Biological evaluation of stabilized full fat black rice bran in hypercholesterolemic rats

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ABSTRACT

This study was performed to investigate the effect of feeding on stabilized full fat black rice bran (S–BRB) in hypercholesterolemic rats. Chemical composition and total phenolic compounds (TPC) of (S–BRB) was determined. Results revealed that (S–BRB) is a good source of crude protein being 15.45%, and crude fat was 16.31%, while the amount of ash and crude fiber were 9.10 and 11.01% respectively. Also, total phenolic compounds of (S–BRB) contained (5.08 mg Tannic acid equivalent/g), obtained results illustrated that substitution of feeding hypercholesterolemic diet with (S–BRB) led to improvement the High lipoprotein cholesterol HDL-C. Furthermore, hypercholesterolemic diet with (S–BRB) replacement at 75% and 100% also recorded the best and nearest of HDL-C to the negative control. It was observed also that LDL-cholesterol value of negative control diets (G1) was 33.92 mg/dl, but also the value of the hypercholesterolemic in control (G2) was 179.80 mg/dl. On the other hand, the LDL cholesterol of rats fed on hypercholesterolemic diet substitution with (S–BRB) 25%, 50, 75 and 100% (G3, G4, G5 and G6) being 77.04, 61.68, 54.51 and 50.98 mg/dl, respectively. At the final of experiment (10 weeks), ALT was significantly increased for the hypercholesterolemic control (G2) was 53.76 U/L, while negative control (G1) was 29.38 U/L respectively. Feeding on hypercholesterolemic diets substituted with (S–BRB) (G3, G4, G5 and G6) led to a more reduction at level 25, 50, 75 and 100% were 36.36, 34.35, 33.25 and 31.15 U/L, respectively comparing with hypercholesterolemic control (G2). Briefly it be could conclude that (S–BRB) has pronounced effect in lowering cholesterol serum levels and may be useful for patients suffering from liver and cholesterol diseases.

Keywords: Black rice bran, Hypercholesterolemic

1. Introduction

Hypercholesterolemia (Hyper-C) is the leading cause of coronary heart disease, which mainly causes death all over the world. Several methods and factors have been used to treat hypercholesterolemia, including dietary fat restriction (Tuma et al., 2014). Hypercholesterolemic (Hyper-C) rats showed a marked increase in serum total lipid, TC, LDL-C, albumin, uric acid, glucose, and reduces the antioxidant defense system and reduce also, the activities of catalase and superoxide dismutase, and increase the content of lipid peroxide (Anila and Vijayalakshmi, 2003). Rice bran is the outer layer of raw rice that is obtained as a by-product of paddy milling include protein, fat, carbohydrate, dietary fiber, ash, vitamin, mineral and phytochemical compounds (Chinma et al., 2015 and Tan and Norhaizan 2017). Black rice is an excellent source of anthocyanidins, in addition to dietary fiber, flavonoids, and other polyphenols. Rice oil contains nonatherogenic fatty acids (Ausman et al., 2005). Anthocyanins are naturally occurring phenolic compounds that provide color and bioactive properties, such as antioxidants, which lowering of serum cholesterol and triacylglycerides in rats, when fed to rats on a daily basis (Graf, et al., 2013). Black rice is also a good source of plant sterols, such as oryzanol. These bioactive components are unsaponifiable, nonglyceride components, which contribute to cholesterol-lowering effects reported in many animal and human studies (Novotny et al., 2015). In addition, RBO also contains tocotrienols, which were reported to inhibit cholesterol synthesis by inhibiting the key enzyme HMGCo A reductase activity through a posttranscriptional mechanism in HepG2 cells (Most et al., 2005 and El-Bana et al., 2008). Several researches has verified that elevation
in serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) and reduction in high-density lipoprotein cholesterol (HDL-C) augmentation the danger of atherosclerosis and coronary heart disease. In addition, Oxidative damage initiated by free radicals is a primary contributor to CVD development (Ausman et al., 2005 and Revilla et al., 2005). The current investigation was designed to study the effect of stabilized black rice bran for oil on hypercholesterolemic rats.

