https://doi.org/10.26682/ajuod.2019.22.2.18

Journal of University of Duhok., Vol. 22, No.2 (Agri. and Vet. Sciences), Pp 189-195, 2019

EFFECT OF GARLIC OIL ON SOME BIOCHEMICAL PARAMETERS IN RATS EXPOSED TO HYDROGEN PEROXIDE

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(Received: September 18, 2019; Accepted for Publication: November 28, 2019)

ABSTRACT

Introduction: Medicinal plants have therapeutic impacts in both modern and traditional medicines. Therefore, some biochemical parameters were investigated in rats fed with garlic oil daily for three weeks.

Methods: A total of 20 healthy adult Wister albino male rats aged eight weeks and older were allocated into four equal groups as follow. Group I or control treated with normal diet and water. Group II/ administrated by 1% hydrogen peroxide (H_2O_2) with drinking water in a dark bottle prepared daily with normal diet. Group III administrated with 5 ml garlic oil added to 25 grams of their ratio for each rat (25oil/125g) also prepared daily with normal drinking water. Group IV also administrated with 5 ml of garlic oil added to 25 gram of their ratio with drinking water that contained1% hydrogen peroxide in a dark bottle.

Results: The subjects in the garlic group had significantly lower total cholesterol (54.30 vs. 68.90 mg/dl); VLDL (12.64 vs. 14.62 mg/dl); LDL (32.27 vs. 46.75 mg/dl); and higher HDL (9.39 vs. 7.53 mg/dl)) compared to the control group. The difference in liver and kidney parameters between the control and H2O2 + garlic was not significant. Similarly, no significant difference was found between the garlic and control group in GPT (20.23 vs. 23.38); urea (40.80 vs. 37.10); uric acid (0.53 vs. 0.59); and creatinine (0.57 vs. 0.78); except for GOT (15.80 vs. 30.04).

Conclusions: The present study showed the garlic extract is effective to reduce total cholesterol, LDL, VLDL, GOT and increase HDL levels.

KEYWORDS: lipid profile, liver parameters, garlic, antioxidant
https://doi.org/10.26682/ajuod.2019.22.2.18

INTRODUCTION

Medicinal plants have valuable therapeutic impacts in both modern and traditional medicines. Garlic (Allium sativum) is being used as food, as well as it has been used as a medicinal plant for more than 4000 years for a wide range of purposes (Block, 1985). The garlic is being used in headache, treating bites, and in intestinal worms and tumors (Block, 1985; Amagase et al., 2001; Rahman et al., 2006). Garlic and its supplements are used as food in several cultures for their hypo-lipidemic, antiplatelet, and beneficial circulatory advantages. Also, it appears that garlic has the role of hepatoprotective, immune-enhancing, anticancer, chemopreventive, and antioxidant activities (Amagase et al., 2001; S. K. Banerjee et al., 2002).

While, the investigations conducted on garlic powder and garlic oil have failed to confirm its role as lipid-lowering or hypoglycemia effects (Kwon et al., 2003; Amagase, 2006). The findings from a meta-analysis showed that garlic is effective in decreasing total serum cholesterol by 17±6 mg/dL and low-density lipoprotein cholesterol by 9±6 mg/dL in subjects with elevated total cholesterol levels (>200 mg/dL) in the case of using garlic for 2 months (Ried et al., 2013).

Lee et al. (2009) examined the antioxidant effect of garlic in an animal model of type 2

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diabetes mellitus. In this regard, they measured the antioxidant activity of garlic and aged black garlic as the activity in scavenging free radicals. The three-week-old mice were fed AIN-93G diet or diet containing 5% freeze-dried garlic or aged black garlic for seven weeks. The antioxidant activity of garlic and aged black garlic were 13.3 ± 0.5 and 59.2 ± 0.8 μmol/g wet weight, respectively. Moreover, they found that aged black garlic consumption reduced the hepatic thiobarbituric acid reactive substances (TBARS) level in a significant way compared to the garlic group. Other studies have reported similar findings on the effect of garlic on the antioxidant level. For example, S. Banerjee et al. (2003) reviewed the current evidence on the effect of garlic on the antioxidant level and showed that garlic has a significant effect on the antioxidative effect.

Eidi et al. (2006) investigated the antidiabetic effect of garlic ethanolic extract in normal and streptozotocin-induced diabetic rats. They fed the rats with oral administration of garlic extract (0.1, 0.25 and 0.5 g/kg body wt.) for two weeks. They found that oral administration of the garlic extract substantially reduced the serum glucose, total cholesterol, triglycerides, uric acid, creatinine, AST (GOT) and ALT (GPT) and urea levels. Importantly, the study found that the antidiabetic effect of the extract was more effective than that observed withglibenclamide. However, still, there are not sufficient clinical trials on the impact of garlic extraction on the antioxidative impact on animals to make a conclusive decision.

