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RESEARCH ARTICLE

THE EFFECTS OF GRAPE SEEDS EXTRACT ON HYPERLIPIDEMIA IN RATS: I. ANTIOXIDANTSTATUS, LIVER ENZYMES, AND HISTOPATHOLOGICAL CHANGES

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ABSTRACT

Objectives We aimed to verify the benefits of grape seeds extract (GSE) on hyperlipidemic rats induced byhigh cholesterol diet. The efficacy of GSE was evaluated by antioxidant status, liver enzymes, and liver histological changes.

Introduction Prevalence of hyperlipidemia and lifestyle-related disorders prompted the search for non-conventional therapy.

Methods Forty male Wistar rats were divided into five equal groups as follows: group1: negative control group, group II: positive control (hyperlipidemic) groups III,IV and V (hyperlipidemic) orally given GSE in doses of 200, 400 and 600 mg/kg body weight, respectively

Discussion The results showed that oral administration of GSE extract to hyperlipidemic rats for one month significantly elevated serum levels of Aspartate Amino-transaminase (AST) and Alanine Aminotransferase (ALT) when compared to the control positive group. Antioxidant profile Catalase (CAT); Superoxide Dismutase (SOD), and Glutathione peroxidase (GSH-px) serum level were significantly increased as compared to the non-treated groups. The changes in liver histology were related to increasing concentrations of GSE.

Conclusion The study outcome suggests that GSE may help in the protection against hyperlipidemia-induced diseases, with further investigation need to be carried.

INTRODUCTION

Hyperlipidemia is a major risk factor for cardiovascular diseases, and is of global health impact (Auti *et al*., 2013), and Saudi Arabia is no of exception (Health Statistical Year book, 2012). There is increasing interest in the introduction of functional food using plant extract in the alleviation of hyperlipidemia. Of these, grape seed proanthocyanidin extract (GSPE) is regarded as a potent antioxidant derived from grape seeds, provides a concentrated source of polyphenols.The aim of this study was to assess the effects of grape seed extract on selected antioxidant indicators and liver enzymes in hyperlipidemic-rats.

MATERIALS AND METHODS

Animal Feeding Studies and Diet

The study protocol was approved by the research ethics committee-Faculty of Medicine King Abdul-Aziz University, Saudi Arabia.Forty adult male albino rats of Wistar strain weighed 120-150g each, were obtained from the Experimental Animal Unit of King Fahd Medical Research Center, King Abdul-Aziz University, Jeddah, Saudi Arabia. After the acclimatization period rats were allocated in to the following groups: Group I (negative control group (n=8 rats) were fed on basal diet. Group II (hyperlipidemic rats n= 32 rats) were fed on high cholesterol and high fat diet (cholesterol + 0.2% (w/w) bile salts + 20% (w/w) saturated fat) HCD. After hypercholestrol induction period (4 weeks), a blood samples were taken and analyzed for HDL-c and total cholesterol to calculate the atherosclerotic index according to (Hanglund *et al*.,1991).Rats with blood cholesterol level ≥5.2mmol/L were considered to be

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hyperlipidemic (Iqbal *et al*., 2011). Then atherosclerotic rats were subdivided into four subgroups each with eight rats, as follow:

Group II (positive control group) rats were fed on basal diet and HCDand received oral gavage of distilled water
Group III Rats were fed on basal diet and HCD and orally given GSE in a dose of 200 mg/kg body weight.
Group IV Rats were fed on basal diet and HCD and orally given GSE in a dose of 400 mg/kg body weight.
Group V Rats were fed basal diet and HCD and orally given GSE in a dose of 600 mg/kg body weight.

After four weeks, all rats were fasted overnight then sacrificed. Blood samples were immediately collected from the retro orbital plexus with capillary tubes under mild ether anesthesia, into clean dried centrifuge tubes. The tubes were then centrifuged at 3000 rpm for 15 minutes. Clear serum samples were carefully separated using Pasteur pipettes, and frozenat -200C until biochemical analysis for biochemical analysis (Salem and Salem, 2011).

