Influence of dietary potato peel and cysteine on oxidative stress in rats

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ABSTRACT

Forty nine albino male rats were randomly classified into seven groups. The first group kept as control negative fed basal diet only. The other six groups fed on basal diet with 10% carbonated grilled meat and classified into control (+ve), and treated groups which were potato peel powder (PP), potato peel extract (PE), cysteine (C), potato peel powder with cysteine (PPC) and potato peel extract with cysteine (PEC) groups. The experiment continued for eight weeks. Control (+ve) group had significant lower values of final weight, body weight gain, food intake, food efficiency ratio (FER), protein efficiency ratio (PER); blood hemoglobin (HG), packed cell volume (PCV), serum superoxide dismutase (SOD), glutathione-peroxidase (GPX) & catalase and liver SOD, glutathione (GSH) & GPX but showed a significant increase in serum malondialdehyde (MDA), nitric oxide (NO), alanine and aspartate aminotransferase (ALT, AST), creatinine & urea ; and liver MDA when compared with control (-ve) group. PP, PE, C, PPC and PEC groups showed a significant higher values of final weight, body weight gain, food intake, PER & FER; blood HG & PCV ; serum SOD, GPX & catalase and liver SOD, GSH and GPX compared with control (+ve). All treated groups showed a significant decrease in serum MDA, NO, ALT, AST & creatinine and liver MDA compared with control (+ve) group. PP, PE and C, groups showed improvement of histological pictures in liver but PPC and PEC showed normal liver section.

Key words: Potato peel, cysteine, carbonated meat& rats.

Introduction

Reactive oxygen and nitrogen species are essential to energy supply, detoxification, chemical signaling and immune function. They are continuously produced by body and controlled by endogenous enzymes (superoxide dismutase, glutathione peroxidase, and catalase). When there is an over-production of these species, an exposure to external oxidant substances or a failure in the defense mechanisms, damage to valuable biomolecules (DNA, lipids, proteins) may occur. This damage has been associated with an increased risk of cardiovascular disease, cancer and other chronic diseases (Aruoma 1998).

Frying at higher temperature and for longer times produce greatest mutagenic response and concomitantly the largest amounts of heterocyclic amines (Knize et al., 1999). The nitrated polycyclic aromatic hydrocarbons are oxidative stress and mutagenic contaminants which may occur in foods; as a result of environmental contamination, heat treatment and smoking (Schlemits and Pfannhauser 1996). Heterocyclic aromatic amines that form on the surface of grilled or fried meat and fish have been shown to form the covalent DNA adduct considered to be the first stage of the process of carcinogenesis (Abd El-Ghany et al., 2007).

Peels are often the waste part from vegetables and fruits processing. These wastes have not generally received much attention with a view to being used or recycled rather than discharged. This might be due to their lack of commercial application (Soong and Barlow 2004). Potato peel, a waste by-product from potato processing, could be considered as a new source of dietary fiber, polyphenols and natural antioxidant. Recently, the antioxidant activity of potato peel extract has been studied in food systems (Rodriguez de Sotillo et al., 1994&1998).

Cysteine is an amino acid, a building block of proteins that are used throughout the body. When taken as a supplement, it is usually in the form of N-acetyl-L-cysteine (NAC). The body makes this into cysteine and then into glutathione, a powerful antioxidant. Cysteine can help prevent side effects caused by drug reactions and toxic chemicals, and helps break down mucus in the body. NAC help prevent or reduce liver and kidney damage (Ozkilic et al., 2006).

The study aimed to investigate the potential of dietary potato peel (PP) powder and extract with or without cysteine in ameliorating oxidative stress induced by consumption of carbonated meat in rats.

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Materials And Methods

A – Materials:

1- Carbonated meat, basal diet and potato peel:

Meat was extremely grilled in hot oven until be carbonated then crushed and mixed in basal diet in 10% all over the period of experiment. Fresh potato (*Solanum tuberosum* L., Solanaceae) peel obtained from local restaurant in Riyadh and washed with tap water. Part of fresh potato peel was subjected for gross chemical analysis. The other part was dried at 60°C, and then crushed to a fine powder. Potato peel powder (PP) Samples were stored in freezer until use. The rat basal diet was performed according to NRC (1995).

