ملخص البحث

المؤثر الواقعي لمستخلص بذور العنب وفيتامين H على الإجهاد التأكسدي الكبدى في ذكور الفئران

هذا البحث يستهدف دراسة التأثير الواقعي لمستخلص بذور العنب مع فيتامين H ضد الإجهاد التأكسدي الكبدى المحدث ببرومات البروتامين في ذكور الفئران. واستخدمت هذه الدراسة عدد 45 فئرًا ذكرًا تم توزيعها على خمس مجموعات كل منها عدد 9 فئران. كانت المجموعة الأولى ضابطة سلبية (فئران غيرمصابون)، وتم حقن فئران المجموعات الأخرى بجرعة إضافية في التوالي بجرعة مفردة (25 مجم/كم) من برومات البروتامين لإحداث الإجهاد التأكسدي. وتركت المجموعة الثانية ضابطة موجبة (فئران مصابون وغيرمصابون). وتم إعطاء فئران المجموعات الثالثة والرابعة والخامسة خلاصة بذور العنب بجرعة 100 ججم/كم أو فيتامين H بجرعة 50 مجم/كم أو الخلاصة والفيتامين معا بنفس الجرعات عن طريق الفم يومياً على التوالي لمدة ثمانية أسابيع. وفي نهاية فترة التجربة تم حساب عينات من الدم لفصل المصل لإجراء التحليلات البيوكيميائية، وفي الوقت الذي أخذ الفئران لإجراء التحليلات البيوكيميائية والفحص الهيستوپاتولوجي. وأوضحت النتائج أن الإجهاد التأكسدي المحدث ببرومات البروتامين في الفئران أدى إلى نقص في وزن الجسم ومعدل التحويل الغذائي. وأدى أيضاً إلى زيادة مستويات إنزيمات الكبد، وتركيزات البيروكسيد، حمض البوليك، الكرياتين، المالمونيديالدهيد، الكولستيروال الكلى والدهون الثلاثية في المصل. وكذلك أدى إلى زيادة أكسدة الدهون ونقص نشاط الإنزيمات المضادة للأكسدة ووجود تغيرات مرضية دهنية بأساسية للكبد. وفقًا لأول الإعطاء المتلازم لخلاصة بذور العنب مع فيتامين H إلى زيادة وزن الجسم ومعدل التحويل الغذائي في الفئران المصابين بالإجهاد التأكسدي. كما أدى إلى عودة مستويات إنزيمات الكبد وتركيزات البيروكسيد، حمض البوليك، الكرياتين، المالمونيديالدهيد، الكولستيروال والدهون الثلاثية إلى المستوى الطبيعي لها في المصل. وكذلك أدى إلى نقص أكسدة الدهون وزيادة نشاط الإنزيمات المضادة للأكسدة بأساسية الكبد إخفاء التغيرات المرضية الدهنية بأساسية الكبد. وتوضح النتائج أن أعطاء خلاصة بذور العنب مع فيتامين H يحدث تأثير مضاد للأكسدة في ذكور الفئران. ونوصي الدراسة أن تناول خلاصة بذور العنب مع فيتامين H قد يكون مفيداً للمرضى الذين يعانون من الإجهاد التأكسدي.
Abstract

Protective Effect of Grape Seed Extract and Vitamin E against Hepatic Oxidative Stress in Male Rats