2. Materials and Methods

2.1. Materials

Black rice bran was obtained from the milling of black rice variety (Oryza sativa L.). Black rice bran sample was obtained during the season of 2019 from Rice Research and Training Center (RRTC) at Sakha, Kafrelsheikh Governorate, Egypt. Other chemicals substances had been of analytical reagent grade were used.

2.2. Methods

2.2.1. Stabilization of rice bran

Black rice bran was steaming by autoclaved under atmospheric pressure for 10 min (Rosniyana et al., 2009).

2.2.2. Determination of gross chemical composition:

Moisture, crude protein (N × 5.95), ether extract, crude fiber and ash contents were performed according to A.O.A.C (2005). Total carbohydrates was calculated by difference.

Total phenolics compounds (TPC) was extracted by the method described by Nara et al., (2006). Total phenolic compounds of the extract was determined spectrophotometrically using Folin-ciocalteau reagent according to the method described by Bonoli et al., (2004). Phenolic acid was estimated by a standard curve prepared using Tannic acid.

2.2.3. Animal and experimental design:

A total of 42 male Albino rats, with average weight (138.15-140.65g) were used for biological evaluation of Hyper-C diets. All animals were housed individual cages with screen bottoms and fed on a basal diet for 7 days, under laboratory condition. Rats had been given free access to food and water the 12-week trial period after acclimation period. All 42 rats were divided into two main groups; the first group (7 rats) was fed on basal diet and kept as a negative control (C-ve). The second group (35 rats) was fed on basal diet mixed with cholesterol at 2 % concentration for 4 weeks before feeding the tested sample supplemented diets for accumulation of hyper-C described in table (A) as mentioned by Lanepeter and Person, (1971). Hyper-C rats were divided into 5 groups and fed experimental diets for 7 weeks.

Table A: Composition of various Hyper-C diets (g/Kg).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-BRB</td>
<td>0</td>
<td>0</td>
<td>153.30</td>
<td>306.60</td>
<td>459.90</td>
<td>613.20</td>
</tr>
<tr>
<td>Corn starch</td>
<td>660.5</td>
<td>598.0</td>
<td>508.73</td>
<td>419.46</td>
<td>329.54</td>
<td>223.39</td>
</tr>
<tr>
<td>Casein</td>
<td>140</td>
<td>140</td>
<td>117.85</td>
<td>95.70</td>
<td>73.56</td>
<td>51.41</td>
</tr>
<tr>
<td>Corn oil</td>
<td>100</td>
<td>100</td>
<td>75.0</td>
<td>50.0</td>
<td>25.0</td>
<td>0</td>
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<tr>
<td>Cellulose</td>
<td>50</td>
<td>50</td>
<td>33.12</td>
<td>16.24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Beef tallow</td>
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<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Bile salt</td>
<td>0</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>L-cystine</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

S-BRB: Stabilized black rice bran. G1: Feeding on the basal diet (negative control). G2: Feeding on (hyper-c) diet (Positive control). G3: Feeding on (hyper-c) diet replaced 25% (S-BRB) for oil. G4: Feeding on (hyper-c) diet replaced 50% (S-BRB) for oil. G5: Feeding on (hyper-c) diet replaced 75% (S-BRB) for oil. G6: Feeding on (hyper-c) diet replaced 100% (S-BRB) for oil.
2.2.4. Blood sampling
At the end of the experiment, blood samples are taken from all the experimental rats. After 12 hours of fasting, blood samples were obtained from the Vein plexus eye and then centrifuged at 3000 r.p.m. for 15 minutes to extract the aspired serum into dry, plastic pipes and kept frozen by -180°C for biochemical analysis (El-Khamissy, 2005).

