

Towards Understanding The Hepatoprotective effect of Grape Seeds Extract on Cholesterol-Fed Rats

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Abstract: In our previous studies, a phenolic-rich extract of grape seed was prepared under optimal conditions. The antioxidant activity of grape seed extract (GSE) was determined in addition to determination of acute oral LD₅₀ toxicity. The current work studies the protective effect of GSE on hypercholesterolemia, where, Wistar rats fed a standard laboratory diet (control group-CG) or a cholesterol-rich diet (hypercholesterolemic group-HCD) and to see the effect of GSE, another group fed on cholesterol-rich diet enriched with 0.3% GSEW/W-PG) for 8 weeks. Serum lipid levels, serum antioxidant status, Liver and kidney function were analysed in addition to histopathological examination of the liver. The hypocholesterolemic effects of GSE is confirmed by lowering the serum total cholesterol (TC) by 31%, low-density lipoprotein cholesterol LDL-C by 41% and elevated the high-density lipoprotein cholesterol HDL-C by 25% compared to TC, LDL-C and HDL-C of HCD group . Furthermore, the liver function expressed as glutamic pyruvate transaminase (GPT) and Albumin serum levels, decreased significantly and reached to normal level in case of oral administration of GSE. The kidney function showed no adverse effect in all groups. In addition, the antioxidant status serum level was increased as compared to those of rats fed only on cholesterol-rich diet. Histological examination of liver sections confirmed the serum analysis where GSE had a protective effect on animals fed on HCD, the liver of these animals showed mild affection in the form of microvesicular vacuolation of hepatocytes in the peripheral zone of the hepatic lobule (<50%) in comparison to the fatty change observed as microvesicular and macrovesicular vacuolation in >50% and <70% of the liver sections in HCD group. These results suggested that the GSE has a hypocholesterolemic effect which might be due to its ability to lower serum TC and LDL-C levels as well as slowing the lipid peroxidation process by enhancing antioxidant enzyme activity.

Key words: Grape seed extract, hypercholesterolemia, liver, drugs, hepatoprotective

INTRODUCTION

Despite the investment of billions of dollars in research and the development of numerous cholesterol-lowering drugs, coronary heart disease is still a leading cause of death in developed countries. Familial hypercholesterolemia (FHC) is a major contributor to this deadly killer (Marks, D., *et al.*, 2003). Unfortunately, there are many problems associated with the use of cholesterol-lowering drugs, including poor quality of life, severe rhabdomyolysis (breakdown of muscle fibers), renal failure, and death (Chung, N., *et al.*, 2001; Law, M.R., *et al.*, 1994). There may be alternative approaches to the management of FHC to consider. Accumulating studies have demonstrated a relationship between flavonoid consumption (from food) and reduced risk of death from coronary heart disease (Kris-Etherton P.M., *et al.*, 2002; Temple, N.J., and K.K. Gladwin, 2003). Grape Seeds are one of the richest sources of proanthocyanidins; a class of biologically active flavonoids found throughout the plant kingdom. Grape seed extract (GSE) has received much attention due to its numerous biological activities, such as antioxidant effects (Koga, T., *et al.*, 1999; Bagchi, D., *et al.*, 2000), protection against X-ray and ultraviolet rays (Castillo, J., *et al.*, 2000; Carini, M., *et al.*, 2000),

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chemoprevention (Sun, G.Y., *et al.*, 1999; Joshi, S.S., *et al.*, 2000), anti-cancer or anti-tumor effects (Bomser, J.A., *et al.*, 1999; Agarwal, C., *et al.*, 2000), and inhibitory effects against atherosclerosis and hypercholesterolemia (Tebib, K., *et al.*, 1994; El-Adawi, H., *et al.*, 2006). Recently, GSE has been considered as a potential health –food ingredient because of these beneficial properties. The extract has received the GRAS(generally recognized as safe) certification from FDA and Has no known side effects (Ray, S., *et al.*, 2001; Wren, A.F., *et al.*, 2002). To date, literature survey shows that no sufficient work has been done to study the hepatoprotective effect of GSE on hypercholesterolemia. The present study was planned to evaluate the effect of GSE on liver function and also to unravel its role on tissue peroxidation and antioxidant levels in treated rats. The findings are compared with those of the control and unsupplemented GSE Rats. This study is promising and can help in the management of familial hypercholesterolemia.

