Treatment Effect of Red Cabbage and Cysteine Against Paracetamol Induced Hepatotoxicity In Experimental Rats

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Abstract: Forty eight male of white albino rats (Sprague dawley strain), weighing 105± 5g received a single dose of 2g/kg paracetamol in 1 % methyl cellulose orally to each animal and divided into six groups of 8 animals each. Group I served as control positive group. Groups II, III and IV received cabbage powder (10% in diet), cabbage extract (100 mg kg/body weight by stomach tube) and cysteine (1.2 g/kg, p.o), respectively. Group V and VI received both cabbage powders with cysteine and cabbage extract with cysteine, respectively. The experiment continued for 60 days. Administering of red cabbage powder or extract with or without cysteine could significantly increase body weight gain, food and protein efficiency ratio but showed significant decrease in the activities of alanine aminotransferase, aspartate aminotransferase, gamma glutamyle transferase and alkaline phosphatase and also decrease cholesterol, triglycerides, low density lipoprotein cholesterol and very low density lipoprotein cholesterol in serum compared with control positive group. On the other hand, the activities of liver glycogen, superoxide dismutase, glutathione peroxidase and glutathione S-transferase in liver tissue were increased while hepatic cholesterol and malondialdehyde were reduced in rat groups administering cabbage powder, cabbage extract, and cabbage powder with cysteine and cabbage extract with cysteine when compared to control positive group. This study confirmed that administering of cabbage either powder or extract in combination with cysteine has improvement of nutritional results and showed treatment effect against paracetamol induced hepatotoxicity.

Key words: liver – paracetamol- red cabbage- cysteine- rats

Introduction

Liver is one of the important organs of the body which plays a major role in the metabolism of proteins, carbohydrates and lipids. It is also having a wide range of functions including detoxification, storage of glycogen, production of several coagulation factors and growth factors hormones. Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorders. Hepatotoxicity in most cases is due to free radicals that are fundamental to many biochemical processes and represent an essential part of aerobic life and metabolism (Wolf 1999 and Abd El-Ghany and Nanees 2010).

Paracetamol hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzo quinoneimine, which causes oxidative stress and glutathione depletion. It is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses (Vermeulen et al., 1992).

The red cabbage (Brassica oleracea var. capitata f. rubra) belonging to the family Brassicaceae, is an herbaceous, biennial, dicotyledonous flowering plant. Its leaves are coloured dark red/purple and have been mostly consumed as salad, coleslaw, and beverage. Red cabbage is also known as Red Kraut or Blue Kraut after preparation and is endemic to the Mediterranean region (Rimm et al., 1996, Fahey et al., 1997 and Lynn et al., 2006). The red cabbage is excellent sources of fibers. Insoluble fiber helps to prevent constipation and reduce colorectal cancer risk. Soluble fiber helps to reduce blood cholesterol and blood sugar, thereby reducing the risk of heart disease and diabetes. Red cabbage has been found to have antioxidant, antihyperglycemic, anticancer and hypcholesterolemic (Aml et al., 2010 and Neelufar et al., 2012).

Cysteine is one of two sulfur-containing amino acids. Cysteine is found in a variety of foods including poultry, yogurt, egg yolks, red peppers, garlic, onions, broccoli, oats, and wheat germ. Cysteine also might prevent or reduce liver or kidney damage caused by an acetaminophen overdose. It also might reduce the symptoms of influenza, acute respiratory distress syndrome, asthma, cystic fibrosis and emphysema. It also has a preventative effect against colon cancer and cataracts (Quig 1998 and Lai et al., 2012).

The present study aimed to investigate the treatment effect of red cabbage and cysteine against paracetamol induced hepatotoxicity in experimental rats.
Materials and methods

2.1. Plant material:

Red cabbage was obtained from local market and was authenticated in the Botany Department, Faculty of Agriculture, Cairo University. The outer layer was removed but the inner were cut into small pieces, dried at 60°C in hot oven and crushed to a fine powder. Red cabbage powder was added as 10% of basal diet.