The protective effect of grape seed extract (GSE) with vitamin E (Vit E) against potassium bromate (KBrO₃)-induced hepatic oxidative stress in rats was investigated. Forty five mature male rats were divided into 5 groups (n=9). Group (1) was negative control and the other 4 groups were injected with a single intraperitoneal dose of KBrO₃ (125 mg/kg) to induce oxidative stress. Group (2) was used as a positive control, while groups (3), (4) and (5) were orally given GSE (100 mg/kg), Vit E (50 mg/kg) and GSE and Vit E daily for 8 weeks, respectively. Blood samples were collected for separating the serum for assessment of hepatorenal function. Livers were taken for preparing of homogenate for biochemical analysis. Histopathology of liver was also carried out. The results showed that oxidative stress induced by KBrO₃ in rats caused significant decreases in body weight gain and feed efficiency ratio. It also passively altered serum biomarkers of hepatorenal function, increased lipid peroxidation, decreased the activity of hepatic antioxidant enzymes glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) and induced hepatic fatty degenerative changes. Oral coadministration of GSE and Vit E increased body weight gain and feed efficiency ratio. It improved hepatorenal function, decreased hepatic lipid peroxidation, normalized biomarkers of oxidative stress in liver and ameliorated fatty degenerative changes seen in hepatic tissue of rats given KBrO₃. In conclusion, GSE and Vit E had good antioxidant activity and alleviate oxidative stress in rats. The study suggests that intake of grape seed extract with vitamin E is beneficial as a food supplement for patients who suffer from oxidative stress.

Keywords: Grape seed extract; Vitamin E; Oxidative stress; Antioxidant enzymes; Hepatorenal function; Histopathology; Rats.


References


Results of the current study revealed that oral administration of grape seed extract (GSE) to rats with oxidative stress improved hepatorenal function, reduced lipid peroxidation and produced high antioxidant activity. The antioxidant effect of GSE could be attributed to its content of polyphenols, proanthocyanidins and anthocyanidins, which are thought to be beneficial for reducing cell damage induced by oxidative stress. The phenolic compounds have the ability to scavenge the free radicals by presence of hydroxyl groups in these compounds (Djeridane et al., 2006 and Belviranli et al., 2013).

The hepatoprotective action of grape seed extract reported in the present study was similar to that obtained by Hamlaoui-Gasmi et al. (2012) and Belviranli et al. (2013). This effect was confirmed by the alleviation of fatty degeneration seen in liver of rats given grape seed extract in this study. The cholesterol lowering effect of grape seed extract agreed with that reported by Jiao et al. (2010). The previous authors concluded that the hypocholesterolemic effect of grape seed extract is most likely mediated by enhancement of bile acid excretion and up-regulation of cholesterol 7-alpha-monooxygenase(hydroxylase) or cytochrome P450 7A1(CYP7A1). The nephroprotective effect of grape seed extract that reported in this study was similar to that reported by Yousef et al. (2009) and Salem and Salem (2011). The renal protective activity of grape seed extract could be due to its antioxidant effect.

The present results also showed that the antioxidant effect of grape seed extract was amplified by its coadministration with vitamin E. It is well known that vitamin E (alpha-tocopherol) is a powerful antioxidant. Previous studies showed that intake of vitamin E could normalize the damaging effect of oxidative stress induced by free radicals (Shalaby et al., 2004; Ramachandran and Raja, 2010 and Iranloye and Oludare, 2011). Coadministration of grape seed extract and vitamin E ameliorated potassium bromate- induced hepatic oxidative stress as evident by decreasing the levels of MDA, increasing hepatic reduced glutathione content and enhancing the activities of hepatic antioxidative enzymes (GPx, SOD and CAT). These biochemical data were supported by the histopathological findings seen in liver tissue. These results agreed with that obtained by Adaramoye et al. (2012). The potentiating of antioxidant activity of grape seed extract by co-administration of vitamin E could be due to the addition effect.

In conclusion, the results suggest that oral administration of grape seed extract and vitamin E to rats with oxidative stress improves hepatorenal function, reduces lipid peroxidation and produces high antioxidant activity. Therefore, intake of grape seed extract with vitamin E is beneficial for reducing the oxidative stress which is a major predisposing risk factor of many chronic diseases.
Photo. (1): Showing normal histological architecture of hepatic lobe of a normal control rat. (H&E, x100)

Photo. (2): Showing severe fatty degeneration of in centrilobular portion of liver of a rat injected intraperitoneally with potassium bromate (C+ve group). (H&E, x100)

Photo. (3): Showing infiltration of leukocytes in liver of a rat with oxidative stress and given orally grape seed extract (100mg/kg). (H&E, x100)

Photo. (4): Showing nearly normal histological structure of liver of a rat given orally combination of grape seed extract and vitamin E(50mg/kg). (H&E, x100)

Discussion

The present study was carried out to investigate the effect of coadministration of grape seed extract (GSE) with vitamin E (Vit.E) on hepatic oxidative stress induced by potassium bromate (KBrO₃) in male rats.