2.2.5. Biochemical Analysis and Enzymes Assays
Triglycerides were performed following the method of Fossati and Prancipe (1982). In accordance with the Richmond (1973) method total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) was studied. Mathematical calculation was conducted using the method Friedwald et al. (1972), for low lipoprotein cholesterol concentration (LDL-C) and very low (VLDL-C) cholesterol density.

\[ \text{LDL-Cholesterol} = \text{Total cholesterol} - (\text{HDL-Cholesterol} - \text{Triglycerides} / 5). \]
\[ \text{VLDL-C} = \text{Triglycerides} / 5. \]

The activity of serum glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) were measured according to Oyanatui (1984).

2.2.6. Liver function tests
The activity of Glutamic pyruvic transaminase (GPT) or alanine amino transferase (ALT) and glutamic-oxaloacetic transaminase (GOT) or aspartate amino transferase (AST) activities were calculated in accordance with the procedure mentioned in Varley et al. (1980) using commercial Pasteur Lab kits, Paris, France and alkaline phosphatase enzymes (ALP) as outlined by King, (1965).

2.2.7. Determination of kidneys functions
In the method described by Chaney and Marbach (1962), Urea was determined by using a commercial kit from the (Biomed Company, Germany). Uric acid, according to the Trinder (1969) method has been determined using a commercial kit (Biomed Company, Germany).

Creatinine concentrations were calculated in accordance with the procedure Fabiny and Ertingshausen (1971) using a colorimetric enzyme kit (Biolabo, Maizy, France).

2.3. Statistical analysis
The received data were statistically analyzed according to Steel and Torrie (1980). Data was analyzed by one way analysis of variance (ANOVA) at 5% (p< 0.05) probability, using the program Spss (Windows, Version 22, 2013).

3. Results and Discussion
Recently, increasing attention has been focused on the health-benefits of phenolic compounds. As an important subclass of phenolics, anthocyanins have been reported to have many bioactivities including antioxidant, anti-inflammatory and anti-carcinogenic properties. Some foods containing abundant anthocyanins, such as blueberry, are becoming extremely popular among ordinary consumers. Furthermore, they have been used as predominant materials for functional foods. As mentioned previously, black rice, whose bran fraction contains abundant anthocyanins is being favored by an increasing number of consumers (Zhang et al., 2011).

3.1. Chemical composition % and total phenolic compounds (mg tannic acid/100g of dry weigh) of stabilized black rice bran
The results of chemical composition of stabilized black rice bran (S – BRB) was recorded in table (1). Data indicated that (S – BRB) is a good source of crude protein being 15.45%, and ether extract was 16.31%, while the amount of ash and crude fiber were 9.10 and 11.01% respectively. These results are in good agreement with those obtained by (El-Bana et al., 2015 and Patil et al., 2016 and Saleh et al., 2017). The results in the same table revealed that, total phenolic compounds of (S–BRB) contained (5.08 mg Tannic acid equivalent/g). These were nearly in the same content by (Arab et al., 2011 and El-Bana, 2012).
Table 1: Gross chemical composition (%) of stabilized black rice bran and total phenolic compounds (mg Tannic acid/g of dry weight basis).

<table>
<thead>
<tr>
<th>Components</th>
<th>Moisture</th>
<th>Protein</th>
<th>ether extract</th>
<th>Ash</th>
<th>crude Fiber</th>
<th>T.C *</th>
<th>Total phenolic compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>S - BRB</td>
<td>8.90</td>
<td>15.45</td>
<td>16.31</td>
<td>9.10</td>
<td>11.01</td>
<td>59.14</td>
<td>5.08</td>
</tr>
</tbody>
</table>

Each value is an average of three determinations.
*T.C: Total carbohydrates was calculated by difference.
S-BRB: Stabilized black rice bran.