MATERIALS AND METHODS

Chemicals:

All diagnostic kits were purchased from Bio-Diagnostic, Cairo- Egypt.

Animals and Diet:

Eighty four pathogen-free male Wistar-albino rats(four weeks) were obtained from, and approved by, Tudor Bilharz institute (Cairo, Egypt). Rats were housed in specific standard laboratory conditions for one week. The conditions were kept in a temperature- controlled environment (18-26°C), a relative humidity (30-70%), and with a regular 12 h light/ 12 h dark cycle. All animals were fed with a standard rat chow diet and water ad libitum, and then rats weighing 100-120 g were used for induction of hypercholesterolemia.

The experiment was conducted according to the procedures described previously (El-Adawi, H., *et al.*, 2006). Rats were randomly divided into Three groups, one group (12 rats) were fed a high Cholesterol Diet (HCD). Another group (12 rats) received the same HCD supplemented with 0.3% GSE w/w (one fifth of the LD 50) to test the preventive effect of GSE on hypercholesterolemia (P-G). The third group (12 rats) was given the basic diet and served as controls (C-G). The lipid profiles were assayed for all groups till we got marked hypercholesterolemia in HCD group. In order to test the hypothesis that the GSE could protect from the hypercholesterolemia than the basic diet or not, we started to feed HCD group with the basic diet for four weeks in parallel with C-G group which received the basic diet and the P-G group which received the basic diet enriched with 0.3% GSE [w/w].

Serum Analysis:

The blood was centrifuged at 3000 rpm at 4°C for 10 min to separate the serum.

Lipid Profile:

- Total Cholesterol (TC) was assayed according to the method of Richmond (1973) and Allain *et al.* (1974). In brief, after enzymatic hydrolysis and oxidation of cholesterol, the resultant hydrogen peroxide reacts with 4-aminoantipyrine and phenol in the presence of peroxidase to form a quinonimine, which was measured colormetrically at 500 nm.
- Total Triglycerides (TG) were determined following the method of Fossati and Prencipe (1982). This method resides simply on the enzymatic hydrolysis of triglycerides to glycerol, which reacts with ATP to form hydrogen peroxide; in turn the resultant hydrogen peroxide reacts with 4-aminoantipyrine in the presence of p-chlorophenol to form a quinonimine, which was measured colormetrically at 505 nm.
- High-Density Lipoprotein (HDL-C) and low- Density Lipoprotein (LDL-C) cholesterol fractions were determined according to Burstein *et al.* (1970) and Lopez-Virella *et al.* (1977). In which phosphotungstic acid and magnesium ions selectively precipitating all lipoproteins except the HDL fraction-cholesterol present in the supernatant can be determined by the same method used for total cholesterol. LDL-C was computed mathematically according to Friedwald's equation (1972): $LDL=TC-[HDL+ (TG/5)]$

Measurement of Liver Function Markers:

- Albumin was assayed according to the method of Dumas *et al.* (1997), where, a green complex of an albumin/bromocresol formed at pH 4.2 and measured spectrophotometrically at 630 nm.

- Glutamic pyruvate transaminase (GPT) was determined following the method of Reitman and Franke (1957). Alanine aminotransferase (GPT) catalyzes the transfer of the amino group from alanine to 2-oxoglutarate, forming pyruvate and glutamate. The catalytic activity was measured by spectrophotometry at 505nm.

Measurement of Kidney Function Markers:

The Kidney function markers including Creatinine and urea were measured in serum by Colorimetric method.

- Creatinine in the serum determined according to the method of Schirmeister *et al.* (1964), Where creatinine reacted with picrate in alkaline medium forming a colored complex. The amount of the complex formed is directly proportional to the creatinine concentration and could be measured at 500nm.
- Urea in the serum originated, by means of the coupled reactions described by Fawcett and Soctt (1960). The blue dye indophenol product reaction absorbs light between 530 nm and 560 nm proportional to initial urea concentration.

Serum Antioxidant Status:

The determination of the antioxidative capacity is performed by the method of Koracevic *et al.* (2001). The antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. The residual H₂O₂ is determined Colorimetrically at 505 nm by an enzymatic reaction which involves the conversion of 3,5, dichloro -2- hydroxyl benzensulphonate to a colored product.