This study aimed to examine the effectiveness of garlic extraction on some biochemical parameters in rats fed with garlic oil daily for three weeks.

MATERIALS AND METHODS

In this experimental study, a total of 20 healthy male adult rats were assigned into three experimental groups along with its control group. The lipid profile parameters, including triglyceride (TG), high density lipoprotein (HDL), and total cholesterol (T-CH) along with some liver and kidney function indicators including GOT, GPT, uric acid, urea, alkaline phosphatase. Creatinine was measured two times, once after 30 seconds and another time after 2 minutes in three groups. These parameters were compared with those in the control group.

The study was conducted at the Department of Physiology, College of Veterinary Medicine in Duhok city from period March 2018 to May 2018.

EXPERIMENTAL GROUPS

A total of 20 healthy adult Wistar albino male rats aged more than 8 weeks weighted 250 ± 10 gm were allocated into four equal groups as follow. Group I or control treated with normal diet and water. Group II/ administrated by 1% hydrogen peroxide (H2O2) with drinking water in a dark bottle prepared daily. Group III treated with 5 ml of garlic oil. The garlic oil was added to 25 grams of this ratio for each rat along with normal drinking water. Finally, group IV was administered with 5 ml of garlic oil that was added to 25 grams of their ratio together with drinking water along with 1% hydrogen peroxide in a dark bottle.

The rats were in the light/dark cycle for one week prior to study commencement. The healthy rats were purchased from the College of Science, University of Duhok.

GARLIC EXTRACTION TECHNIQUE

To prepare garlic extract, the fresh garlic bulbs were obtained from retail local food store. The garlic bulbs cut by a sharp knife into small pieces then were weighed and blended by an electrical grinder ,one litter of ethanol 70% was added to each 1000 g of garlic extract, it was kept at room temperature for 48 h and then sieved by gauze. The alcoholic garlic mixture was collected in a flask and concentrated in die rotary flash evaporator Sushama et al.,(2012) The amount of garlic oil about 3-10 ml of were prepared for feeding the rats

BLOOD COLLECTION AND MEASUREMENT

Blood samples (5 ml) were taken from the subjects through the retro-orbital venous plexus and were entered to non-heparinized tubes after 21 days of the study the blood samples were collected to measure the biochemical parameters following fasting 12-16 hours. The serum

samples were centrifuged at 3500 rpm using cooling centrifuge at 4c° for 10 minutes. Accordingly, the separated sera were stored at -28C° to estimate biochemical parameters. To assess liver, kidney, and lipid profile levels in subjects, the specific enzymatic kit was used using spectrophotometer according to the Biorex diagnostics. The lipid profile parameters included cholesterol (T-CH mg/dL), high-density lipoprotein (HDL mg/dL), and triglyceride (TG mg/dL) were measured, using Biolab Kit (Germany). The liver and kidney functions which were measured included GOT, GPT, urea, uric acid (UA), and creatinine following 30 minutes and 2 minutes.

STATISTICAL METHODS

The normality of the lipid profiles and kidney parameters among study groups was tested using drawing a histogram. The descriptive purposes of the study were presented in mean and standard deviation. The lipid profile parameters, liver, and kidney functions were compared between control and H_2O_2, garlic, and H_2O_2+ garlicgroups was examined in independent t-test. The P-value of less than 0.05 was considered a significant difference. The statistical calculations were performed by Statistical Package for Social Sciences 24 (SPSS 24; IBM; USA).

RESULTS

The comparison of lipid profile parameters between the subjects in the control and other groups was presented in Table 1. The study showed that the subjects in H2O2 group had significantly ($P<0.01$) worse level of lipid profile compared to the subjects in the control group; including TG (146.79 vs. 73.09 mg/dl); total cholesterol (123.60 vs. 68.90 mg/dl); HDL (5.79 vs. 7.53 mg/dl); VLDL (29.36 vs. 14.62 mg/dl); and LDL (88.45 vs. 46.75 mg/dl). However, no significant difference was seen in the lipid parameters between the control and H2O2+garlic group (Table 1).

The subjects in the garlic group had significantly lower concentration total cholesterol (54.30 vs. 68.90 mg/dl); VLDL (12.64 vs. 14.62 mg/dl); and LDL (32.27 vs. 46.75 mg/dl); and higher concentration of HDL (9.39 vs. 7.53 mg/dl) compared to control group, respectively. But, there was no statistically significant difference in the TG (63.21 vs. 73.09; $P>0.05$),respectively as shown in Table 1.