2- Cysteine powder:

N-acetyl-cysteine is the acetylated form of cysteine which is more efficiently absorbed and used. It was purchased from Somatco Co., in Riyadh. The rat received 25 mg /kg body weight of freshly dissolved in distilled water by oral administration daily.

3-Experimental animals:

49 adult male of white albino rats (Sprague dawley strain), weighing 103± 5g, provided from experimental animals center in Medicine collage of King Saud University in Riyadh. Rats were housed as groups in wire cages under the normal laboratory conditions.

II- Methods:

1- Chemical analysis:

Moisture, ash, crude protein, and crude fat contents were determined in fresh potato peel and total carbohydrates were calculated according to the method of the A.O.A.C. (2000). Calcium, zinc, iron, selenium and copper were determined in ash of potato peel by using atomic absorption spectrophotometer according to Pupsa et al., (1994).

2 - Potato peels extract:

Hundred grams of potato peel powder was extracted with 400 ml of ethyl alcohol (50%) at room temperature with continuous stirring by a magnetic stirrer for 4 h, incubated over night and then filtered in Whatman filter paper no.1. The filtrate was then dried at 37°C and stored for future use. Each peel extract powder was dissolved in double distilled water (DW) for final administration (WHO 1983). The rat dose of peel extract was 25 mg /kg body weight daily.

3- Biological deign:

Experimental animals fed on basal diet for a week as adaptation period. Food and water were provided ad-libitum. The rats were randomly classified into seven groups (7 rats each). The first group kept as control (-ve) fed basal diet only. The other six groups fed basal diet with 10% of carbonated grilled meat and classified into control (+ve), and treated groups which were potato peel powder (PP), potato peel extract (PE), cysteine(C), potato peel powder with cysteine (PPC) and potato peel extract with cysteine (PEC) groups. The experiment continued for eight weeks. The food intake was calculated daily and the body weight gain was recorded weekly. Food efficiency ratio (FER) and protein efficiency ratio (PER) were determined by Chapman et al., (1950). At the end of experiment, rats were anesthetized, blood sample were collected from hepatic portal vein in clean centrifuge tubes. Liver was removed and blotted on filter paper.

4- Biochemical analysis:

Part of blood was heparinized for estimation of hemoglobin (HG) and packed cell volume (PCV) according to Drabkin (1949) and Mc Inory (1954), respectively. The rest of blood was left to coagulate then centrifuged at 3000 rpm for 15 minutes to obtain serum. Serum alanine and aspartate aminotransferase (ALT, AST) activity, urea and creatinine were estimated according to Bergmeyer and Horder (1980), Kanter (1975) and Bonsens and Taussky (1984), respectively. Serum superoxide dismutase (SOD), glutathione-peroxidase (GPX), catalase,
malondialdehyde (MDA) and nitric oxide (NO) were determined by enzymatic colorimetric procedures according to Dechatelet et al., (1974), Habig et al., (1974), Sinha (1972), Uchiyama and Mihara (1978) and Green et al., (1981), respectively. Liver SOD, glutathione (GSH), GPX and MDA were estimated according to Misra and Fridovich (1972), Lueck (1965), Rotruck et al., (1973) and Ohkawa et al. (1979), respectively.

5-Histopathological examination:

Part of liver used for biochemical analysis but the others were separately immersed in 10% neutral buffered formalin as fixative and sent to Cancer Institute for histopathological examination (Drury and Wallington 1980).

Statistical analysis:

Data are expressed as mean ± SE. Statistical analysis was done by using analysis of variance (ANOVA) followed by student’s t-test and P values of 5% and less were considered to be significant.