2.2. Chemicals:

All the materials used for this experiment were of analytical grade. Paracetamol and cysteine were obtained from Sigma Chemical Co., and Gomoheria Company. Diagnostic kits were manufactured by Ranbaxy Diagnostics Ltd., were purchased from the Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt.

2.3. Test animals:

Forty eight male of white albino rats (Sprague dawley strain), weighing 105± 5g, provided from of National Research Center, Cairo, Egypt. Rats were housed as groups in wire cages under the normal laboratory conditions.

2.4. The basal diet:

The rat basal diet was performed according to Reeves et al., (1993).

2.5. Preparation of red cabbage extract:

The cabbage powdered leaf (100g) was soaked in 600ml of 80% methanol with constant stirring by a magnetic stirrer for 48 hr. The mixture was filtered followed by removal of the solvent on the rotatory evaporator to give a dark-brown crude extract. The rat received cabbage extract in 100 mg kg/body weight by stomach tube.

2.6. Treatment schedule:

Rats received a single dose of 2g/kg paracetamol in 1 % methyl cellulose orally to each animal and divided into six groups of 8 animals each. Group I served as control positive group. Groups II, III and IV received cabbage powder in diet, cabbage extract and cysteine (1.2 g/kg, p.o), respectively. Group V and VI received both cabbage powders with cysteine and cabbage extract with cysteine, respectively. The experiment continued for 60 days. Daily food intake and weekly body weight gain were recorded. After 24 h of last dose, blood was collected from overnight fasted rats of each group by cardiac puncture for estimation of serum. Then the rats were sacrificed for the study of liver biochemical and histopathological parameters.

2.7. Serum biochemical estimations:

Serum alanine and aspartate amino transferase (ALT&AST), serum alkaline phosphatase (ALP) and gamma glutamyle transferase enzymes were estimated by using commercially available kits according to Reitman and Frankel (1957), Draper and Hadley (1990), Kind and King (1954), respectively. Serum cholesterol (CHO), triglycerides (TG) and high density lipoprotein cholesterol (HDL-C) were determined by using enzymatic colorimetric methods (Abell et al., 1952, Buccolo and David 1973 and Kostener 1977, respectively).

2.8. Liver biochemical estimations:

Liver cholesterol (CHO), total lipids, triglyceride and glycogen were determined according to Richmond (1973), Folch et al., (1957), Scheletter and Nussel (1975) and Rerup and Lundquist (1967), respectively.

2.9. Assay of hepatic antioxidants:

The level of liver superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione S-transferase (GST) activity was determined by the method of Misra and Fridovich (1972), Weiss et al., (1980) and Habig et
al., (1974), respectively. The method of Uchiyama and Mihara (1978) was employed in determining malondialdehyde (MDA).

2.10. Calculation of some parameters:

Food efficiency ratio (FER) and protein efficiency ratio (PER) were determined by Chapman et al., (1950). Low density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VLDL-c) and CHO/HDL-c were calculated according to Fruchart (1982) and Castelli and levitar, (1977).

2.11. Histopathological studies:

For histopathological study, the fresh liver tissues were collected and part of it immediately fixed in 10% formalin, dehydrated in gradual ethanol (50-100%), cleared in xylene and embedded in paraffin. Sections (4-5 μm) were prepared and then stained with hematoxylin-eosin dye for photomicroscopic observations (Carleton 1979).

2.12. Statistical analysis:

All the results were expressed as Mean ± SD. The statistical analysis was carried out by one-way ANOVA followed by Dunnett’s multiple comparison tests. P < 0.05 was considered as Significant (Artimage and Berry 1987).

3. Results:

3.1. Nutritional results:

Administration of cabbage powder or extract with or without cysteine and cysteine showed a significant increase in body weight, FER and PER (P<0.05, 0.01 &0.001) compared with control positive group. The best results were appeared in rats consumed cabbage powder or extract with cysteine (P<0.01&0.001) as shown in table 1.

