The Effect Of Garlic Aqueaus Extract And Garlic, Apple And Balsamic Vinegars To Improve The Health Of Ofhypercholesterolemic Obese Rats.

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Abstract

Obesity has become a major worldwide health problem. This study was conducted to investigate the effect of garlic aqueous extract and garlic, apple and balsamic vinegars to improve the health of rats infected with obesity and hypercholesterolemia.

Rats were divided into two main group, the first group negative control group (5 rats) fed on basal diet for all experimental period as control negative group. And obese hypercholesterolemic rats (245–250g) were divided into 4 subgroups (5 per each): subgroup 1 served as positive control group, subgroup 2, 3, 4 and 5 were fed basal diet and given orally one receives a daily gavage of garlic aqueous extract, garlic vinegar, apple vinegar, balsamic vinegar (7mL/kg/d) for 30 days. After the end of experiment period, blood samples were collected for used to the biochemical analysis as kidney functions, lipids profile and antioxidant status. The results indicated that treatments with garlic vinegar, garlic aqueous extract, apple vinegar, balsamic vinegar significantly (p≤0.05) improved lipid profile and kidney and liver functions compared to positive control group. In conclusion: Garlic aqueous extract and garlic, apple, balsamic vinegars had improved lipid profile and atherogenic indexes, kidney and liver functions and antioxidant status in hypercholesterolemic obese rats.
Key words: Antioxidant statues, garlic aqueaus extract, hypercholesterolemicobeserats

1. Introduction:

Obesity is most commonly caused by a combination of excessive food intake, lack of physical activity, and genetic susceptibility. A few cases are caused primarily by genes, endocrine disorders, medications, or mental illness. Evidence to support the view that obese people eat little yet gain weight due to a slow metabolism is not generally supported (Kushner et al., 2007). Obesity is a significant risk factor for and contributor to increased morbidity and mortality, most importantly from cardiovascular disease (CVD) and diabetes, but also from cancer and chronic diseases, including osteoarthritis, liver and kidney disease, sleep apnea, and depression (Sunyer 2009). It increases cardiovascular risk through risk factors such as increased fasting plasma triglycerides, high LDL cholesterol, low HDL cholesterol, elevated blood glucose and insulin levels and high blood pressure (Klop et al., 2013). Hypercholesterolemia, also called dyslipidemia, is the presence of high levels of cholesterol in the blood. It is a form of high blood lipids and "hyperlipoproteinemia" (elevated levels of lipoproteins in the blood) (Durrington, 2003). Vinegar is a liquid consisting of about 5–20% acetic acid (CH3COOH), water, and other trace chemicals, which may include flavorings. The acetic acid is produced by the fermentation of ethanol by acetic acid bacteria. Vinegar is now mainly used as a cooking ingredient, or in pickling. As the most easily manufactured mild acid, it has historically had a great variety of practiced today (AL-Hazzaa, 2004). Vinegar has a positive effect on biomarkers for diabetes, cancer, and heart diseases (Ali et al., 2018). Garlic, a perennial herb, plays an important medicinal and dietary role throughout the history. Garlic is used in numerous forms such as extracted oil, powdered garlic tablets, or raw garlic (Negar et al. 2016). Ancient Indian and Chinese medicine recommend that garlic can be used to help respiration and digestion, and to treat leprosy and parasitic diseases (Bayan et al., 2014). Garlic has attracted particular attention of modern medicine because of its widespread health usage around the world. To date, many favorable experimental and clinical effects of garlic such as hypoglycemic, hypolipidemic, ant atherosclerotic, anticoagulant, antihypertensive, anticancer, antioxidant, hepatoprotective and immune modulation properties have been reported (Keithet et al., 2006). Despite the positive evidence from few studies on the beneficial effect of garlic aqueous extract on hypolipidemic and diabetic properties, the effect on obesity, which is closely associated with diabetes and cardiovascular
disease, has not yet been elucidated. Therefore, the present study was performed to examine the therapeutic effects of garlic aqueous extract and vinegar, apple and balsamic vinegars to improve the health hypercholesterolemic rats.