Results:

The statistical data in table (1) denoted that the percentage values of protein, fat, ash, moisture, and carbohydrate in potato peel were 12.33, 2.11, 6.22, 72.4 and 6.94 %. Values of calcium, zinc, iron, selenium and copper were 37.66, 0.66, 17.88, 0.03 and 0.99 mg/100g.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Initial weight (g)</th>
<th>Final weight (g)</th>
<th>Weight gain (g)</th>
<th>Food intake (g/d)</th>
<th>PER (g/d)</th>
<th>FER (g/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-ve)</td>
<td>103.66± 2.66a</td>
<td>211.33± 14.33a</td>
<td>107.67± 11.24a</td>
<td>16.99± 1.36a</td>
<td>31.76± 3.67a</td>
<td>0.787± 0.031b</td>
</tr>
<tr>
<td>Control (+ve)</td>
<td>104.51± 3.51a</td>
<td>155.16± 11.24a</td>
<td>50.65± 6.03a</td>
<td>13.11± 1.51a</td>
<td>19.33± 2.14a</td>
<td>0.482± 0.011c***</td>
</tr>
<tr>
<td>PP</td>
<td>103.55± 2.25a</td>
<td>201.22± 12.45a</td>
<td>97.67± 8.21a</td>
<td>15.25± 1.01a</td>
<td>32.02± 4.11a</td>
<td>0.800± 0.022a***</td>
</tr>
<tr>
<td>PE</td>
<td>103.11± 2.41a</td>
<td>203.21± 13.27a</td>
<td>100.11± 10.66a</td>
<td>15.66± 1.12a</td>
<td>19.33± 2.14a</td>
<td>0.799± 0.011c***</td>
</tr>
<tr>
<td>C</td>
<td>105.22± 2.51a</td>
<td>199.99± 15.77a</td>
<td>94.77± 1.35a</td>
<td>15.77± 3.08a</td>
<td>30.08± 3.20a</td>
<td>0.751± 0.044b***</td>
</tr>
<tr>
<td>PPC</td>
<td>106.14± 2.50a</td>
<td>208.67± 14.33a</td>
<td>102.53± 3.29a</td>
<td>15.88± 1.03a</td>
<td>32.34± 4.36a</td>
<td>0.807± 0.015a***</td>
</tr>
<tr>
<td>PEC</td>
<td>104.21± 2.33a</td>
<td>209.36± 13.36a</td>
<td>105.15± 4.17a</td>
<td>16.11± 1.50a</td>
<td>32.65± 2.67a</td>
<td>0.815± 0.031a***</td>
</tr>
</tbody>
</table>

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant

Table (3) showed that the values of hemoglobin and packed cell volume in were significantly decreased in control (+ve), PP, PE and C groups at p <0.001, 0.01&0.05, respectively in compared with control (-ve) group. All treated groups showed a significant increase in hemoglobin and packed cell volume compared with control (+ve) group.

<table>
<thead>
<tr>
<th>Groups Variables</th>
<th>Initial weight (g)</th>
<th>Final weight (g)</th>
<th>Weight gain (g)</th>
<th>Food intake (g/d)</th>
<th>PER (g/d)</th>
<th>FER (g/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-ve)</td>
<td>13.36± 1.65a</td>
<td>8.31± 1.11a</td>
<td>10.11± 1.33a</td>
<td>10.55± 1.25a</td>
<td>10.81± 1.31a</td>
<td>11.13± 1.21a</td>
</tr>
</tbody>
</table>

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each row having different superscript (a, b, c, d) are significant
Data presented in table (4) showed that control (+ve) group showed a significant decrease in the values of serum SOD, GPX and catalase at p <0.001 but showed a significant increase in MDA and NO (p <0.001) when compared with control (-ve) group. Also, PP and C groups showed the same results but at p<0.05, 0.01&0.001. PE group showed a significant decrease in the values of serum GPX and catalase at p <0.05 and a significant increase in MDA and NO (p <0.05) when compared with control (-ve) group. PPC group showed a significant decrease in the values of serum GPX and catalase at p <0.05&0.001 and a significant increase in MDA at p <0.05 while PEC group showed a significant decrease in the value of serum catalase at p <0.05 and a significant increase in MDA at p <0.05 when compared with control (-ve) group. All treated groups showed a significant increase in serum SOD, GPX and catalase and a significant decrease in serum MDA and NO compared with control (+ve) group.

