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PROTECTIVE EFFECT OF GRAPE SEEDS POWDER AND OIL AGAINST HYPERCHOLESTEROLEMIA IN RATS

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ABSTRACT

The aim of present study was to evaluate the antihyperlipidemic effect of grape seeds powder (GSP) and oil (GSO) on lipid profile of hypercholesterolemic rats. The chemical properties of grape seed powder (GSP) and grape seed oil (GSO) were determined. Total phenolic compounds of GSP were determined and fractionated .Results showed that grape seeds are considered to be rich source of natural antioxidants and have bioactive compounds that play a great role to protect human body. Biological evaluation also conducted on hypercholesterolemic rats feed on diets supplemented with GSP and GSO at levels of (4, 5%). The supplementation of GSP and GSO decreased markedly serum TC, TG and LDLC. GSO also significantly reduced liver enzymes levels ALT, AST and ALP, and increased the glutathione content of the liver. The histological examination revealed a potent protective action of GSO rather than GSP, on heart, aorta, and liver. Therefore, use of GSP (mainly) and GSO as supplements caused significant improvement in biochemical parameters in favor of hypocholesterolemia in all rats groups and could be protect against hypercholesterolemia and can be also used as effective natural antioxidants.

Keywords: Hypercholesterolemic diet; Grape seed powder; Grape seed oil; Lipid profile; Liver enzymes and Histopathological examination.

INTRODUCTION

In Egypt, grapes are considered the second important fruit crop after citrus. Grapes growing area is about 159,000 faddan (one faddan=0.42ha) producing about 210,000 ton fruits (Anon, 2015). Grape seeds, a byproduct of the wine making or juice processing industry, contains 10-20% of oil with high unsaturated fatty acids content such as linoleic (58-78%), oleic (3-15%), and linolenic acids (0.3%), which are responsible for its values as nutritive edible oils (El- Bastawesy *et al.*, 2007, Bail *et al.*, 2008 and Kim *et al.*, 2010). Grape seeds oil (GSO) is composed of average 90% poly- and monounsaturated fatty acids of which higher nutritive value as including linoleic acid followed by oleic acid and minor amounts of saturated fatty acids (10%) (El-bastawesy *et al.*, 2007, Bail *et al.,* 2008). Also, GSO contains 0.8 to 1.5% unsaponifiables rich in phenols (tocopherols) and steroids (campesterol, beta-sitosterol, stigmasterol) (Jain *et al.,* 2010, Pilehvar *et al.,* 2013). The α-tocopherol is capable of quenching free radicals, which protects phospholipids and cholesterol against oxidation and subsequent breakdown to potentially harmful chemically reactive products (Gray *et al.,* 1996., Guthrie and Kurowska 2001).

Grapes are considered a major source of phenolic compounds comparing to other fruits and vegetables, but the great genetic diversity among varieties results in grapes with different characteristics, flavor, and color, which is associated with content and profile of polyphenols (Abe LT *et al.,* 2007).Therefore, grape seeds mostly have marked protection against free radicals than those of vitamin C and E (Belviranli *et al.*, 2012) . Moreover, resveratrol in grape seed is a natural compound preventing cell proliferation in leukemia, prostate, breast, and other types of cancers. It also possesses antimycotic, antineoplastic, antioxidant, antiproliferative, and anti-inflammatory effects (Feringa *et al.*, 2011).

Grape seeds (*Vitis vinifera* Linn.) contains important vitamins, minerals and polyphenols including flavonoids, proanthocyanidins and procyanidins (Weber *et al.*, 2007). Grape seeds are well known for its pharmaceutical properties including; anti-inflammatory, immunomodulatory, antimicrobial, arcaricadal, antipruritic, remedy of gastrointestinal disorders, lipid and stress lowering effect, anti-allergic and anti-solar agents (Lafka *et al.*, 2007, Pilehvar *et al.*, 2013) .

Hypercholesterolemia is considered one of metabolic disorder associated with hyperlipidemia, and characterized by high levels of cholesterol in the blood. The modern life style especially fast foods, high fat diets and little physical activity significantly contributes to hypercholesterolemia and its cardiovascular complications (Freedman, 2003).

Previous studies have shown that GSO exhibits protection against the oxidation of LDLs, prevention of thrombosis, inhibition of cardiovascular disease (CVD), reduction of cholesterol in serum, dilation of blood vessels, and regulation of autonomic nerve (Oomah *et al.*, 1998). According to Nash (2004) , up to 45 g of GSO per day raised HDL-cholesterol levels by 13% and reduced LDL-cholesterol levels by 7% in three weeks treatment. Therefore, the present study was aimed to investigate the protective effect of GSP , GSO and their combination on induced hypercholesterolemia in rats. A feeding experiment on rats was conducted to measure the effects including growth parameters, lipid profile, liver functions and histological examination of hypercholesterolemia in rats.