2. Materials and Methods

Materials:
Garlic (Allium sativum L.) and Garlic, Grape, Apple, and Balsamic vinegars were purchased from Agriculture Research Center, Giza, Egypt. Casein, cellulose, vitamin mixture, mineral mixture, corn oil, and corn starch were obtained from Morgahn Co., Menoufia, Egypt. Cholesterol was obtained from Winlab (UK), cholic acid was obtained from Biomark (India) and methyl thiouracil was purchased from Sigma– Aldrich (USA). Any other chemical used was of the highest analytical grade. Kits for estimating biochemical analysis were purchased from Alkan Medical company, St. El-Doky, Cairo, Egypt.

Methods:

Preparation of samples:
Garlic aqueous extract: The peeled garlic bulbs were weighed (100 g) and ground thoroughly to obtain fine garlic juice. It was homogenized in 100 ml of 0.9% cold and sterile. The homogenized mixture was filtered through muslin cloth about three times. The resultant aqueous extract of garlic was stored at 20°C until use.

Infection with obesity and hypercholesterolemia:
Rats fed diet containing (fat is 40% of total calories) for four weeks to achieve obesity according to Kang et al. (2005). Hypercholesterolemia was induced in obese rat by feeding high cholesterol diet [4% cholesterol (w/w) and 1% cholic acid (w/w)] for 8-weeks (Kamesh and Sumathi, 2012).

Experimental design
The rats were housed in wire cage under controlled condition. The diet was introduced to rats in special food container to avoid scattering of food and contamination. Tap water were provided to rats by mean of glass tubes projecting through wire cages from inverted bottles supported to one side of the cage. Rats fed standard diet for 7 days for adaptation. The standard diet was formulated according to AIN-93 guidelines (Reeves et al., 1993). Rats were divided into two main group, the first group negative control group (5 rats) fed on basal diet for all experimental period as control negative group. And obese hypercholesterolemic rats (245–250 g) were divided into 4
subgroups (5 per each): subgroup 1 served as positive control group, subgroup 2, 3, 4 and 5 were fed basal diet and given orally one receives a daily gavage of apple cider vinegar (7mL/kg/d) for 30 days of garlic aqueous extract, garlic vinegar, apple vinegar, balsamic vinegar according to Bouderbala et al (2016). After completing the treatment period, animals were sacrificed under diethyl ether anesthesia. Blood samples were collected from the hepatic portal vein, for used to the biochemical assays.

**Biochemical analysis:**
Total cholesterol (TC), Triglyceride (TG), and High-density lipoprotein cholesterol (HDL-c), were determined according to Adaramoye and Akanni (2014). Total lipids determined according to NIHP (1987). Respectively. Low density lipoprotein (LDL), very low density Lipoprotein (VLDL), atherogenic index of Plasma (AIP), cardiac risk ratio (CRR), atherogenic coefficient (AC) were calculated according to Bleichet al. (2008) respectively as the flowing equations:

- LDL-c (mg / dl) = total cholesterol – (HDL-c + VLDL-c).
- VLDL-c (mg / dl) = triglycerides / 5.

The Atherogenic ratios were calculated according to Bhardwaj et al. (2013) as follows:
- Atherogenic Index (AI) = log TG/HDLc
- Cardiac risk ratio (CRR) = TC/HDLc
- Castelli’s Risk Index (CRI) = LDLc/HDLc
- Atherogenic Coefficient (AC) = (TC – HDLc)/HDLc
- Atherogenic fraction (AF) was calculated as the difference between TC and HDL-C according to Aguilar et al. (2011).