3.2. Serum biochemical parameters:

Administering of cabbage and cysteine reduced the levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyle transferase (γGT) at P<0.001. Also, alkaline phosphatase (ALP) was significantly decreased at P<0.001&0.01 in all treated groups compared with control positive group. Consumption of cabbage extract with cysteine showed the lowest values of liver function enzymes (Table 2).

The levels of CHO, TG, LDLc and VLDLc were significantly (P<0.001&0.01) decreased in all treated rats groups when compared to control positive group. Administering of cabbage and cysteine in all treated groups increase the level of HDLc (P<0.001&0.01) but decrease atherogenic index (CHO/HDLc) P<0.001&0.01 when compared to control positive group. Cabbage powder group showed non significant difference in the levels of CHO, TG and VLDLc compared to cysteine group. Cabbage powder with cysteine group showed non significant difference in the levels of CHO, TG, HDLc, LDLc, and VLDLc and also CHO/HDLc compared to cabbage extract with cysteine group (Table 3).

3.3. Hepatic biochemical parameters:

Administering of cabbage either powder or extract with or without cysteine reduced hepatic CHO (P<0.001&0.01) and increase hepatic glycogen (P<0.001&0.01) when compared to control positive group. The level of total lipid was significantly decreased in rat groups administering cabbage powder, cabbage extract, cabbage powder with cysteine and cabbage extract with cysteine while the level of triglyceride was significantly increased in rat groups administering cabbage extract, cysteine, cabbage powder with cysteine and cabbage extract with cysteine (P<0.05, 0.01&0.001) when compared to control positive group (Table 4).

Administering of cabbage either powder or extract with or without cysteine increase hepatic SOD, GPX and GST (P<0.001&0.01) and decrease hepatic MDA (P<0.001&0.01) when compared to control positive group. The highest values of hepatic SOD, GPX and GST and the lowest values of hepatic MDA were appeared in rat groups administering cabbage powder with cysteine and cabbage extract with cysteine (Table 5).
**Table 1:** Mean values ± SD of body weight gain, food intake, FER and PER of the experimental rat groups.

<table>
<thead>
<tr>
<th>Groups Variables</th>
<th>Control positive</th>
<th>Cabbage Powder</th>
<th>Cabbage Extract</th>
<th>Cysteine</th>
<th>Cabbage Powder + cysteine</th>
<th>Cabbage Extract + cysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight gain (g)</td>
<td>60.18±3.14**</td>
<td>88.70±7.61***</td>
<td>93.14±9.10**</td>
<td>87.71±6.21**</td>
<td>105.4±11.21**</td>
<td>103.21±10.15**</td>
</tr>
<tr>
<td>Food intake (g/w)</td>
<td>13.55±1.28**</td>
<td>14.65±1.39*</td>
<td>15.91±1.40**</td>
<td>14.88±1.32**</td>
<td>15.69±1.61**</td>
<td>15.44±1.51**</td>
</tr>
<tr>
<td>FER</td>
<td>0.074±0.001**</td>
<td>0.100±0.002**</td>
<td>0.097±0.004**</td>
<td>0.098±0.003**</td>
<td>0.111±0.005**</td>
<td>0.112±0.001**</td>
</tr>
<tr>
<td>PER</td>
<td>0.370±0.01**</td>
<td>0.504±0.03**</td>
<td>0.488±0.02**</td>
<td>0.492±0.04**</td>
<td>0.561±0.03**</td>
<td>0.558±0.06**</td>
</tr>
</tbody>
</table>

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Values with the same letters indicate non-significant difference (P<0.05) and vice versa.