MATERIALS AND METHODS

Materials

Grape seeds: Seeds of Red Globe grape fruits (*Vitis vinifera* Linn.) were obtained from the Agricultural Research Center, Giza, Egypt.

Animals: Forty five male albino rats (Sprague Dawley) 100-120 g, were obtained from the Agricultural Research Center, Giza, Egypt.

Chemicals : Casein, all vitamins, minerals, cellulose and cholesterol powder were obtained from Algomhoria Co. for Trading in Medicines, Chemicals and Medical Supplies, Cairo, Egypt.

Methods

Preparation of grape seeds powder and extraction of oil:

Grape seeds were washed by tap water and dried in air circulated oven at 60°C for 12 hrs ,up to moisture content to less than 10%. The obtained dried seeds were milled using Braun mill, sieved through 21 mesh screens, packaged in polyethylene bags and stored in refrigerator (4±1°C) for analysis. Oil was extracted from powdered grape seeds, (100 g) using hexane in a Soxhlet apparatus for 20 hrs, according to Molero Gómez *et al.*, (1996).The solvent was evaporated by anhydrous sodium sulfate column and pre-concentrated under reduced pressure to remove all the solvent.

Analytical Methods:

Gross chemical composition

Moisture, ash, crude protein, ether extract, crude fibers, total carbohydrates and hydrocyanic acid contents were determined according to the methods described by A.O.A.C. (2005). Tannins were determined according to the method described by Rangana (1979). Phytic acid was determined according to the method mentioned by Wheeler and Ferrd (1971)

Chemical properties of grape seeds oil:

Acidity % (as oleic acid), peroxide and iodine values were determined according to the methods described by A.O.A.C. (2005).

Fatty acids composition of grape seeds oil:

The methyl esters of extracted crude grape seeds oil were determined and identified using gas liquid chromatography (Agilent 6890 GC., USA) as described by Zygaollo *et al.,* (1994).

Determination of α-tocopherol and total phenol compounds

1-α-Tocopherol

α-tocopherol was determined by HPLC according to the method described by Lampi *et al.* (1999) with direct injection of an oil extract in a mixture of heptane:tetrahydrofuran (THF) (95:5) solution.

2-Total phenol compounds

The total phenolic content was determined using spectrophotometric method (Singleton *et al.,* 1999). The reaction mixture was prepared by mixing 0.5 ml of ethanolic solution (1 mg/ml) of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% NaHCO3. The samples were incubated at 45°C for 15 min. The absorbance was determined at λmax = 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (mg of GaA/g of extract).

Biological Assay

Animals and experimental design:

Animal facility and protocol were approved by the Laboratory Animal Care and Use Committee at Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt.

Forty five rats (100-120 g) were divided into five groups with nine rats per each. The 1st group was fed on the basal diet (Table 1) as a negative control group (-ve), the 2nd G2-5 were fed on high cholesterol diet (1% cholesterol powder and 0.5% bile salt) for 8 weeks as described by (Yokozawa *et al.*, 2006).The 3rd and 4th groups were fed on high cholesterol

diet supplemented with 4 % and 5% of grape seeds oil and powder, respectively. The 5th group(G5), the rats were fed on high cholesterol diet supplemented with grape seeds oil 4 % + grape seed powder 5 %. Water and diets were introduced *ad-Libitum* under hygienic conditions. They were housed in well aerated cages under hygienic condition and fed on basal diet for one week for adaptation. The basal diet consisted from the following components of AIN-93M diet according to Reeves *et al.*, (1993) as shown in Table (1).

Table (1): Composition of basal diet

Ingredient	**%**
Corn Starch	46.569
Casein (85% protein)	14.0
Dextrin	15.5
Sucrose	10.0
Soybean Oil	4.0
Fibers	5.0
AIN 93M Mineral Mix	3.5
AIN 93 Vitamin Mix	1.0
L-Cystine	0.18
Choline Bitartrate	0.25
t-Butylhydroquinone	0.008

At the end of experiment (after 8 weeks), rats were fasted overnight,and anaesthetized by diethyl ether before scarifying. Blood samples were withdrawn in two test tubes. The whole blood in the EDTA tube was used for estimation of some biochemical parameters; the other tubes of blood were left for coagulation then centrifuged at 3000 rpm for 15 min to obtain serum for further analysis. The obtained serum was kept at -20°C for the subsequent analysis. The heart and liver were removed from each rat for histological examination.

Biochemical Assay:

Lipid profile: The serum level of triglyceride was determined as described by Fossati and Prencipe (1982). Serum total cholesterol level was assayed by the method of Naito and David (1984) Serum HDL was determined according to Grove (1979) using enzymatic kits from Quimica Clinica Aplicada S.A. (Amposta, Spain).The LDL and very low density lipoprotein (VLDL) was calculated using the formula of Friedewald *et al.*, (1972).