3.2. Effect of feeding at different levels of stabilized black rice bran (S-BRB) for oil on hypercholesterolemic rats

3.2.1. Serum lipids parameter

Data in table (2) indicated that, total cholesterol content at the end of experimental period for the G1 (control -ve) was 121.22 mg/dl, while total cholesterol contents of G2 (control +ve) was 269.26 mg/dl. Data in the same table showed that, hypercholesterolemia rats which fed on hypercholesterolemia diet which replaced with (S-BRB) at the ratio 25,50,75 and 100% for oil showed significant decreases in serum levels of total cholesterol comparing with positive control group (G2). These results are supported by those of (Wilson et al., 2007; Zigoneanu et al., 2008; Sahar, R. Abd El-Hady 2013 and Kopec et al., 2020) they reported that, full fat rice bran is more effective in cholesterol lowering than either rice bran oil or defatted rice bran, certainly due to the presence of comparatively high levels of tocopherol, tocotrienol and oryzanol as well as unsaponifiables. In addition there are several cholesterol lowering mechanisms coupled with rice bran. It has been observed that rice bran lowers the cholesterol by increasing short chain fatty acid production in the cecum by hindering cholesterol absorption due to a change in intestinal fluid viscosity or by directly inhibiting cholesterol synthesis in the liver (Fukushima et al., 1999). Furthermore, Bran fiber offers a protective effect during cholesterol metabolism and reduces the circulating cholesterol levels (Gerhardt and Gallo, 1998).

Table 2: Effect of feeding at different levels of stabilized black rice bran for oil on serum lipids profile in hypercholesterolemia rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>T.C mg/dl</th>
<th>Triglyceride mg/dl</th>
<th>HDL- C mg/dl</th>
<th>LDL- C mg/dl</th>
<th>vLDL - C mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1(Control-ve)</td>
<td>121.22</td>
<td>116.75</td>
<td>63.95</td>
<td>33.92</td>
<td>23.35</td>
</tr>
<tr>
<td>G2(Control+ve)</td>
<td>269.26</td>
<td>222.93</td>
<td>44.87</td>
<td>179.80</td>
<td>44.59</td>
</tr>
<tr>
<td>G3(S-BRB 25%)</td>
<td>165.20</td>
<td>141.25</td>
<td>59.91</td>
<td>77.04</td>
<td>28.25</td>
</tr>
<tr>
<td>G4(S-BRB 50%)</td>
<td>151.51</td>
<td>137.60</td>
<td>62.33</td>
<td>61.68</td>
<td>27.50</td>
</tr>
<tr>
<td>G5(S-BRB 75%)</td>
<td>145.25</td>
<td>135.71</td>
<td>63.60</td>
<td>54.51</td>
<td>27.14</td>
</tr>
<tr>
<td>G6(S-BRB 100%)</td>
<td>142.66</td>
<td>132.30</td>
<td>65.22</td>
<td>50.98</td>
<td>26.46</td>
</tr>
</tbody>
</table>

Each value is an average of seven determinations.
Values followed by the same letter in column are not significantly different at P < 0.05.
S-BRB: stabilized black rice bran. T.C: Total Cholesterol
G1, G2 … etc. were as given in Table (A).