**Table 2:** The Mean values ± SD of serum ALT, AST, ALP and γGT of the experimental rat groups.

<table>
<thead>
<tr>
<th>Groups Variables</th>
<th>Control positive</th>
<th>Cabbage Powder</th>
<th>Cabbage Extract</th>
<th>Cysteine</th>
<th>Cabbage Powder + cysteine</th>
<th>Cabbage Extract + cysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (µ/ml)</td>
<td>95.3±3.11**</td>
<td>75.3±7.41***</td>
<td>72.14±8.17***</td>
<td>79.80±9.12***</td>
<td>68.96±6.21***</td>
<td>51.71±5.11***</td>
</tr>
<tr>
<td>AST (µ/ml) 103.41±11.41**</td>
<td>85.91±7.97***</td>
<td>70.98±8.61***</td>
<td>80.11±9.11***</td>
<td>60.96±5.99c***</td>
<td>62.21±5.33***</td>
<td></td>
</tr>
<tr>
<td>ALP (µ/ml) 117.61±13.67**</td>
<td>87.74±8.91***</td>
<td>79.33±7.88***</td>
<td>88.22±8.61***</td>
<td>75.14±7.40***</td>
<td>69.31±6.30***</td>
<td></td>
</tr>
<tr>
<td>γGT (µ/ml)</td>
<td>15.41±2.11**</td>
<td>8.97±1.65***</td>
<td>8.61±1.21***</td>
<td>10.21±1.31***</td>
<td>6.65±1.96***</td>
<td>6.34±1.47***</td>
</tr>
</tbody>
</table>

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Values with the same letters indicate non-significant difference (P<0.05) and vice versa.

**Table 3:** Mean values ± SD of serum CHO, TG, HDLc, LDLc, VLDLc and CHO/HDLc of the experimental rat groups.

<table>
<thead>
<tr>
<th>Groups Variables</th>
<th>Control positive</th>
<th>Cabbage Powder</th>
<th>Cabbage Extract</th>
<th>Cysteine</th>
<th>Cabbage Powder + cysteine</th>
<th>Cabbage Extract + cysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO (mg/dl)</td>
<td>195.1±15.67**</td>
<td>120.7±11.15***</td>
<td>110.3±10.10***</td>
<td>133.21±12.4***</td>
<td>106.14±10.36***</td>
<td>107.32±10.25***</td>
</tr>
<tr>
<td>TG (mg/dl) 159.40±15.36**</td>
<td>110.71±12.27***</td>
<td>112.96±13.44***</td>
<td>114.30±17.22***</td>
<td>103.21±13.719***</td>
<td>101.96±10.25***</td>
<td></td>
</tr>
<tr>
<td>HDLc (mg/dl)</td>
<td>21.20±3.33**</td>
<td>32.17±4.35**</td>
<td>33.24±3.21**</td>
<td>31.27±4.76*</td>
<td>34.96±4.11**</td>
<td>36.11±3.61**</td>
</tr>
<tr>
<td>LDLc (mg/dl)</td>
<td>142.03±14.33**</td>
<td>66.39±5.96***</td>
<td>54.51±4.39***</td>
<td>79.05±6.44***</td>
<td>50.54±5.96***</td>
<td>50.81±6.21***</td>
</tr>
<tr>
<td>VLDLc (mg/dl) 31.88±4.11**</td>
<td>22.14±2.10***</td>
<td>22.39±2.71***</td>
<td>22.89±2.14***</td>
<td>20.64±3.10b</td>
<td>20.40±2.11**</td>
<td></td>
</tr>
<tr>
<td>CHO/HDLc</td>
<td>9.22±1.27**</td>
<td>3.75±0.56***</td>
<td>3.31±0.77***</td>
<td>4.25±0.63***</td>
<td>3.03±0.61***</td>
<td>2.97±0.44***</td>
</tr>
</tbody>
</table>

Significant with control (+ve) group * P<0.05 ** P<0.01 *** P<0.001
Values with the same letters indicate non-significant difference (P<0.05) and vice versa.

**Table 4:** The Mean values ± SD of liver cholesterol, total lipid, triglyceride and glycogen of the experimental rat groups.