Histological Analysis:

Soon following the animals sacrifice, the abdomen was opened, the rat livers were removed and immediately fixed in 10% formalin saline solution (pH 7.4) and processed by successive dehydration with a sequence of ethanol solution and embedded in paraffin. The serial sections were cut 5µm thick and stained with haematoxylin-eosin (HE) stain using standard procedures (Drury, R.A.B. and E.A. Willington, 1980). Stained liver sections were examined for structure and architecture changes photomicroscope. The liver sections were examined for the type of the fatty change (macrovesicular: large droplets or microvesicular: small droplets) and the acinar zone involvement considering the three zones (peripheral/periportal, middle and central/perivenular zones) of the hepatic lobule, also assessment for presence of complications such as steatohepatitis, steatohepatitis with cirrhosis or hepatic fibrosis was examined (Scheuer, P.J. and J.H. Lefkowitz, 2005).

Statistical Analysis:

Data were expressed as mean ± SD. Differences between control and other groups were tested for significance using a one-way analysis of variance (ANOVA). *P*-values of 0.05 or less were considered significant.

RESULTS AND DISCUSSION

In our previous study, GSE displayed a marked hypocholesterolic activity and inhibited LDL-C-oxidation (El-Adawi *et al.*, 2006). However, Its hypocholesterolic activity has not been fully elucidated. In this follow up study, we confirm that GSE could protect from the hypercholesterolemia by lowering the TC by 31%, LDL-C by 41% and elevated the HDL-C by 25% compared to HCD-G (Table.1). GSE-supplemented HCD noticeably reduced the serum TG by 18% (39.2 mgdL⁻¹) compared to animals fed on GSE-free HCD (49.8 mgdL⁻¹). The LDL-C/HDL-C risk ratio was more than twice in HCD -G compared to P-G.

The present study showed no significant difference in serum creatinine and urea concentration (indicators of kidney function) in the experimental groups. This suggests that neither GSE nor the HCD has nephrotoxicity effect. Statistical analysis of the total antioxidant capacity indicates that GSE could elevate the antioxidant status in P-G to normal value where it was significantly reduced in HCD-G.

The elevation of GPT activity and Albumin concentration in the blood reflects indirectly the failure of liver function due to APAP-induced hepatotoxicity. In table 1. GPT activity was significantly increased in HCD-G as compared with the control group (*P*< 0.05). Pretreatment with 0.3% w/w ethanolic extract of Grape seeds significantly reduced the elevation of GPT and albumin as well.

Histologic examination of the Liver sections of the studied animal groups revealed normal histology and architecture of the control group (C-G) as shown in Fig1.A However, examination of the livers of the high cholesterol diet fed animals (HCD-G) showed micro and macrovesicular vacuolation of the hepatocytes due