Oxidative stress induced in rats by KBrO₃, in this study, characterized by a reduction in body weight gain and feed efficiency ratio, increase in lipid peroxidation and elevation in serum and liver biochemical markers of oxidative stress. These findings were partially similar to those obtained by Abd El-Ghany et al. (2011) who reported that the serum bilirubin level and activity of AST and ALT enzymes were greatly increased, while the total proteins were significantly decreased in rats with hepatotoxicity due to oxidative stress. However, oral administration of bromobenzene induced hepatic oxidative stress manifested by significant elevation of activities of AST and ALT in male rats (El-Sharaky et al., 2009). These findings indicated impaired function and damage of liver cells by bromobenzene. Lipid peroxidation is a complex process that damages the cell structure and function. Peroxidation of cell lipids membrane initiates a loss of membrane integrity; membrane bound enzyme activity and cell lyses. Low levels of tissue antioxidant enzymes are likely to result in tissue damage by lipid peroxides or protein carbonyls (Pryor and Squadrito, 1995). In the present study, the increased lipid peroxidation due to oxidative stress induced by potassium bromate was previously reported by Kurokawa et al. (1990), Khan and Sultana, (2004) and Abd El-Ghany et al. (2011).

Grape seed extract (GSE) is an extract contains a variety of biologically active species that used for protection against oxidative stress caused by free radicals and reactive oxygen spices (ROS) (Sharma et al., 2004 and Faria et al., 2006). GSE has antioxidant and free radical scavenging activity (Jayaprakasha et al., 2003 and Caillet et al., 2006). Most of the beneficial health effects of GSE are attributed to its antioxidant and free radical scavenging properties (Oliboni et al., 2011 and Belviranli et al., 2013).
compared to the negative control group. Oral coadministration of grape seed extract with vitamin E caused significant ($P<0.05$ and $P<0.001$) increases in the activity of GPx, SOD, CAT enzymes in liver tissues as compared with the positive control group (Table 6).

**Table (6):** Effect of grape seed extract (GSE) and vitamin E (Vit. E) on the activity of antioxidant enzymes in liver tissues of rats.

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Control (-ve)</th>
<th>Control (+ve)</th>
<th>GSE</th>
<th>Vit. E</th>
<th>GSE + Vit. E</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPx (nmol/min/mg protein)</td>
<td>0.70 ±0.02a</td>
<td>0.21 ±0.01d***</td>
<td>0.44 ±0.003b*</td>
<td>0.43 ±0.002b**</td>
<td>0.63 ±0.001c**</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>54.66 ±1.25a</td>
<td>29.83 ±1.25d***</td>
<td>43.42 ±2.71b**</td>
<td>45.22 ±2.66b**</td>
<td>51.42 ±1.28c***</td>
</tr>
<tr>
<td>CAT (nmol/min/mg protein)</td>
<td>0.185 ±0.003a</td>
<td>0.133 ±0.002d***</td>
<td>0.151 ±0.001b**</td>
<td>0.159 ±0.002b**</td>
<td>0.170 ±0.001c**</td>
</tr>
</tbody>
</table>

Mean ± SD values in each row with different superscripts are significant when compared to the control groups at * $P<0.01$ ** $P<0.01$ *** $P<0.001$, n= 9 rats/group.

Histopathological examination of liver of normal control rats showed normal histological structure of hepatic lobule Photo.(1). Intraperitoneal injection of potassium bromate to rats induced severe fatty degeneration (fat droplets) as shown in Photo. (2). Oral administration of grape seed extract to rats with oxidative stress alleviated the fatty degeneration and only few infiltration of leukocytes was seen in Photo. (3). Livers of rats orally given combination of grape seed extract with vitamin E showed nearly normal histological structure Photo.(4).
Mean ± SD values in each raw with different superscripts are significant when compared to the control groups at * $P<0.05$ ** $P<0.01$ *** $P<0.001$, n= 9 rats/group.