<table>
<thead>
<tr>
<th>Variables</th>
<th>SOD (µ/ml)</th>
<th>GPX (µ/ml)</th>
<th>Catalase (µ/ml)</th>
<th>MDA (mmol/l)</th>
<th>NO (µ Mol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (+ve)</td>
<td>52.31±7.21 a 68.36±9.11 b 211.7±20.21 b 2.11±0.45 a 3.21±0.45 a</td>
<td>27.31±5.25 a 31.11±4.25 b 100.21±12.71 b 9.32±2.11 a 12.17±2.11 a</td>
<td>PP</td>
<td>33.18±4.41 a 46.36±5.61 b 109.91±13.21 b 5.39±1.21 a 6.31±1.71 a</td>
<td>PE</td>
</tr>
</tbody>
</table>

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant

Data in table (5) revealed that control (+ve) group showed a significant decrease in the values of liver SOD, GSH, and GPX and a significant increase in MDA at p <0.001 when compared with control (-ve) group. PP and C groups showed a significant decrease in the values of liver SOD, GSH and GPX at p <0.01 and a significant increase in MDA at p <0.05 when compared with control (-ve) group. PE group showed a significant decrease in the value of liver GPX and a significant increase in MDA (p <0.05) when compared with control (+ve) group. PPC group showed a significant decrease in the values of liver SOD, GPX and catalase and a significant increase in liver MDA compared with control (+ve) group.

<table>
<thead>
<tr>
<th>Variables</th>
<th>SOD (µ/mg)</th>
<th>GPX (µg/mg)</th>
<th>Catalase (µ/ml)</th>
<th>MDA (mmol/g)</th>
<th>NO (µ mOl/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (+ve)</td>
<td>41.36±5.11 a 60.24±7.55 b 71.83±8.31 b 5.21±0.88 c</td>
<td>17.87±1.31 c*** 19.61±2.11 c*** 22.41±2.11 c*** 21.36±2.11 c***</td>
<td>PP</td>
<td>25.41±5.21 a 39.96±3.14 b 43.61±3.21 c 8.61±1.79 b</td>
<td>PE</td>
</tr>
</tbody>
</table>

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant

Data recorded in table (6) showed that control (+ve), PP, PE, C and PPC groups showed a significant increase in the values of serum ALT, AST, creatinine and urea at p <0.001,0.01&0.005 compared with control (-ve) group. PEC group showed a significant increase in the values of serum ALT and creatinine at p <0.05 compared with control (+ve) group. All treated groups showed a significant decrease in serum ALT, AST and creatinine compared with control (+ve) group. PEC group showed a significant decrease in the values of serum urea compared with control (+ve) group.

<table>
<thead>
<tr>
<th>Variables</th>
<th>ALT(µ/ml)</th>
<th>AST(µ/ml)</th>
<th>Creatinine (mg/dl)</th>
<th>Urea (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (+ve)</td>
<td>9.81±1.71 a 27.28±5.67 b 0.51±0.3 a 3.04±1.22 a</td>
<td>41.61±3.21 b 53.41±5.14 a 1.71±0.33 a 49.31±6.41 a</td>
<td>PP</td>
<td>27.33±2.7 a 40.31±4.21 a 1.01±0.22 a 45.33±4.98 a</td>
</tr>
</tbody>
</table>

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant
Liver histopathology results:

Microscopically, liver of rat from control (-ve) group revealed the normal histological structure of hepatic lobule (Photo 1). Conversely, Liver of rat of control (+ve) group showed congestion of hepatoporal blood vessels, portal edema and focal hepatic necrosis associated leucocytic cells infiltration (Photo 2). However, Liver of rat from PP group showed congestion of hepatoporal blood vessels with portal oedema (Photo 3). Liver of rat PE group showed slight congestion of central vein (Photo 4). Examined sections from liver of rat from C group showed sinusoidal leucocytosis (Photo 5). No histopathological changes observed in liver of PPC rat group (Photo 6). Also, Liver of PEC rat group revealed no histopathological changes (Photo 7).