Results also illustrated that, total triglycerides (TG) for the G1 (control -ve) was 116.75 mg/dl after 12 weeks. Observed increased to 222.93 mg/dl in hyper-c rats which fed on hyper-c diet (G2) while, the total triglycerides contents for G3, G4, G5 and G6 fed on hyper-c diet replacement with (S-BRB) (25,50,75 and 100%) showed values of 141.25, 137.60, 135.71 and 132.30 mg/dl respectively. These results agreement with (Ha et al., 2005, Ronis et al., 2010 ;Wang et al., 2014; Saleh et al., 2017 and Kopec et al., 2020). Results in the same table showed that, supplementation of feeding hyper-c diet with (S-BRB) led to improvement the HDL-C. Furthermore, hyper-c diet with (S-BRB) replacement at 75% and 100% also recorded the best and nearest of HDLC to the negative control. These values were significantly different from that recorded in positive control. It was observed also that, LDL-C value of G1 (control -ve) was 33.92 mg/dl, but the value of the hypercholesterolemic G2 (control +ve) was 179.80 mg/dl. On the other hand, the LDL-C of rats fed on hyper-c diet substitution with (S-BRB) at the ratio of 25, 50, 75 and 100% (G3, G4, G5 and G6) being 77.04, 61.68, 54.51 and 50.98 mg/dl, respectively. Data of vLDL cholesterol for fed rats on basal, hyper-c diets summarized in table.
was observed that, vLDL cholesterol value of G1(control -ve) was 23.35 mg/dl, while the value of G2 (control +ve) was 44.59 mg/dl. On the other hand, the vLDL cholesterol rats fed on hyper-c diet substitution with (S-BRB) 25, 50, 75 and 100% (G3,G4,G5 and G6) produced 28.25, 27.50, 27.14 and 26.46 mg/dl , respectively.

It could be observed that, hyper-c rats fed on hyper-c diet substitution with (S-BRB) 25, 50, 75 and 100% relative to basal diets had a significant lower serum total cholesterol, total triglycerides, LDL cholesterol and VLDL cholesterol compared with hyper-c G2 (control +ve). In contrary, these groups had significantly high level HDL-C at (P<0.05). The obtained results are in agreement with Ronis et al. (2010) and Wang et al. (2014); Karthika and Devi (2016) and Nie et al. (2017).

3.2.2. Liver function activities

The effect of feeding on (S-BRB) for oil at the level of, alanine amino transferase (ALT), aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) enzymes in serum of hyper-c rats for 12 weeks recorded in table (3). At the final of experiment (12 weeks), the concentration value of ALT was significantly increased for the hyper-c control G2 (control +ve) .The concentration value of (ALT) enzyme in hyper-c rats (G2) was 53.76 U/L, while G1 (control -ve) was 29.38 U/L.

Data in the same table showed that, the feeding on hyper-c diets substitution with (S-BRB) (G3, G4,G5 and G6) led to a more reduction at level 25,50,75 and 100% were 36.36, 34.35, 33.25 and 31.15 U/L, respectively comparing with hyper-c control (G2). AST activity was significantly increased for hyper-c control G2. The liver AST activity of hyper-c rats was 76.75 U/L relative to G1 (control -ve) was 53.30 U/L . Data in the same table showed that, the rats fed on substitution of (S-BRB) for oil at 25,50,75 and100% were 59.01, 57.90, 54.87 and 53.25 U/L, respectively for (G3, G4, G5 and G6). On the other hand, (ALP) activity of the negative control (G1) was 93.50 U/L while, (ALP) activity of hyper-c diets positive control (G2) was 129.10 U/L. hyper-c rats fed on (S-BRB) which substitutes 25, 50, 75 and 100% for oil showed significant decreases comparing with hyper-c control (G2). The results were in a good agreement with those many authors (Ha et al., 2005; Houa el al. 2013; Saleh et al., 2014 and Saleh et al., 2017), reported that Feeding of the high cholesterol control diet increased the activities of AST, ALT, and ALP. This result may be due to an increase of lipid peroxidation in liver tissue associated with hypercholesterolemia. The reduction of AST by S-BRB indicates that black rice bran oil (BRBO), anthocyanin and dietary fibers including natural antioxidant can alleviate the liver damage induced by hypercholesterolemia.