The liver was removed and rinsed with a phosphate buffered saline solution, wiped with a paper towel, weighed quickly, frozen in liquid nitrogen, and stored at 80 until assayed (Park *et al*.,2008) washed with coldsaltine solution then weighed and kept for histopathological examination.The antioxidant profile were assayed in the liver tissues after homogenization and supernatant extraction to measure glutathione peroxidase (Paglia and Valentaine,1979), superoxide dismutase (Kakkar *et al*., 1984) and catalase (Sinha, 1972). Liver enzymes Aspartate Aminotransaminase (AST), and Alanine Aminotransferase (ALT) were assayed using the method adopted by Bergmeyer *et al*. (978). Histological examination of liver were done according to (Bancroft and Cook,1998), and examined microscopically.

Statistical Analysis

Data were presented as mean±standard error of mean. Analysis of Variance (ANOVA) test was used for determining the significances among different groups according to Armitage and Berry (1987). All differences were consider significant if $P < 0.05$.

RESULTS AND DISCUSSION

There is an increasing interest in lifestyle-related conditions such as hyperlipidemia. Hyperlipidemia is one of the principal causes of cardiovascular disease, and proanthocyanidins (PAs) regulate lipid homeostasis (Margalef *et al*., 2014). The increase of liver enzymes due to free radical production (FRT), theory cause release of oxidant in favor of antioxidant, and thus caused oxidative stress.

The histological changes of macrovascularsteatosis were observed in the periportal areas. This result was in agreement with the previous study of Jeong *et al*. (2005)

Oxidative stress plays an essential role by causing peroxidation of lipids and free radical generation (Stark, 2005). In line with the current study, hyperlipidemia increased serum levels of AST and ALT enzymes (Saki *et al*. (2011; Otunola *et al*., 2010). On the contrary, AL-Ahmadi *et al*. (2014) reported that there were no changes in the serum levels of AST and ALT.

As indicated in the present study, the untreated hyperlipidemic rats had a significant decrease in the level of antioxidant enzyme system (CAT, SOD and GPx). Consistent with our resultsSaki *et al*. (2011) who reported the effectiveness of the antioxidant defense system and the decrease in the activities of catalase(CAT), superoxide dismutase(SOD) and glutathione peroxidase (GPx).

Table 1 Effect of Oral Administration of Grape Seeds Extract at Three Concentrations on Serum Levels of Liver Function Enzymes in Hyperlipidemic Rats

Parameter / Groups	AST(U/L)	ALT(U/L)
Negative control	50.16±0.33 ^{c}	30.43±0.19 ^{d}
Positive control	80.74±0.45 ^{a}	51.06±0.46 ^{a}
Grape seeds extract 200mg /kg	66.27±0.73 ^{b}	42.76±0.77 ^{b}
Grape seeds extract 400mg /kg	66.08±0.33 ^{b}	42.13±0.33 ^{c}
Grape seeds extract 600mg /kg	59.48±0.18 ^{b}	38.69±0.33 ^{c}

Data are presented as means ± standard error of mean, (n = 8 for each group). Values with different superscripts within the column are significantly different at P< 0.05.: Values with similar or partially similar superscripts are non-significant.

Table 2 Effects of oral Administration of Grape Seeds Extract at Three Concentrations on Liver Homogenates Levels of Catalase (CAT), Glutathione Peroxidase (GPx) and Superoxide Dismutase (SOD) Enzymes in Hyperlipidemic rats

Parameter / Groups	CAT (U/ml)	SOD (U/ml)	GPx (U/ml)
Negative control	1.57±0.06 ^{a}	1.85±0.14 ^{a}	30.25±0.22 ^{a}
Positive control	0.99±0.04 ^{c}	0.99±0.24 ^{d}	24.06±0.34 ^{c}
Grape seeds extract 200mg /kg	1.35±0.03 ^{b}	1.30±0.01 ^{a}	25.18±0.20 ^{b}
Grape seeds extract 400mg /kg	1.41±0.01 ^{b}	1.53±0.12 ^{b}	26.33±0.19 ^{b}
Grape seeds extract 600mg/kg	1.52±0.02 ^{a}	1.74±0.06 ^{a}	29.79±0.18 ^{a}

Data are presented as means ± standard error of mean, (n = 8 for each group). Values with different superscripts within the column are significantly different at P< 0.05.: Values with similar or partially similar superscripts are non-significant.

In recent years, there is an increasing interest on the health potentials of GSE (Su *et al*., 2016; El-Awda *et al*., 2013; Varadharaja *et al*. 2016; Yong *et al*., 2012). There is a well-documented work on hypolipidemic agents from the plant and synthetic materials. It is of interest here to extend the knowledge on the possible effects of GSE.