Discussion:

The analysis of potato peel was interested by many authors. Toma et al., (1979) reported that potato peel contained 4.9 % moisture, 17.1 % protein, 8.4 % ash, 9.5 % crude fiber and 68% dietary fiber. Mahmood et al., (1998) recorded that potatoes peel were about 12.0% of their fresh weight, was discarded as waste (peels and trimmings). Approximately 63% (on a dry weight basis) of the potato waste was alcohol-insoluble which was separated into pectic substances, hemicellulose, cellulose, and lignin. This fraction consisted of 3.4% pectin, 2.2% cellulose, 14.7% protein, 66.8% starch, and 7.7% ash. The sugars in the alcohol-soluble fraction consisted of 1.4% total soluble sugars and 0.9% reducing sugars. The predominant minerals in potato peel were potassium with measurable levels of magnesium, phosphorus, sulfur, and chlorine in addition of significant concentration of silicon. El-Anany and Rehab (2007) recorded that moisture content of potato peel was 5.61% while the protein, crude fat, crude fiber and ash content were 13.51, ND, 12.70 and 8.6 %, respectively.

It is known that reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated as a result of the inflammatory process are believed to play a role in the pathobiology. The nutritional and biochemical results were agreed with results of Freidmen (1997) who suggested that potato peel provides an excellent source for the recovery of phenolic compounds, since almost 50% of phenolics are located in the peel and adjoining tissues and decrease toward the center of the tuber. Aqueous extract of potato peel was shown to provide a good source of phenolic acids and exhibited good antioxidant activity (Rodriguez de Sotillo et al., 1994). The total amount of
chlorogenic acid and caffeic acid in the free-form phenolics from the peel was highly correlated with the DPPH radical scavenging activity. Ferulic acid was identified as the active radical scavenging compound in the bound-form phenolics from the peel. The potato peel may therefore offer an effective source of an antioxidative (Kazuhiro et al., 2006). Incorporation of PP powder reduced significantly the hypertrophy of both liver and also normalized the activities of serum ALT and AST, hepatic and renal MDA and GSH, as well as activities of various antioxidant enzymes in liver and kidney of diabetic rats (Singh et al., 2005).

The obtained results which investigated the effects of cysteine were revealed to the scientific facts of antioxidant action. Cysteine is important for cell growth and it is an amino acid that has high sulfur content and is formed in the liver from L-Methionine . Cysteine plays a role in the sulfation cycle, acting as a sulfur donor in phase II detoxification and as a methyl donor in the conversion of homocysteine to methionine.

Cysteine is also critical to the metabolism of a number of essential biochemicals including coenzyme A, heparin, biotin and lipid acid. It can then form into the glutathione that is a small protein produced naturally in body when the right circumstances are available. It aids the body as an antioxidant and antitoxic and is a prevalent defense against sickness and aging (Mardikian et al., 2007). As an antioxidant, cysteine works to fight free radicals and is known to detoxify the body as well as protect it from radiation damage caused by radiation cancer treatments. N-acetyl cysteine NAC, a potent antioxidant, inhibited induction of NO production in peritoneal macrophages (Pahan et al., 1998). Acetylcysteine protects the liver from drug's toxicity because of an anti-inflammatory and antioxidant effects and also has positive inotropic effects and increases local nitric oxide concentrations, and this vasodilatory effect on microcirculatory blood flow enhances local oxygen delivery to peripheral tissues. These vasodilating effects decrease morbidity and mortality even in the setting of established liver damage (Silva et al., 2008).

It is concluded that the improvement of the obtained results is related to the direct or indirect antioxidant activity of potato peel and cysteine. The combination of potato peel extract and cysteine gave the best favorable effects and that were confirmed by histopathological results.

From this study, it is recommended to pay attention of potato peel extract as antioxidant which has therapeutic and preventive effects in the treatment of side effects of free radicals oxidative stress. Potato peel can be used as a dietary fiber supplement in bakery products, breakfast cereals and pasta products.

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References