<table>
<thead>
<tr>
<th>Groups Variables</th>
<th>Control positive</th>
<th>Cabbage Powder</th>
<th>Cabbage Extract</th>
<th>Cysteine</th>
<th>Cabbage Powder + cysteine</th>
<th>Cabbage Extract + cysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/g)</td>
<td>5.88±0.77**</td>
<td>4.01±0.61**</td>
<td>3.51±0.49***</td>
<td>4.21±0.55**</td>
<td>3.99±0.66**</td>
<td>3.76±0.55**</td>
</tr>
<tr>
<td>total lipid (mg/g)</td>
<td>49.14±4.19**</td>
<td>38.22±4.96**</td>
<td>37.74±3.24**</td>
<td>41.40±3.90b</td>
<td>35.01±4.21***</td>
<td>36.11±5.10**</td>
</tr>
<tr>
<td>Triglyceride (mg/g)</td>
<td>1.59±0.04**</td>
<td>1.96±0.10**</td>
<td>2.40±0.35**</td>
<td>2.11±0.34**</td>
<td>2.49±0.85a***</td>
<td>2.99±0.45**</td>
</tr>
<tr>
<td>glycogen (mg/g)</td>
<td>3.81±0.25**</td>
<td>5.83±0.99***</td>
<td>6.88±1.10a***</td>
<td>6.01±1.11b**</td>
<td>6.17±1.30ab***</td>
<td>7.21±1.45**</td>
</tr>
</tbody>
</table>

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Values with the same letters indicate non-significant difference (P<0.05) and vice versa.

**Table 5:** The Mean values ± SD of liver SOD, GPX, GST, catalase and MDA of the experimental groups.

<table>
<thead>
<tr>
<th>Groups Variables</th>
<th>Control positive</th>
<th>Cabbage Powder</th>
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<th>Cysteine</th>
<th>Cabbage Powder + cysteine</th>
<th>Cabbage Extract + cysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (mmol/l)</td>
<td>20.90±2.99**</td>
<td>32.41±3.89***</td>
<td>45.98±4.69***</td>
<td>35.40±3.78***</td>
<td>46.71±4.21***</td>
<td>53.21±4.99***</td>
</tr>
<tr>
<td>GPX (mmol/l)</td>
<td>17.15±2.10**</td>
<td>28.71±3.20**</td>
<td>40.36±3.96**</td>
<td>31.63±3.21**</td>
<td>43.21±5.10**</td>
<td>50.14±5.11**</td>
</tr>
<tr>
<td>GST (mmol/l)</td>
<td>2.05±0.77**</td>
<td>7.11±1.21*</td>
<td>10.36±1.14*</td>
<td>8.45±1.31*</td>
<td>11.21±1.20*</td>
<td>13.47±1.34*</td>
</tr>
<tr>
<td>MDA (mmol/l)</td>
<td>17.78±1.98**</td>
<td>9.36±1.51**</td>
<td>7.20±1.41**</td>
<td>9.96±1.51**</td>
<td>8.67±1.24**</td>
<td>7.31±1.30**</td>
</tr>
</tbody>
</table>

Significant with control (+ve) group * P<0.05 ** P<0.01 *** P<0.001
Values with the same letters indicate non-significant difference (P<0.05) and vice versa.
3.4. Histopathological examination:

Liver sections from control positive rat group showed inflammatory infiltration of lymphocytes with massive fatty changes and cell necrosis (pict 1). Liver section of cabbage powder treated rats showed moderate inflammatory cells around central vein and absence of necrosis (pict 2) while liver of rats treated with cabbage extract showed slight hydropic degeneration of hepatocytes (pict 3). However, mild kupffer cells activation was observed in liver section of rat group treated with cysteine (pict 4). Cabbage powder with cysteine and cabbage extract with cysteine treated rats groups exhibited significant treatment against paracetamol induced hepatotoxicity as evident by presence of normal architecture hepatic cords and absence of necrosis with minimal inflammatory conditions around the central vein (pict 5& 6)