Table 3: Effect of feeding at different levels of stabilized black rice bran for oil on Liver function activities in hypercholesterolemia rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT U/L</th>
<th>AST U/L</th>
<th>ALP U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (Control-ve)</td>
<td>29.38</td>
<td>53.30</td>
<td>93.50</td>
</tr>
<tr>
<td>G2 (Control+ve)</td>
<td>53.76</td>
<td>76.75</td>
<td>129.10</td>
</tr>
<tr>
<td>G3(S-BRB 25%)</td>
<td>36.36</td>
<td>59.01</td>
<td>113.10</td>
</tr>
<tr>
<td>G4(S-BRB 50%)</td>
<td>34.35</td>
<td>57.90</td>
<td>107.60</td>
</tr>
<tr>
<td>G5(S-BRB 75%)</td>
<td>33.25</td>
<td>54.87</td>
<td>101.00</td>
</tr>
<tr>
<td>G6 (S-BRB 100%)</td>
<td>31.15</td>
<td>53.25</td>
<td>97.55</td>
</tr>
</tbody>
</table>

Each value is an average of seven determinations.
Values followed by the same letter in column are not significantly different at P < 0.05.
G1, G2 … etc. were as given in Table (A).

3.2.3. Antioxidant enzymes

Results given in table (4) showed that, hyper-c rats had significantly lower levels of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) antioxidant enzymes activity in compared with (G1). The data in the aforementioned Table clearly show that substitution of (S-BRB) at level 25, 50, 75 and 100% in hyper-c diet improved the activity levels of (GPx), (SOD) and (CAT) antioxidant enzymes in compared with positive group (G2). Aforementioned results coincide with those obtained by Hsu et al. (2010); Houa el al. (2013) Wang et al. (2014) and Saleh, el al. (2017). They reported that, hyperlipidemia could destroy the antioxidant defense system; decrease the activity of SOD, GPx and CAT and elevate the lipid peroxide level.
G1, G2 … etc. were as given in Table (A).

Each value is an average of seven determinations.
Values followed by the same letter in column are not significantly different at P < 0.05.
G1, G2 … etc. were as given in Table (A).

3.2.4. Kidney functions

The results of urea, uric acid and creatinine in serum of negative control (G1) and hyper-c positive control (G2), at the end of experimental period after feeding for 12 weeks are shown in table (5). The obtained results illustrated that, urea at the end of experimental period for (G1) was 20.60 mg/dl. The same table presented that urea content of hyper-c positive control (G2) showed that value of 57.72 mg/dl in serum, while the hyper-c rats fed on (S-BRB) at 25, 50, 75 and 100 % (G3, G4, G5 and G6) gave 39.72, 37.45, 35.60 and 33.71 mg/dl respectively. The results showed that, the urea contents was decreased in rats fed on hyper-c diets substitution with (S-BRB) 25,50,75 and 100% compared to hyper-c control (G2). The obtained results (table 5) illustrated that, uric acid contents at the end of experimental period for negative control (G1) was 1.82 mg/dl . The same Table presented that, uric acid contents of hyper-c positive control (G2) showed a value 4.75 mg/dl. while the hyper-c diets of G3,G4,G5 and G6 substitution with (S-BRB) 25, 50, 75 and 100 % were 3.55, 2.95, 2.02 and 1.91 mg/dl, respectively. The results showed that creatinine contents at the end of experimental period for negative control (G1) was 0.88 mg/dl, while hyper-c diets G3, G4, G5 and G6 fed on (S-BRB) 25, 50, 75 and 100 % were 0.88, 0.80, 0.77 and 0.74 mg/dl, respectively. It could be seen from the data present in table (5) illustrated that hyper-c rats fed (S-BRB) 25,50,75 and 100% had significantly lower serum urea ,uric acid and creatinine compared with hyper-c group G2 (P≤0.05). Meanwhile, negative group G1 fed on basal diet had a significantly lower mean value for urea, uric acid and creatinine. These results were in a agreement with by Karthika and Devi (2016) and Nie et al. (2017).

4. Conclusions

Stabilized black rice bran (S-BRB) has significant practical value for protecting against the alterations caused by a hypercholesterolemic diet, and antioxidative ingredients which suppress lipid peroxidation play a key role.

References