Data in Table (4) showed that intraperitoneal injection of potassium bromate to rats caused an elevation in serum level of lipid peroxide malondialdehyde (MDA) and lowering the level of reduced glutathione (GSH) compared with the negative control rats. Grape seed extract and vitamin E when concomitantly given orally to rats with oxidative stress induced a significant ($P<0.05$ and $P<0.001$) decrease in MDA and an increase in GSH levels in the serum when compared with the positive control group.

**Table (4):** Effect of grape seed extract (GSE) and vitamin E (Vit E) on serum malondialdehyde (MDA) and reduced glutathione (GSH) in rats.

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Control (-ve)</th>
<th>Control (+ve)</th>
<th>GSE</th>
<th>Vit E</th>
<th>GSE + Vit E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDA (mmol/ml)</td>
<td>34.11 ± 2.21&lt;sup&gt;d&lt;/sup&gt;</td>
<td>57.33 ± 3.61&lt;sup&gt;a***&lt;/sup&gt;</td>
<td>44.55 ± 2.53&lt;sup&gt;b*&lt;/sup&gt;</td>
<td>43.19 ± 2.46&lt;sup&gt;b*&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>GSH (mmol/ml)</td>
<td>64.36 ± 3.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.11 ± 2.91&lt;sup&gt;d***&lt;/sup&gt;</td>
<td>59.21 ± 3.11&lt;sup&gt;c**&lt;/sup&gt;</td>
<td>60.31 ± 2.98&lt;sup&gt;b***&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean ± SD values in each raw with different superscripts are Significant when compared to the control groups at * $P<0.05$ ** $P<0.01$ *** $P<0.001$, n= 9 rats/group.

In liver tissues of rats with oxidative stress, there was a significant ($P<0.05$ and $P<0.001$) increase in levels of MDA and decrease in levels of GSH when compared with the negative control group. Oral administration of grape seed extract and vitamin E significantly reduced liver MDA level and increased GSH level when compared with the positive control group as shown in Table (5).

**Table (5):** Effect of grape seed extract (GSE) and vitamin E (Vit.E) liver malondialdehyde (MDA) and reduced glutathione (GSH) in rats.

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Control (-ve)</th>
<th>Control (+ve)</th>
<th>GSE</th>
<th>Vit E</th>
<th>GSE + Vit E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDA (nmol/min/mg protein)</td>
<td>0.24 ±0.002&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.71 ±0.003&lt;sup&gt;a***&lt;/sup&gt;</td>
<td>0.54 ±0.001&lt;sup&gt;b**&lt;/sup&gt;</td>
<td>0.44 ±0.003&lt;sup&gt;b**&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>GSH (nmol/min/mg protein)</td>
<td>22.50 ±1.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.66 ±1.15&lt;sup&gt;d***&lt;/sup&gt;</td>
<td>18.95 ±2.71&lt;sup&gt;b*&lt;/sup&gt;</td>
<td>19.46 ±2.66&lt;sup&gt;b**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean ± SD values in each raw with different superscripts are significant when compared to the control groups at * $P<0.01$ ** $P<0.01$ *** $P<0.001$, n= 9 rats/group.

Concerning the activity of tissue antioxidant enzymes, the rats with oxidative stress had decreased activity of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) enzymes in liver tissues as
Mean ± SD values in each raw with different superscripts are significant when compared to the control groups at * $P<0.05$ ** $P<0.01$ *** $P<0.001$, n= 9 rats/group.