Uric acid was determined according to Hill et al. (2003). Creatinine was determined according to AL-Hazzaa (2004). Urea was determined according to Puglisi and Gullo, (2002). SGOT and SGPT was determined according to Piva, et al. (2008). ALP was determined according to Maokiet al. (2010). Superoxide dismutase (SOD) enzyme was determined according to Nakayama (1959). Malondialdehyde (MDA) was estimated according to Ostman et al. (2005).

**Statistical analysis:**
Results were expressed as the mean ± SD. Data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). For the comparison of significance between groups, Duncan’s test was used as a post hoc test according to the statistical package program (Lisa and Paolo, 2009).

3. Results and Discussion:
The effect of garlic aqueus extract, garlic vinegar, apple viniger and Balsamic vinegar on body weight of negative and hypercholestreolemic obese group showed in Table (1). No significant (p > 0.05) difference was
found in initial weight between negative control group and obese hypercholesterolemic groups. A significant reduced in final weight were observed in obese hypercholesterolemic groups which treated compared to untreated group (positive control group). Rats which treated with garlic vinegar had lower significantly (p≤0.05) final weight than other groups, followed by apple vinegar, followed by balsamic vinegar and garlic extract, their values still higher than negative control group. This may be due to the short of the experimental period. These findings are supported by Kondo et al., (2009) who reported that the vinegar administration has favorable effects on body weight regulation. Wei et al. (2005) reported that fruit vinegars can significantly reduce the body weight, lipid content, and total cholesterol and triglyceride contents of animals. Also, Liu and Yang (2015) suggest that the long-term intake of grain vinegars may help weight loss in obese people.

Table (1): Effect of garlic aqueous extract and garlic, apple and balsamic vinegars on weights of normal and hypercholesterolemic obese groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Negative Control group</th>
<th>obese hypercholesterolemic groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial weight (g)</td>
<td>Positive control</td>
</tr>
<tr>
<td>Initial</td>
<td>155.30±2.3</td>
<td>153.80±2.7</td>
</tr>
<tr>
<td>Final</td>
<td>167.60±5.46</td>
<td>259.60±8.47</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. Values within a row having different superscripts are significantly different (p ≤ 0.05).

The results of Table (2) show the effect of garlic aqueous extract and garlic, apple and balsamic vinegars on weights of normal and hypercholesterolemic obese groups. The results revealed that there were significant increase (p≤0.05) in serum levels of total lipid (T.L), total cholesterol, (T.C) triglycerides (T.G), LDL and VLDL and decrease in HDL in hypercholesterolemic obese group (positive control) compared to negative control group and other treated group. Cholesterol-cholic acid feeding has often been used to raise cholesterol levels in plasma and tissues of experimental animals (Chen et al., 2004). It has been shown by other investigators that an increase in dietary cholesterol intake in animals led to hypercholesterolemia (Kishida et al., 2002). In the same table, a significant (p≤0.05) reduction were observed in hypercholesterolemic obese groups which treated with garlic extract and vinegar, apple and balsamic
vinegar than positive control group in TL, TG, TC, LDL and VLDL, while HDL-c had an opposite trend. Treatments with balsamic vinegar group was more effective (P≤0.05) in reducing total lipids, cholesterol, triglyceride, VLDL-c and LDL-c levels than other treated groups. Similar results were obtained by Maoki et al. (2010) who suggest that the concentrations of intracellular triglycerides and total cholesterol were reduced in the presence of balsamic vinegar. Followed by apple vinegar which were (328.58, 128.49, 137.7, 24.8 and 27.5) respectively. These results were agreed with Ostman et al. (2005) who indicated that consumption of apple vinegar can reduce the LDL, triglyceride, and cholesterol levels in patients with hyperlipidemia. Moreover, apple cider vinegar can be used to prevent and even treat the complication and probably other heart problems. Followed by garlic vinegar and garlic aqueous extract. These results also agreed with Wolfe et al. (2003) who showed that garlic reduced blood lipid profiles in high-fat induced obesity mice, garlic reduced hyperlipidemia and hypercholesterolemia in garlic supplemented groups. Soltan and Shehata (2012) reported that fruit vinegars can significantly reduce the concentration of total cholesterol, triglycerides, and low-density lipoprotein (LDL) cholesterol and increase the concentration of high-density lipoprotein (HDL) cholesterol. A significant increase (P≤0.05) in atherogenic index (AI), cardiac risk ratio (CRR%), castelli’s risk index (CRI), and atherogenic coefficient (AC) were observed in hypercholesterolemic obese rats compared to normal rats and other treated groups. These results could be attributed high serum levels of TC, TG, LDL-c and VLDL-c as well as lower level of HDL-c which was observed in hypercholesterolemic obese rats. The present results were in accordance with those of Adaramoye and Akanni (2014) who showed that hypercholesterolemic rats had high serum, hepatic and cardiac TC, TG and LDL-c. Atherogenic indices are powerful indicators of the risk of cardiovascular diseases and the higher the risk for developing cardiovascular diseases reported by Chigozie and Chidinma (2013). On the other hand, atherogenic index (AI), cardiac risk ratio (CRR%), castelli’s risk index (CRI), and atherogenic coefficient (AC) were significantly reduced (P≤0.05) in hypercholesterolemic obese rats which treated with balsamic vinegar, followed by apple vinegar, followed by garlic aqueous extract group, and garlic vinegar compared with untreated hypercholesterolemia obese rats (positive control group) but their level still higher (p≤0.05) than the level of the negative control rats. This may be due to the short of the experimental period. These results could be attributed low serum levels of
TC, TG, LDL-c and VLDL-c as well as higher level of HDL-c which was observed in hypercholesterolemic obese rats which treated with vinegars and garlic aqueous extract. Similar trend was observed for Liu and Yang (2015), the regulation of lipid metabolism by vinegars was also observed in obese mice.