Table 3 Effect of oral Administration of Grape Seeds Extract at Three Concentrations on Liver Relative Weight in Hyperlipidemic Rats

Groups	Relative liver weight
Negative control	3.13±0.07^{c}
Positive control	4.23±0.01^{a}
Grape seeds extract200mg /kg	3.71±0.01^{a,b}
Grape seeds extract400mg /kg	3.59±0.02^{b}
Grape seeds extract600mg /kg	3.30±0.08^{b}

Data are presented as means ± standard error of mean, (n = 8 for each group). Values with different superscripts within the column are significantly different at P< 0.05.: Values with similar or partially similar superscripts are non-significant.

The antioxidant properties of GSE could possibly explain the improvement of CAT, SOD and GPx enzyme activities due to the presence of bioactive polyphenoliccompounds which play a role in scavenging free radicals (Almajwal *et al*., 2015).

Concerning relative weights of liver, there were significant increases in liver relative weights of hyperlipidemic rats as compared to the average group table 3. These results might be due to the accumulation of fat in the liver cells leading to an increase in their weight.

The histopathological examination of the liver of the negative control group demonstratednormal histological pattern where hepatic lobule slides revealed typical structure (Figure 1). As shown in Figure 2, hyperlipidemia caused a marked impairment of the normal structural organization of hepatic.

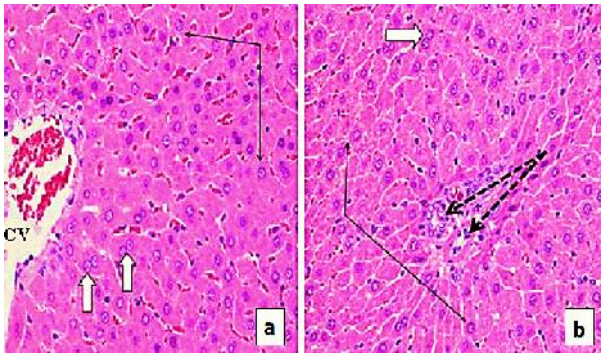

Figure 1 Cross Section in the Liver of a Normal Rat (Negative Control)

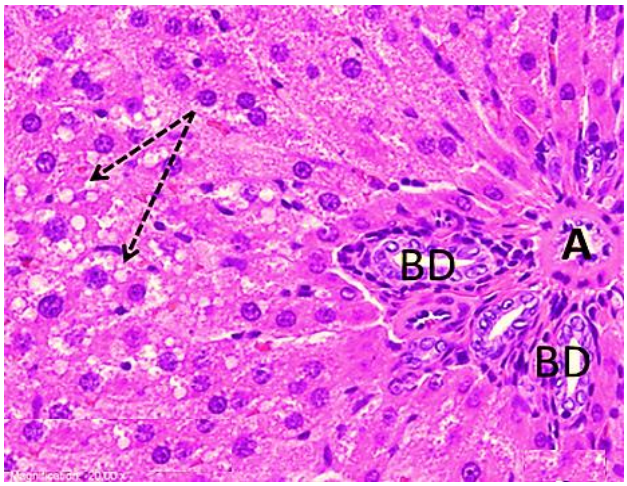

Figure 2 liver of hyperlipidemic rat (positive control)

In the current study, histopathological examination of the liver of hyperlipidemic rats dealt with by 200 mg GSE/kg body weight revealed a marked improvement with normal hepatocytes, with few scattered cells still showed tiny fat droplets when compared to the positive control group (Figure 3).Histopathological examination of the liver of hyperlipidemic rat treated by grape seeds extract in a dose of 400mg/kg BWT showed marked lipid deposition (Figure 4). Sections from liver of hyperlipidemic rat after treated by GSE at a dose of 600 mg/kg body weight, showed tiny lipid droplets as presented in Figure 5.