4. Discussion:

It is known that oral paracetamol administration induced hepatotoxicity in rats. Elevated levels of serum enzymes (ALT, AST, ALP& γ GT) activities are inductive of cellular leakage and loss of functional integrity of cell membrane in liver. Elevated serum cholesterol and triglyceride levels in paracetamol challenged rats indicated impaired fat metabolism due to hepatic damage (Black 1984 and Abd El-Ghany and Nanees 2010). Elevated lipid peroxidation causes tissue injury and damage to cellular macromolecules by generation of reactive oxygen species increasing the risk of tissue damage. Lipid peroxidation is usually measured through its
catabolite malondialdehyde as a marker of oxidative stress. The enzymatic antioxidant defense system is the nature protector against lipid peroxidation. SOD, catalase and glutathione peroxidase enzymes are important scavengers of superoxide ion and hydrogen peroxide. These enzymes prevent generation of hydroxyl radical and protect the cellular constituents from oxidative damage (Janero 1990, Scott et al., 1991 and O'Brien et al., 2000).

The results of the previous studies of Xue et al., (2008) and Wu and Juurlink (2001) supported the results of the current study which confirmed that, Brassica vegetables are excellent sources of fibers. Soluble fiber helps to reduce blood cholesterol and blood sugar, thereby reducing the risk of heart disease and diabetes. Brassica vegetables have a potent antioxidant and previously been linked to the lower risk of heart attacks and strokes. Sulforaphane (the active component in Brassica members) could up-regulate the impaired glutathione system in vascular smooth cells of spontaneously hypertensive rats. Igarashi et al., 2000 and Jagdish et al., 2006 recorded that presence of flavonoids was reported in red cabbage species and flavonoids have good therapeutic potential in inflammation and pain. Red cabbage extract has prevented oxidative stress induced in livers and brains of animals because of presence isothiocyanates (glucosinolate), vitamins A, B, C and anthocyanins which were found to have the strongest antioxidant power. Anthocyanin in cabbage extract significantly attenuated alterations in the cardiac and hepatic antioxidants and lipid peroxidation, and histopathological changes in cardiac and hepatic tissue (Sankhari et al., 2012).

Previous studies recorded that cysteine is a key constituent of glutathione which formed from cysteine, glutamic acid, and glycine. Although cysteine is classified as a non essential amino acid but it may be essential for infants, the elderly, and individuals with certain metabolic disease or who suffer from malabsorption syndromes. Cysteine has many important physiological functions. Glutathione is a potent antioxidant, protecting fatty tissues from the damaging effects of free radicals. The antioxidant activity of glutathione is attributed specifically to the presence of cysteine in the compound (Breuille and Obled, 2000 and Puerto et al., 2002).

Cysteine can be converted into a powerful antioxidant that can prevent this damage, which might lower a person's risk for heart disease and certain types of cancers. It is useful to detoxify the body from harmful toxins and help protect the brain and liver from damage from alcohol, drugs etc. Cysteine is also critical to the metabolism of a number of essential biochemicals including coenzyme A, heparin, biotin and lipid acid. Cysteine is used to counteract acetaminophen and treats acetaminophen poisoning by binding the poisonous forms of acetaminophen that are formed in the liver. It is also an antioxidant, so it may play a role in preventing cancer. Cysteine is given intravenously to prevent or reduce liver damage and help restore the glutathione level (Droge 1999 and Lai et al., 2012).

While co-treatment with ribose cysteine had previously protected against acetaminophen-induced liver and kidney injury, ribose cysteine serves as a cysteine prodrug that facilitates GSH biosynthesis and protects against acetaminophen-induced target organ toxicity (Smilkstein et al., 1999). Levels of AST and, ALT were decreased in groups treated with N-Acetyl cysteine and erdosteine after acetaminophen administration, but the levels of glutathion was increased with improvements of liver histopathology (Kandis et al., 2011). N-acetyl-L-cysteine has preventive effect on oxidative stress and cognitive impairment in hepatic encephalopathy following bile duct ligation (Dhanda et al., 2012).

From the present investigation, it can be concluded that the cabbage powder or extract with cysteine offered potential hepatotreatment effect against paracetamol induced hepatotoxicity in rats.

References