Table (2): Effect of garlic aqueous extract and garlic, apple and balsamic vinegars on lipid profile and atherogenic indices of normal and hypercholesterolemic obese groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Negative Control group</th>
<th>Obese hypercholesterolemic groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive control</td>
<td>Garlic extract</td>
</tr>
<tr>
<td>T. L (mg/dl)</td>
<td>207.95±2.4</td>
<td>637.69±4.9</td>
</tr>
<tr>
<td>T.C (mg/dl)</td>
<td>99.04±0.44</td>
<td>257.01±4.4</td>
</tr>
<tr>
<td>T.G (mg/dl)</td>
<td>71.8±1.7</td>
<td>261.93±5.5</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>79.31±0.77</td>
<td>50.27±1.3</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>5.37±0.95</td>
<td>154.35±3.7</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>14.36±0.34</td>
<td>52.38±1.0</td>
</tr>
<tr>
<td>AI (mg/dl)</td>
<td>0.248±0.0122</td>
<td>4.113±0.08</td>
</tr>
<tr>
<td>CRR (mg/dl)</td>
<td>1.24±0.012</td>
<td>5.11±0.08</td>
</tr>
<tr>
<td>CRI (mg/dl)</td>
<td>0.067±0.010</td>
<td>3.07±0.05</td>
</tr>
<tr>
<td>AC (mg/dl)</td>
<td>0.248±0.0122</td>
<td>4.11±0.08</td>
</tr>
</tbody>
</table>

Data recorded in Table (3) showed effect of garlic aqueous extract and garlic, apple and balsamic vinegars on kidney and liver function in normal and hypercholesterolemic obese groups. The results indicated that there were significant elevated (p≤0.05) in urea, creatinine and uric acid (U.A) in positive control group compared to negative control and other treated groups. This in agreement with Li et al. (2014) who demonstrated that obesity is an independent risk factor associated with increasing serum creatinine levels in children aged more than 10 years and weight control is important in the protection of renal function. In the same table, rats which received with balsamic vinegar group was more effective (P≤0.05) in reducing the level of urea, creatinine, followed by apple vinegars, followed
by garlic extract and garlic vinegar. Furthermore, the apple vinegar group is the lower in U.A value than other treated groups.