As shown in Table (2) the rats injected intraperitoneally with potassium bromate had significant increases ($P \leq 0.05$ and $P \leq 0.001$) in serum levels of AST, ALT compared to the negative control group, while the total cholesterol (TC) and triglycerides (TG) also had a significant ($P \leq 0.05$ and $P \leq 0.001$) increases in serum levels if compared to the negative control group. Oral administration of grape seed extract and vitamin E, alone and in combination, to rats with oxidative stress induced significant ($P \leq 0.05$ and $P \leq 0.01$) decreases gradually the elevated serum levels of AST, ALT, and the same effect showed in the TC and TG when compared with the positive control group.

**Table (2):** Effect of grape seed extract (GSE) and vitamin E (Vit. E) on some liver enzymes and lipid profile concentration in rats.

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Control (-ve)</th>
<th>Control (+ve)</th>
<th>GSE</th>
<th>Vit. E</th>
<th>GSE + Vit. E</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/dl)</td>
<td>63.56 ±2.61c</td>
<td>85.11 ±4.66***</td>
<td>78.33 ±4.11b*</td>
<td>76.55 ±3.88b*</td>
<td>66.22 ±3.98b*</td>
</tr>
<tr>
<td>ALT (U/dl)</td>
<td>35.22 ±2.11c</td>
<td>57.66 ±3.71***</td>
<td>47.66 ±3.71b*</td>
<td>44.19 ±5.66b*</td>
<td>38.49 ±4.28b*</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>111.9 ±3.22d</td>
<td>131.5 ±2.44***</td>
<td>125.4 ±3.15b**</td>
<td>123.3 ±2.13b*</td>
<td>117.4 ±1.16***</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>96.6 ±2.12d</td>
<td>120.3 ±3.14***</td>
<td>115.4 ±2.52b**</td>
<td>113.7 ±2.41b*</td>
<td>103.3 ±1.90***</td>
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</table>

Mean ± SD values in each raw with different superscripts are significant when compared to the control groups at * $P<0.05$ ** $P<0.01$ *** $P<0.001$, n = 9 rats/group.

Rats with the intraperitoneal injection of potassium bromate in a single (125mg/kg b.w.) dose to rats caused a significant ($P \leq 0.05$ and $P \leq 0.001$) increases in urea nitrogen (UN), uric acid (UA) and creatinine (Cr) levels in the serum when compared to the negative control rats. Oral administration of grape seed extract and vitamin E, alone and in combination, to rats with oxidative stress produced significant ($P<0.01$) decreases gradually the elevated serum urea nitrogen (UN), uric acid (UA) and creatinine (Cr) concentration when compared with the positive control group (Table 3).

**Table (3):** Effect of grape seed extract (GSE) and vitamin E (Vit. E) on kidney functions in rats.

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Control (-ve)</th>
<th>Control (+ve)</th>
<th>GSE</th>
<th>Vit. E</th>
<th>GSE + Vit. E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea nitrogen (mg/dl)</td>
<td>35.50 ±2.34d</td>
<td>67.63 ±2.54***</td>
<td>41.55 ±4.11b*</td>
<td>39.65 ±3.13b*</td>
<td>37.44 ±2.11b*</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>1.65 ±0.12d</td>
<td>2.85 ±0.17a**</td>
<td>1.90 ±0.15b**</td>
<td>1.85 ±0.13b**</td>
<td>1.72 ±0.16b**</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.20 ±0.02d</td>
<td>1.70 ±0.04**</td>
<td>1.50 ±0.02b**</td>
<td>1.45 ±0.01b**</td>
<td>1.30 ±0.02b**</td>
</tr>
</tbody>
</table>
Lipid peroxide (MDA) was determined according to Ohkawa et al. (1979). The activities of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) antioxidant enzymes were determined chemically according to Paglia and Valentaine (1979), Spitz and Oberley (1989) and Sinha (1972), respectively.

9. Histological procedure

The fixed specimens of livers were trimmed, washed and dehydrated in ascending grades of alcohol. The specimens were then cleared in xylene, embedded in paraffin, sectioned at 4-6 microns thickness and stained with Hematoxylin and Eosin (H&E) and examined microscopically Carleton (1976).

