinomoto et al. (2000) evaluated the toxic effect of some chemicals on male reproductive organs and considered that, necrosis of spermatocytes and presence of spermatid giant cells as lesions indicating toxicity to male reproductive organs. On the other hand, decreased number of mature sperms in the lumen of seminiferous tubules that observed in our study indicated that ACR induces impairment of spermatogenesis either by direct action on spermatogonia or indirectly by affecting the integrity of the Sertoli cells. However, the presence of spermatid giant cells indicated faulty in spermatid cell divisions. The faulty in the spermatid cell divisions and decreased fertility by ACR were mentioned by Chapin et al. (1995). In addition, Sakamoto and Hashimoto (1988) found that the main effects of ACR on testicular tissue were decreased sperm count and decreased number of spermatocytes.

The directed action of ACR on the testicular tissue could be explained according to the findings of Dearfield et al. (1988) who reported that ACR rapidly metabolized, primarily by glutathione conjugation and its toxic metabolites mainly persist in testis and skin.

In our study, the presence of residual bodies indicated degeneration of germ cells and spermatids where the residual cytoplasm of such cells phagocytosed by Sertoli cells and appeared as residual bodies. The presence of residual bodies was completely explained by Gary et al. (1990). These pathological alterations were decreased when animals were treated with either barley or sage.

Concerning the histopathological findings in the renal tissue, the lesions were not severe in ACR- treated animals or the combined treatment with protective agents. These findings could be explained by the rapid excretion of the ACR (24 hrs.) post administration where the residues were detected in testis and skin only as mentioned by Dearfield et al. (1988). Adversely, previous studies indicated that the kidney is one of the organs affected by the higher dose of ACR in animal studies (EU, 2002).

In conclusion, the present study revealed that ACR induced toxic, clastogenic, and histological alterations typically to those reported in the literature. Both barley and sage have a protective role against these deleterious effects possibly due to their higher contents of antioxidant substances which can modulate the metabolism of ACR resulting in the reduction of its toxicity and/or increase the GSH production by the target organs which involved in the detoxification of ACR. These plants may be useful when add to certain foods cooked in a higher temperature.

References


Chemoprevention of Barley and Sage against


الحماية من التغيرات الوراثية والبيوكيميائية والهستوتأثيثولوجية الناتجة من الأكريلاميد باستخدام الشعير والمرمية

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إن ظهور الأكريلاميد حديثاً في الأغذية المعبأة أو المقلية يعتبر دليلاً على مدى انتشارها و تعرض الإنسان له على نطاق واسع. ورغم أن الأكريلاميد من الكيمياء الصناعية التي تسبب تسمم الجهاز العصبي في الإنسان كما قد صنف بواسطة الهيئة الدولية للسرطان بأنه من المواد التي يحمل أن تسبب سرطان للإنسان.

تهدف الدراسة الحالية إلى تقييم التأثير الوقائي للشعير والمرمية ضد التسمم الوراثي و التغيرات البيوكيميائية والهستوتأثيثولوجية في الفئران المغذية بالأكريلاميد، حيث قسمت الفئران (عدد 40 فار) إلى ثمانية مجموعات تم تغذيتها على الشعير فقط أو بغذاء مدعوم بالمرمية (5%) و عاملت الالحويات بالأكريلاميد (50 مجم / کجم من وزن الجسم).

وأوضح النتائج البيوكيميائية أن الأكريلاميد سبب زيادة معنوية في الإنيزيمات الناقلة للأمين والجسيمات الثلاثية والكولسترول وحمض الربوك. كما سبب زيادة معنوية في التغيرات النووية (نوبات صغيرة) في كرات الدم الحمراء لنضاع العظام كما سبب زيادة التشوهات الكروموسمية في الخلايا الجسدية والجزئية للخضرة. كما سبب الأكريلاميد تغيرات حادة في التركيب النسيجي للخضرة والكلية. و من الخصائص النسيجية ظهر وجود تأثيرات بايثولوجية للخلايا المكونة للخضرة حيث لوحظ وجود تكرار في الخلايا المكونة للخلايا المنوية، وكذلك حدوث ارتفاعات سائدة في الألياف المكونة للخضرة في حالة الالحويات بالأكريلاميد أما النسيج الكلوي فلم يحدث به تأثيرات خطيرة.

كذلك أثبتت الدراسة أن إضافة كلاً من الشعير أو المرمية بغيرهما أو معًا إلى أذينة الحيوانات المغذية بالأكريلاميد تنتج عنها تحسن معنوي في كل الصفات المدروسة. و نستنتج من هذه الدراسة أن إضافة الشعير أو المرمية للأذينة المغذية باقيًا باستخدام درجات حرارة عالية يمكن أن يكون له تأثير فعال في حماية الإنسان من الأخطار الناتجة عن الأكريلاميد.