Obese hypercholesterolemic rats showed fat deposition in the liver and eventually developed and hepatic damage. Hepatic damage was assessed by measuring the activities of enzyme markers of liver function. Serum ALT, AST and ALP activities are increased when hepatic damage occurs, and we measured the activities of these enzymes in control and obese hypercholesterolemic rats. In the same table the obtained result indicated that T.B, ALT, ALP, AST and GGT in positive control group had higher significantly (p≤0.05) than negative control and other treated groups. While, albumin had an opposite trend. These results were agreed with Durrington, (2003) who reported that serum enzymes including AST and ALT are used in the evaluation of hepatic disorders. An increase in these enzyme activities reflects active liver damage. On the other hand, this study showed significant reduction (P≤0.05) of AST, ALT, GGT, ALP and TB in hypercholesterolemic obese rats after treated them with balsamic vinegar than other treated groups Durrington, (2003) indicated that the garlic alcoholic extract significantly decreased AST and ALT.

Table (3): Effect of of garlic aqueous extract and garlic, apple and balsamic vinegars on kidney and liver functions in normal and hypercholesterolemic obese groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Negative control</th>
<th>hypercholesterolemic obese groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive control</td>
</tr>
<tr>
<td>Urea(mg/dl)</td>
<td>27.09±0.95</td>
<td>41.93±1.05</td>
</tr>
<tr>
<td>Creatinin(mg/dl)</td>
<td>0.59±0.08</td>
<td>1.79±0.04</td>
</tr>
<tr>
<td>UA(mg/dl)</td>
<td>0.75±0.02</td>
<td>2.88±0.12</td>
</tr>
<tr>
<td>GGT(U/L)</td>
<td>37.16±4.79</td>
<td>96.36±3.04</td>
</tr>
<tr>
<td>T.B(U/L)</td>
<td>3.40±0.12</td>
<td>8.67±0.96</td>
</tr>
<tr>
<td>Albumin(U/L)</td>
<td>8.99±0.75</td>
<td>1.89±0.12</td>
</tr>
<tr>
<td>ALT(U/L)</td>
<td>57.20±1.49</td>
<td>201.26±5.41</td>
</tr>
<tr>
<td>ALP(U/L)</td>
<td>109.0±6.55</td>
<td>270.00±3.61</td>
</tr>
<tr>
<td>AST(U/L)</td>
<td>134.66±4.1</td>
<td>258.41±3.65</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. Values within a row having different superscripts are significantly different (p ≤ 0.05).
Effect of of garlic aqueous extract and garlic, apple and balsamic vinegar on antioxidant status in normal and hypercholesterolemic obese rats are presented in Table (4). Superoxide dismutase (SOD), catalase and reduced glutathione (GSH) are naturally produced cellular antioxidants that are responsible reducing oxidative stress. These cellular antioxidant activities were compromised due to the increase in oxidative stress in obese rats. A significantly (P≤ 0.05) decrease in the levels of reduced glutathione (GSH.Rd), superoxidase dismutase (SOD), total antioxidant capacity (TAC) and catalase (CAT) and a significant increase (P≤ 0.05) in the levels of malondialdehyde (MDA) was observed in positive control when compared to negative control group and hypercholesterolemic obese treated groups. Treating obese hypercholesterolemic rats with balsamic vinegar was more (p≤0.05) effective in increasing the levels of GSH-RD, SOD, TAC and CAT and reducing MDA level as compared with positive control rats. This effect is attributed to its content of phenolic compounds, polyphenols. Similar trend was observed for Liu et al. (2019) who reported that fruit vinegars are rich in polyphenols and organic acids and can be a good dietary source of antioxidants. Followed by garlic vinegar and apple vinegar and finally garlic aqueous extract. These findings are supported by Abdulrauf et al. (2018) who reported that apple cider vinegar treatment decreased lipid peroxidation (MDA) and serum catalase activity. Fruit vinegars are popular condiments worldwide. Antioxidants and organic acids are two important components of the flavors and health benefits of fruit vinegars. Liu et al. (2019).
Table(4): Effect of garlic aqueous extract and vinegar, apple and balsamic Vinegars on antioxidant status in normal and hypercholesterolemic obese groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Negative control group</th>
<th>Obese hypercholesterolemic groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive control</td>
</tr>
<tr>
<td>SOD(mg/dl)</td>
<td>67.03±2.69</td>
<td>17.12±8.2</td>
</tr>
<tr>
<td>MDA(nmol/ml)</td>
<td>38.40±2.16</td>
<td>97.00±1.00</td>
</tr>
<tr>
<td>GSH.Rd(u/ml)</td>
<td>43.07±2.68</td>
<td>10.90±1.65</td>
</tr>
<tr>
<td>TAC(mg/dl)</td>
<td>8.23±0.41</td>
<td>2.47±0.15</td>
</tr>
<tr>
<td>CAT(u/ml)</td>
<td>162.0±7.54</td>
<td>45.00±6.25</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. Values within a row having different superscripts are significantly different (p ≤ 0.05); (SOD): Superoxide dismutase, (MDA): Malondialdeyde, (GSH): Glutathion, (TAC): Triacetyl cellulose, (CAT): Catalase.