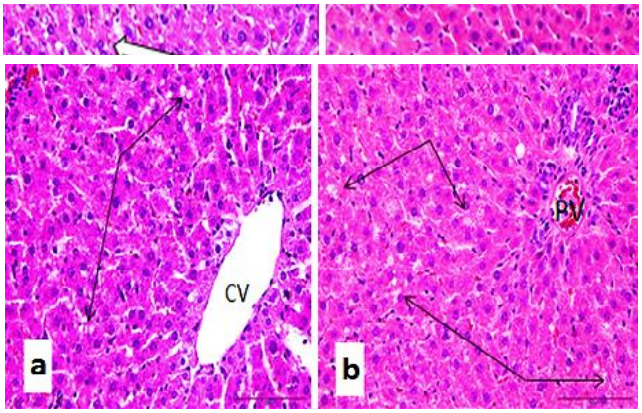

Figure 4 Liver histology of hyperlipidemic rats after treatment with 400mg GSE/ kg body weight

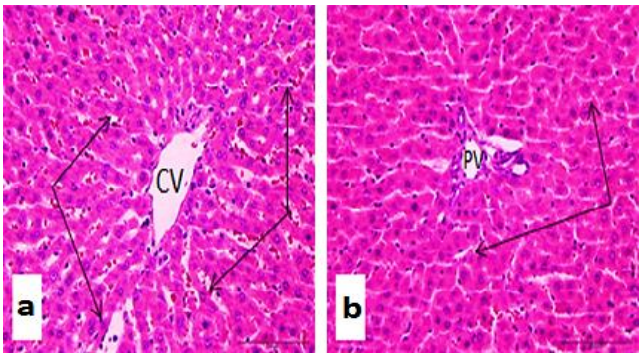

Figure 5 Liver histology of hyperlipidemic rats after treatment with 600mg GSE/ kg body weight

Similar effects were obtained by Olorunnisola *et al*. (2012), in which the liver histology showed that the extract markedly protected against hypercholesterolemia induced micro-vesicular steatosis. The combination of metformin and Grape seed proanthocyanidins improved the metabolism of lipids efficiently (Yogalakshmi *et al*., 2013; Vinson *et al*., 2002; (Downing *et al*., 2015).

In brief, more studies could be conducted to isolate and characterize functional bioactive materials in GSE which is responsible for hypolipidemic effects.

References

Al-Ahmadi, A.A., Soad, SH.A.,Nasra, N.A. and Abeer, K. A. (2014) Amelioration of hypercholesterolemia induced hepatic changes with red grape juice: A histopathological study. Histology Histopathology, vol. 5(29): 1169-1183.

Almajwal, A.M. and Elsadek, M.F. (2015) Lipid lowering and hepatoprotective effects of (VitisVinifera) dried seeds on paracetamol induced hepatotoxicity in rats, Nutrition Research and Practice, vol. 9(1): 37- 42.

Auti, S.S., Thakar,A. B., Shukla,V. J., Ravishankar, B.(2013) Assessment of Lekhana Bastiin the management of hyperlipidemia. Clinical Research, AYU Journal, vol. 34(4): 339-345.

Bancroft, J.D. and Cook, H.C. (1998) Manual of histotechnological techniques. Edited by: Churchill Livingstone., New York: 243.

Downing, L.E., Heidker, R.M., Caiozzi, G.C., Wong, B.S., Rodriguez, K., Rey, F.D., Ricketts, M.L., 2015. A grape seed procanidin extract ameliorates fructose-induced

hyperglyceridemia in rats via enhanced fecal bile acid cholesterol excretion and inhibition of hepatic lipogenesis. PLoSOne, 10(10), In Press.

El-Awdan, S.A., Abdel Jalee, G.A., Saleh, D.O., 2013. Grape seed extract attenuates hyperglycaemia-induced in rats by streptozotocin. Bulletin of Faculty of Pharmacy, Cairo University, 51, 203-209.

Hanglund,O., Luostarinen, R., Wallin,R .,Wibell, L. and Saldeen, T.(1991) The effect of fish oil on triglyceride, cholesterol,fibrinogen and malnodiahyde in humans supplement with vitamin E. *Journal of Nutrition*, vol.121:165-169.

Iqbal, M., Kalsoom and Jafri, S. A. (2011) Effect of punicagranatum flowers extract on hypercholesterole mic and alloxan induced diabetic rats, Global *Journal of Biotechnology and Biochemistry*, vol. 6(2):83-86.

Salem, N.A. and Salem, E. A. (2011) Renoprotective effect of grape seed extract against oxidative stress induced by gentamicin and hypercholesterolemia in rats.Renal Failure, vol. 33(8):824-832.