Table (1): Comparison of lipid profile parameters between control and H_2O_2, garlic, and H_2O_2+garlicgroups

	Lipid Profile				
Study Groups	**Triglyceride (mg/dL)**	**Cholesterol (mg/dL)**	**HDL (mg/dL)**	**VLDL (mg/dL)**	**LDL (mg/dL)**
Control	73.09 ± 12.74	68.90 ± 6.80	7.53 ± 0.39	14.62 ± 2.55	46.75 ± 6.28
H_2O_2	146.79 ± 5.81*	123.60 ± 17.34*	5.79 ± 0.48*	29.36 ± 1.16*	88.45 ± 18.16*
Garlic	63.21 ± 2.28	54.30 ± 3.37*	9.39 ± 0.54*	12.64 ± 0.46	32.27 ± 3.74*
H_2O_2+ garlic	73.93 ± 2.64	67.80 ±10.25	18.69±14.70	14.79 ± 0.53	34.32±23.67

Independent t-test was performed for all statistical analysis.
Point: *Significant at 0.01 level. The non-significant difference between control and other groups was not presented by an asterisk.

The study showed that the subjects included in the H2O2 group had significantly higher concentrations of GOT (76.64 vs. 30.04); GPT (64.94 vs. 23.38); Urea (59.68 vs. 37.10); uric acid (0.92 vs. 0.59); but no difference in creatinine (1.26 vs. 0.78) compared to the subjects in control group (table 2)

Whereas, the difference in liver and kidney parameters between the control and H2O2 + garlic was not significant. Similarly, no significant difference was found between the garlic and control group in GPT (20.23 vs. 23.38); urea (40.80 vs. 37.10); uric acid (0.53 vs. 0.59); and creatinine (0.57 vs. 0.78); except for GOT (15.80 vs. 30.04), as shown in Table 2.

Table (2): Comparison of kidney and liver function parameters between control and H_2O_2, garlic, and H_2O_2+garlic study groups

	Liver and Kidney Parameters				
Study Groups	**GOT (U/L)**	**GPT (U/L)**	**Urea (mg/dl)**	**Uric Acid (mg/dl)**	**Creatinine (mg/dl)**
Control	30.04 ± 3.46	23.38 ±11.09	37.10 ± 8.39	0.59 ± 0.05	0.78 ± 0.26
H_2O_2	76.64 ± 9.19*	64.94 ±10.53*	59.68 ±1.30*	0.92 ± 0.07*	1.26 ± 0.49
Garlic	15.80 ± 1.44*	20.23 ± 6.98	40.80 ± 9.84	0.53 ± 0.01	0.57 ± 0.15
H_2O_2+ garlic	29.60 ± 5.30	30.73 ± 6.29	34.10 ± 4.06	0.58 ± 0.03	0.68 ± 0.34

Independent t-test was performed for all statistical analysis.
Point: *Significant at 0.01 level.The non-significant difference between control and other groups was not presented by an asterisk.

DISCUSSION

The present interventional investigation showed that the subjects in the garlic group had significantly lower concentration of total cholesterol, LDL, VLDL, and higher concentration of HDL compared to the subjects included in the control group.

Also, it was shown that the garlic extraction is effective to reduce the level of GOT compared to the control. However, it has an antioxidant effect on the subjects underwent H2O2.

The effect of garlic extraction on lipid profile and biochemical parameters have been investigated by other studies as well. For example, Ha et al. (2015)included thirty-two male Sprague-Dawley rats aged four weeks into four groups in a random way. The rats were fed for five weeks. The groups were fed with normal food diet; a high-fat diet; a high-fat diet along with 0.5% black garlic extract; and a high-fat diet along with 1.5% black garlic extract (HFBG1.5). The authors measured body weight, biochemical parameters, and expression of genes associated with lipid metabolism. The study showed that the subjects who were included in the HFBG1.5 had significantly lower concentrations of blood biochemical parameters compared to those included in the high-fat group. In addition, the significant improvements were observed in the plasma lipid profiles as well as fecal excretions of total lipid and triglyceride in the HFBG1.5 group compared to the high-fat diet group. The HFBG1.5 group had significantly lower body weight and weight gain compared to other groups.

The levels of AST in the high-fat group were substantially elevated in contrast with a lower level in the HFBG1.5 group. Similarly, the subjects in both HFBG0.5 and HFBG1.5 groups had significantly lower levels of ALT (Ha et al., 2015). The similar results were reported by other studies as well (Yeh et al., 2001; Jang et al., 2008).

The studies conducted on the effect of garlic powder or oil were not successful to show any lipid-lowering or hypoglycemia effects (Kwon et al., 2003; Amagase, 2006).