10. Statistical analysis

Data were presented as means ± SD and statistically analyzed using one way analysis of variance (ANOVA) test followed by Duncan’s multiple range test. Differences among control and treated groups were considered significant at $P<0.05$ level (Snedecor and Cochran, 1986) using computerized SPSS program.

Results

Intraperitoneal injection of potassium bromate in a single dose (125mg/kg b.wt.) dose to rats caused significant ($P \leq 0.05$ and $P \leq 0.001$) decreases in body weight gain, feed intake and feed efficiency ratio (FER) compared to the negative control group. In rats with oxidative stress induced by potassium bromate, the oral administration of grape seed extract and vitamin E alone and in combination for 8 weeks caused significant ($P \leq 0.05$ and $P \leq 0.001$) increases in body weight gain, feed intake and FER compared to the positive control group (Table 1).

Table (1): Effect of grape seed extract (GSE) and vitamin E (Vit E) on body weight gain, feed intake and feed efficiency ratio (FER) in rats with oxidative stress

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Control (-ve)</th>
<th>Control (+ve)</th>
<th>GSE</th>
<th>Vit. E</th>
<th>GSE + Vit. E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight (g)</td>
<td>175.00 ± 5.24a</td>
<td>180.50 ± 6.31a</td>
<td>175.00 ± 4.55a</td>
<td>179.50 ± 4.36a</td>
<td>180.00 ± 4.66a</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>264.25 ±14.20a</td>
<td>215.50 ±12.71b</td>
<td>242.06 ±12.98a</td>
<td>244.19 ±14.66a</td>
<td>255.50 ±13.28c</td>
</tr>
<tr>
<td>Weight gain (%)</td>
<td>51.00 ± 6.65a</td>
<td>19.39 ± 3.21c***</td>
<td>38.32 ± 7.11b</td>
<td>34.92 ± 7.13b</td>
<td>41.94 ± 8.11c**</td>
</tr>
<tr>
<td>Feed intake (g)</td>
<td>19.30 ± 1.26a</td>
<td>18.74 ± 1.21b</td>
<td>19.25 ± 1.31b</td>
<td>18.88 ± 1.21b</td>
<td>19.20 ± 1.25c**</td>
</tr>
<tr>
<td>FER</td>
<td>0.216 ± 0.003a</td>
<td>0.032 ± 0.004b***</td>
<td>0.051 ± 0.001c**</td>
<td>0.055 ± 0.002c**</td>
<td>0.071 ± 0.004b***</td>
</tr>
</tbody>
</table>
Food intake was recorded daily and body weight gain (g) was calculated. Feed efficiency ratio (FER) was calculated using this formula:

$$\text{FER} = \frac{\text{feed intake (g)}}{\text{weight gain (g)}}.$$ 

At the end of experiment, rats were anesthetized using ether anesthetic and blood samples were collected into clean centrifuge tubes to obtain the serum which used for biochemical analyses. Halves of livers were immediately removed, rinsed with 10% saline solution, blotted on filter paper and stored at -70°C pending for the preparation of liver homogenate for biochemical assays. The other half of livers was preserved in 10% neutral formalin solution till histological examination.

6. Biochemical analyses

Activities of serum liver enzymes aspartate and alanine aminotransferases (AST and ALT) were chemically determined according to Bergmeyer et al. (1978). Serum total cholesterol (Ratliff and Hall, 1973) and triglycerides (Jacob and Van-Denmark, 1960) were chemically determined. Blood urea nitrogen (BUN) was determined using BioMérieux kits according to Patton and Crouch (1977). Serum uric acid was determined using the enzymatic colorimetric method as described by Fossati et al. (1980). Serum creatinine concentrations were colorimetrically determined by the Jaffé reaction (Husdan and Rapoport, 1968). Serum lipid peroxide malondialdehyde (MDA) and reduced glutathione (GSH) contents were estimated according to methods described by Placer et al. (1966) and Afzal et al. (2002), respectively.