4. References:
Aguilar, E.C.; Queiroz, M.G.M.N. and Oliveira, D.A. (2011): Serum lipid profile and hepatic evaluation in mice fed diet containing...


تأثير المستخلص المائي للثوم و خل الثوم والتفاح و البلسمك على تحسين حالة الصحة للفنان

عنوان البحث:
عبير أحمد خضر، شيماء المسيلمي، زينب الرفاعي عبد الناصر

قسم التغذية وعلوم الأطعمة، كلية الاقتصاد المنزلي - جامعة المنوفية - مصر

الملخص العربي:

أصبحت السمنة مشكلة صحية عالمية كبيرة، أجرت هذه الدراسة لتعريف تأثير مستخلص الثوم و خل الثوم و خل التفاح و البلسمك على صحة الفناء و ارتفاع الكوليسترول بالدم، تم تقسيم الفناء إلى مجموعتين، مجموعة 1: المجموعة الأولى، المجموعة الثانية (ن = 25) التي تم اتباعها بالسمنة و ارتفاع كوليسترول الدم و التي تتم تقسيمها إلى 4 مجموعات فرعية (5 فناء لكل مجموعة) ثم المجموعة الفرعية 1 كمجموعة ضابطة موجبة، المجموعة الفرعية 2 و 3 و 4 و 5 و تم تغذيةهم على الوجبة الأساسية و أعطى لهم من طرق الوجبة يومية بمقدار (7 مل/ كجم/ د) لمدة 30 يومًا، و كنستخلصات الثوم، خل الثوم، خل التفاح، البلسم، في نهاية التجربة، تم جمع عينات الدم لاستخدامها في التحليل الكيميائي كوظائف الكلى، صوره دهون الدم، و الحالة التناكشية، أشارت النتائج إلى أن المدعم بخل الثوم، و خل التفاح، و خل البلسم، قد حسنت معنويً من مستوى الدهون، و مؤشرات تصلب الشرايين، مقارنة بالجميع، و حصة والتغذية وتحسين وظائف الكلى و الكبد، الخلاصة: المستخلص المائي للثوم و خل الثوم و خل التفاح، و خل البلسم، قد حسنما عملية التمثيل الغذائي للدهون، و معاملات الجلطة، و وظائف الكلى و الكبد، ومنع حدوث تصلب الشرايين في الفناء، و ارتفاع الكوليسترول.

الكلمات المفتاحية: حالة التناكشية - مستخلص الثوم - ارتفاع كوليسترول الدم