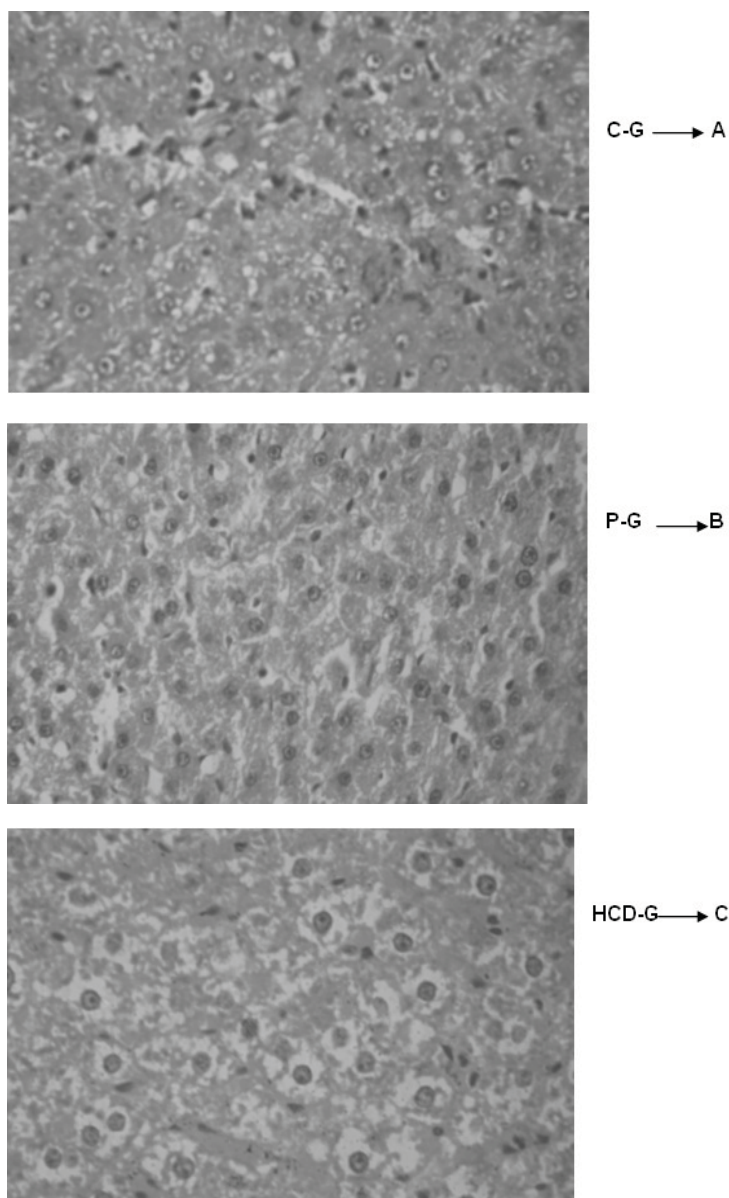


Fig. 1: shows H&E stained liver sections of control group (C-G) -A, preventive group (P-G) -B and High Cholesterol Diet group (HCD-G) -C. Images are 400x magnified. Note the occasional vacuolated hepatocytes in the P-G liver section.

to fatty change in the peripheral zone (Zone 1) and middle zone (zone 2) of the hepatic lobule, and the central zone (zone 3) in some hepatic lobules. Using an arbitrary subjective scoring, the extent of involvement of livers of the HCD-G was scored as >50% and <70% of the liver sections, and was therefore considered as moderately affected (Fig 1. C). Whereas the preventive group animals showed milder affection of their liver sections where the microvesicular vacuolation involved occasional scattered hepatocytes within the lobule mostly in the peripheral zone of the hepatic lobule (zone 1) and the degree of affection was therefore scored as 25% of the liver section (Fig1. B). There were no complications detected in any of the sections examined. So far, the results have been very promising. In addition, this medication-free approach enables us to avoid the dangerous side effects that are so prevalent with prescription drug use.

Table 1: Effect of GSE on serum GOT, Albumin, Creatinine, Urea and Total antioxidant capacity.

| Parameters | | Groups | | |
|----------------------------------|------|--------|--------|--------|
| | | C-G | P-G | HCD-G |
| Total cholesterol (mg/dl) | Mean | 80.2 | 146.2* | 210* |
| | SD | 5.2 | 4.8 | 3.9 |
| Tiglyceride (mg/dl) | Mean | 79.6 | 39.2* | 49.8* |
| | SD | 3.4 | 2.5 | 4.1 |
| LDL (mg/dl) | Mean | 25.22 | 103.3* | 172.3* |
| | SD | 4.0 | 1.9 | 3.4 |
| HDL (mg/dl) | Mean | 38.86 | 34.8 | 27.7* |
| | SD | 2.11 | 1.5 | 1.8 |
| GPT(I.U/L) | Mean | 21.7 | 23.2 | 38.1* |
| | 1.9 | 0.84 | 4.0 | |
| Albumin (g/dl) | Mean | 2.3 | 2.37 | 3.7* |
| | SD | 0.19 | 0.25 | 0.28 |
| Creatinine (mg/dl) | Mean | 0.63 | 0.52 | 0.63 |
| | SD | 0.06 | 0.22 | 0.3 |
| Urea (mg/dl) | Mean | 17.7 | 4.3 | 20.1 |
| | SD | 4.6 | 2.8 | 3.12 |
| Total Antioxidant capacity(mM/L) | Mean | 0.84 | 0.82 | 0.64* |
| | SD | 0.025 | 0.011 | 0.016 |

P < 0.05, significant difference from control group.

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