The lipid-lowering role of garlic extract is so important, as the excessive fat intake changes the lipid metabolism and expose the persons to the various diseases; such as fatty liver disease, hypertension, hyperlipidemia, and arteriosclerosis (Larsson, 2013). The effects of garlic extract on lipid profile parameters have been supported by other studies as well (Lin et al., 2002; Jung et al., 2011). The amount of the garlic supplements were given to the animals were different among investigations; including 3-5% fresh garlic or black garlic extract (Seo et al., 2009; Jung et al., 2011), while 3-5T garlic in the rat's diet was so high elsewhere (Gardner et al., 2001).

The changes in the AST and ALT as serum biomarkers of liver change are observed in high-fat diets (Shin et al., 2014). The effects of the intake of 0.2-1.0% fresh garlic extract for a short period on AST and ALT lowering have been reported in repeatedly in the literature (Chen et al., 1999; Yeh et al., 2001; Jo et al., 2002).

STRENGTHS AND LIMITATIONS

The strong point of the study may back to the number of groups who were included in the study. However, the findings reported in this study may not be consistent with some

investigations due to discrepancies in the types of garlic studies, included raw garlic, cooked garlic, or black garlic.

Conclusions and recommendations

The present study showed the garlic extract could play a lipid-lowering role and antioxidant against H2O2 and reducing the level of GOT.

Acknowledgment: the authors would like to present their deep thanks to Mohammed Saleem Mohammed,Megdad S. Ahmed for their kind assistance.

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المقدمة: النباتات الطبية لها تأثيرات طبية في مجال الطب الحديث والتقليدي, ولذلك اجريت هذه الدراسة لمعرفة تاثير المستخلص الكحولي لنبات الثوم (زيت الثوم)على بعض الفحوصات الكيموحيوية للجرذان البالغة لمدة ثلاث اسابيع متتالية حيث غذيت الجرذان يوميا بعلف مخلوط بزيت الثوم

المواد وطرق البحث: اجريت الدراسة على 20 من ذكورالجرذان البالغة من صنف ويستر البينو بعمر 8 اسابيع وتم تقسيمها على اربعة مجاميع متساوية في العدد وكالاتي:-

المجموعة الاولى: هي مجموعة السيطرة والتي عوملت وغذيت بعلف وماء خالي من اي اضافة.

المجموعة الثانية: عوملت الجرذان ب1% من بيروكيد الهيدروجين المخفف بالماء والمحضر يوميا +علف خالي من اي اضافة.

المجموعة الثالثة: تم اعطاء25 غرام العلف المخلوط ب 5 مل من زيت الثوم للجرذان يوميا (125غرام/25 زيت) مع ماء خالي من اضافة.

المجموعة الرابعة: جرذان معاملة ب5 مل من زيت الثوم مضاف اليها 25 غم من الغذاء المحضر يوميا + ماء يحتوي 1% من بيروكسيد الهايدروجين وتعباء في قناني غامقة اللون .

النتائج: اثرت المعاملة بزيت الثوم لوحده تاثيرا معنويا على قياسات الدهون مقارنة بمجموعة السيطرة على مستوى كل من الكوليسترول الكلي (54.30 vs68.90mg/dl)والكوليسترول الجيد (9.39 vs 7.53 mg/dl)وكوليسترول البروتبن الشحمي سى الكثافة(12.64 vs 14.62 mg/dl))والكوليسترول السيء (32.27 vs 46.75 mg/dl)ماعدا الشحوم الثلاثية (63.21 vs73.09; p>0.05) ولم تظهر اي فروقات معنوية في قيم فحوصات الكبد والكلى .

الفروقات بين فحوصات الكبد والكلى لم تكن معنوية بين المجموعتين السيطرة والمجموعة الرابعة وكذلك لم تكن هناك فروقات معنوية للمجموعتيين السابق ذكرهما في قيم انزيمات الكبد GPT(23.38 vs 20.23) و اليوريا (37.10 vs 40.80), حامض اليوريك(0.59 vs 0.53) وبالاضافة الى قيم الكرياتنيين (0.78 vs 0.57) ما عدا قيم انزيم ناقلة امين الاسبارتات GOT(30.04 vs 15.80) .

الاستنتاجات:- اظهرت الدراسة الحالية ان مستخلص الثوم كان له دور في خفض مستوى الدهون ومضادات الاكسدة مقارنة بيروكسيد الهايدروجين واختزال مستوى انزيم الكبدGOT(AST).

الكلمات الدالة: فحص الدهون, فحص انزيمات الكبد, الثوم: مضادات الاكسدة.