7. Preparation of liver homogenate

One gram of frozen liver tissue was collected, washed in ice-cold 0.9% NaCl and homogenized in ice-cold 1.15% solution of potassium chloride and 50 mM potassium phosphate buffer solution (pH 7.4) to yield 10% homogenate (W/V). Homogenization was performed using ultrasonic homogenizer (Sonicator, model 4710, Cole-Parmer Instrument Company). The homogenate was then centrifuged at 4000 rpm for 15 minutes at 4°C and the supernatant was collected for further use.

8. Lipid peroxidation and tissue antioxidant enzymes

Lipid peroxidation (LPO) was determined by quantifying malondialdehyde (MDA) that formed in terms of thiobarbituric acid reactive substances (TBARS). Liver homogenate was used for determination of tissue lipid peroxide (MDA), enzymatic (GPx, SOD and CAT) and non enzymatic (GSH) antioxidants. The reduced glutathione (GSH) content in liver homogenate was determined colorimetrically by the method modified by Bulaj et al. (1998).
Helwan, Egypt. Rats were housed at a controlled room temperature of 23 ± 1°C, 55 % humidity and under a 12-hr light / hr dark cycles. The animals were fed on basal diet and water was provided ad libitum for one week before the start of experiment for acclimatization.

3. Preparation of grape seed extract

Ripe grapes (Vitis vinifera L., Vitaceae) were obtained from Giza government, Egypt. Undamaged and disease-free berries were snipped from clusters. Following manual separation of the seeds from whole berries, the seeds were oven dried at 30 - 40 °C. The dried grape seeds were grinded into fine powder using an electric grinder. The ethanol extract was prepared by soaking 100 gm of powdered grape seeds in 300 ml of 95 % ethanol and kept in refrigerator with daily shaking for 5 days. The ethanol was evaporated using a rotatory evaporator apparatus (manufactured in West Germany) attached with a vacuum pump. The 100 gm of dried grape seeds powder yield 26.9 gm of methanol extract. Twenty grams of the obtained semisolid extract were suspended in distilled water with 2ml of Tween 80 (suspending agent) and 80 ml of distilled water was gradually added to prepare a 20% liquid grape seed extract. The rats will be housed in the cages in the central animal house of the Agriculture Research Center.

4. Preparation of basal diet

The basal diet was prepared using AIN-93 according to Reeves et al. (1993). It consists of 20 % protein (casein), 10 % sucrose, 4 % corn oil, 2% choline chloride, 1% vitamin mixture, 3.5 % salt mixture and 5% fibers. The remainder was corn starch up to 100%.

5. Experimental design and grouping of rats

After one week adaptation period, the animals were randomly distributed into five equal groups, of 9 rats each. Group (1) was fed on basal diet and kept without any treatment as a negative control. Rats of the other four groups were injected by a single intraperitoneal dose of potassium bromate at 125 mg/kg b.wt. for induction of oxidative stress (Khan and Sultana 2004). Group (2) was left as a positive control and groups (3), (4) and (5) were given grape seed extract (GSE) by oral gavage (0.6 ml/rat) in a dose of 100 mg/kg (1ml/rat), vitamin E (Vit. E) in a dose of 50 mg/kg (0.5ml/rat) and GSE in combination with Vit. E at the same doses daily, respectively for 8 weeks. The dose of grape seed extract was selected according to Chis et al. (2009) and the dose of vitamin E according to Ganie et al. (2013).
Grapevine (Vitis vinifera L.), one of the most important widely consumed fruits, is cultivated today in all temperature regions of the world. Its seeds contain several active components including flavonoids, polyphenols, anthocyanins, proanthocyanidins, procyanidines, and the stilbene derivative resveratrol. Grape seed extract is a rich source of beneficial groups of plant flavonoids and proanthocyanidins oligomers (El-Ashmawy et al., 2007). Grape seed extract is widely consumed as a dietary supplement and has been reported to possess several effects such as antioxidative, anti-inflammatory, and antimicrobial, cardioprotective, hepatoprotective, and nephroprotective effects (Shenoy et al., 2007; Oliboni et al., 2011 and Belviranli et al., 2013). Several studies have indicated that grape seed extract inhibits enzyme systems that are responsible for the production of free radicals, so it has antimutagenic and anticarcinogenic effects (Aysun et al., 2008; Maier et al., 2009 and Hamlaoui-Gasmi et al., 2012).