Kakkar, P., Das, B. and Viswanathan, P. N. (1984) A modified spectrophotometric assay of superoxide dismutase, *Indian Journal of Biochemistry and Biophysics*, vol. 21(2):130-132.

Margalef, M., Guerrero, L., Pons, Z., Bravo, F.I., Arola, L., Muguerza, B., Arola-Arnal, A., 2014.A dose-response study of the bioavailability of grape seed proanthocyanidin in rat and lipid-lowering effects of generated metabolites in HepG2 cells. Food Research International, 64, 500-507.

Olorunnisola, O.S., Bradley, G., and Afolayan, A.J., 2012. Anti-hyperlipidemic and biochemical effect of extract of Tulbaghiaviolacea rhizomes on high cholesterol diet fed rats. *African Journal of Biotechnology*, 11(7), 13498-13505.

Otunola, G.A., Oloyede, O. B., Oladiji, A.T. and Afolayan, A.A. (2010) Effects of diet induced hypercholesterolemia on the lipid profile and some enzyme activities in female Wistar rats. *African Journal of Biochemistry* Research, vol, 4 (6):149-154.

Paglia, D.F. and Valentaine, W.N. (1979) Studies on glutathione and glutathione characterization of erythrocytes glutathione peroxidase. *Journal Lab Clinical Medicine*, vol.4 (70) :158-169.

Pandey, K.B. and Rizvi,S.I.(2009) Plant polyphenols as dietary antioxidants in human health and disease. Oxidative medicine and Cellular Longevity, vol. 2: 270-278.

Pataki, T., Bak, I., Kovacs, P., Bagchi, D., Das, D.K. and Tosaki,A.(2002) Grape seed proanthocyanidins improved cardiac recovery during reperfusion after ischemia in isolated rat hearts. *American Journal of Clinical Nutrition*, vol. 75: 894-899.

Saki, N., Saki,Gh., Rahim, F., khoozani, A.S. and Nikakhlagh ,S.(2011) Modulating effect of soy protein on serum cardiac enzymes in cholesterol fed rats. *International Journal of Medicine and Medical Sciences*, vol. 3(14): 390-395.

Singh, J. and Singh, A.K. (2009) Analgesic and anti-inflammatory activity of methanolic extract of (VitisVinifera) leaves. Pharmacology online, vol. 3: 496-504.

Sinha, A. K. (1972) Colorimetric Assay of Catalase, Analytical Biochemistry, vol. 47(2): 389-394.

Stark, G. (2005) Functional consequences of oxidative membrane damage. *The Journal of Membrain Biolgy*, vol. 20(5):1-16. Huang, W.C., Chen, Y.M., Kan, N.W., Ho, C.S., Wei, L., Chan, C.H., Huang, H.Y., Huang, C.C., 2015. Hypolipidemic effects and safety of Lactobacillus Reuteri 263 in a Hamster model of hyperlipidemia. Nutrients 2015, 7(5), 3767-3782.

Sun, Y., Xiu, C., Liu, W., Tao, Y., Wang, J., Qu, Y., 2016. Grape seed proanthocyanidin extract protects the retina against early diabetic injury by activating the Nrf2 pathway. Experimental and Therapeutic Medicine, 11, 1253-1258.

Varadharajan, V., Shanmugam, S., Ramaswamy, A., 2016.Correlation between the total phenolics, proanthocyanins content and anti-oxidant activity of grape seed extract. Bangladehs *J. Pharmacol*., 11, 165-167.

Vinson, J.A., Mandarano, M.A., Shuta, D.L., Bagchi, M., and Bagchi, D., 2002. Beneficial effects of a novel IH636 grape seed proanthocyanin extract and a niacin-bound chromium in a hamster atherosclerosis model. Molecular and Cellular Biochemistry, 240, 99-103.

Yang, Y., Piao, X., Zhang, M., Wang, X., Xu, B., Zhu, J., 2012. Bioactivity guided fractionation of the triglyceride-lowering component and in viv and in vitro evaluation of hypolipidemic effects of Calyx seufructus physalis. Lipids in Health and Disease, 11(1), 380-45.

Yogalakshmi, B., Sreeja, S., Geetha, R., Radika, M.K., Anuradha, C.V., 2013. Grape seed proanthocyanidin rescues rats from steatosis: A comparative and combination study with metform in. *Journal of lipids*, 11.

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