Vitamin E (alpha-tocopherol) is a fat soluble vitamin which regulates oxidation processes in the body as it acts as a powerful antioxidant. Previous studies showed that intake of vitamin E could normalize the damaging effect of oxidative stress induced by free radicals (Sanoka et al., 1997; Shalaby et al., 2004; Ramachandran and Raja, 2010 and Iranloye and Oludare, 2011).

The present study was designed to investigate the effect of grape seed extract in combination with vitamin E on hepatic oxidative stress induced by potassium bromate in male rats.

Materials and Methods

1. Chemicals

Potassium bromate (KBrO3) in the form of white powder (soluble in boiling water) was purchased from El-Gomhoryia Company, Cairo Egypt. Vitamin E was supplied from Pharco Company for Pharmaceutical, Alexandria, Egypt. It is dispensed in the form of soft gelatinous capsules each containing 1000 mg of dl-alpha tocopherol acetate. Biochemical kits for the determination of liver enzymes AST, ALT and urea nitrogen, uric acid, creatinine, total cholesterol and triglycerides were purchased from Alkan Company for Chemicals and Biodiagnostics, Dokki, Cairo, Egypt. The other kits used for biochemical analyses were purchased from Gamma Trade for Company Pharmaceutical and Chemicals, Dokki, Egypt.

2. Rats

Forty five mature male rats of Sprague Dawley strain weighing 175 ± 5 g each and 11-12 weeks old were obtained from the Laboratory Animals Farm,
Protective Effect of Grape Seed Extract and Vitamin E against Hepatic Oxidative Stress in Male Rats

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Introduction

Oxidative stress is a state in which oxidation exceeds the antioxidant systems in the body. Oxidative stress arises from an imbalance between the production of reactive oxygen species (ROS) and the body’s antioxidant defenses against them, which induces cellular damage. The antioxidant defenses enable the body system to remove ROS, restore the prevailing reducing environment and repair the tissue damage (Halliwell and Gutteridge, 1999). Free radicals such as nitric oxide (NO) and superoxide ions are produced as second messengers, particularly by immune cells. Superoxide reacts rapidly with nitric oxide by nitric oxide synthase to produce peroxynitrite, whereas hydrogen peroxide (H₂O₂) slowly decomposes to the highly reactive hydroxyl radical. Both peroxynitrite and hydroxyl radicals are highly reactive oxidizing agents, capable of damaging proteins, lipids, and DNA (Pryor and Squadrito, 1995 and Beckman and Koppenol, 1996). Oxidative stress plays an important role in the etiology and pathogenesis of many chronic diseases such as atherosclerosis, hypertension, diabetes mellitus and cancer (Reuter et al., 2010 and Krajcovicova et al., 2012).

Potassium bromate (KBrO₃) is widely used as a food additive in the bread making processes and found in drinking water samples as a by-product of ozone disinfection. KBrO₃ causes renal cell cancer and act as a tumor promoter in carcinogen-initiated animals. Renal cell tumors have been observed in rats after exposure to this compound due to oxidative stress induced by KBrO₃ (Fuji et al., 1984 and Kurokawa et al., 1990).

Dietary intake of antioxidants can inhibit or delay the oxidation of susceptible cellular substrates so prevent oxidative stress. Phenolic compounds such as flavonoids, phenolic acids, diterpenes, saponins and tannins have received much attention for their high antioxidative activity (Rice-Evan et al., 1996). Therefore, it is important to enrich the diet with antioxidants for protection against many chronic diseases related to oxidative damage (Rubio et al., 2008). Moreover, antioxidants play an important role in food quality preservation due to their ability to prevent oxidative deterioration of lipids (Erukainure et al., 2012).