Liver functions: The activities of alanine and aspartate aminotransferases (ALT and AST) were assayed by the combined methods of Bergmeyer *et al.*, (1976). Alkaline Phosphatase (ALP) was determined by the method of Rec, (1972).Malondialdehyde and glutathione-S-transferase (GST) were measured according to Kei, (1978) and Mannervik and Danielson, (1988) respectively, using commercial assay kits (Diamond Diagnostics, Egypt).

Histolost

Samples were taken from the heart; aorta and liver of the scarified rats were collected and immersed in 10 % neutral buffered formalin as fixative agent the fixative the heart; aorta and liver samples were histologically examined in Histology Dept., Veterinary Medicine Fac , Cairo Univ. according to Bancroft *et al*., (1996)

Statistical analysis

Statistical analysis was carried out using analysis of variance (ANOVA) (Sas and Guide, 1990, Institute, 2000). Results were expressed as mean ± SD at $P < 0.05$ significance.

RESULTS AND DISCUSSION

Gross chemical composition

Moisture, ash, protein, ether extract, crude fibers, total carbohydrates, phytic acid and hydrocyanic acid (HCN) contents were determined in grape seed powder GSP and the results are given in Table (2).It could be noticed that the moisture content of (GSP) was found less than 10% to avoid the growth of microorganisms. Grape seed powder contained 3.86% ash, 13.003 % protein, 14.94 %, fat, 32.75 % crude fibers and 26.92 % carbohydrates. These data are in accordance with those reported by Badr *et al.,* (1994) and Abou Rayan *et al.,* (1998). Generally, from these results, it could be observed that GSP had considerable content of fat and crude fibers.

Some anti-nutritional substances in grape seed powder also determined to evaluate this waste as a safety food ingredient for human utilization and the results are presented in Table (1). As show in the same table hydrocyanic acid was not detected in GSP. On the other hand GSP contained 6.37% tannins and lower content of phytic acid (0.18%). These results are in agreement with those of Youssef (1999).

Table (2): Gross chemical composition of grape seeds powder

Constituents %	**Grape seeds powder**
Moisture	8.531
Protein	13.003
Fat	14.935
Crude fiber	32.747
Ash	3.864
Carbohydrates	26.992
Tannis (as tannic acid)	6.373
Phytic acid	0.180
HCN	o.ooo

Chemical properties of grape seeds oil:

Some chemical properties of GSO were determined and results are presented in Table (3).From these results it was found that grape seed powder had oil content of 14.935% this high content of oil in grape seeds encouraged, researchers to evaluate and utilize this extracted oil. As shown in Table (3), grape seeds crude oil had acidity of 1.93 while refined sunflower oil acidity was 0.164%. The acidity of grape seeds oil was near to the reported by Han-Chul-Kang *et al,* (1999) but less than the value stated by Badr *et al,* (1994).

From results of acid value it could be observed that oil acid value was higher than those found in refined SFO (0.164%) It is well known that the refining process leads to decrease the acid value.

Peroxide number of extracted crude oil was 1.43 (meq.O2/kg). This value showed that no oxidation has taken place in the extracted crude oil .From the same table, Iodine value of grape seeds oil was 135.45. These results show that the grape seeds oil contains a large amount of unsaturated fatty acids. From aforementioned data it could be concluded that, grape seeds oil could be considered as a drying oil.

The iodine value of grape seeds oil is in accordance with Badr *et al.,* (1994). Generally, it could be concluded that the extracted grape seeds oil had chemical properties in the normal range of edible oils.

Table(3): Crude oils content and some chemical properties of grape seeds oil.

Samples / Analysis	Grape seeds oil	*SFO
Oil content %	14.935	-
Total acidity % (as oleic acid)	1.93	0.164
Peroxide value (meq.O2/kg)	1.43	4.9
Iodine value	133.45	133.346

***SFO= Refined sunflower oil.**

Fatty acids composition of grape seeds oil

The fatty acids composition of extracted grape seeds crude oil was separated and identified using GLC and results are given in Table (4). From data presented in table (4), it could be noticed that, 6 fatty were identified in grape seeds oils. Grape seeds oil had high content of total unsaturated fatty acids (80.158%), since polyunsaturated fatty acids (PUSFA) represent the major part (56.8%) of total unsaturated fatty acids. Linoleic acid (C18:2) was the predominant fatty acid (56.33%), followed by oleic acid (23.47% in grape seeds oil. On other hand,grape seeds oil was characterized by low level of total saturated fatty acids (13.39%), mainly, palmitic acid (9.46%) and stearic acid (4.05%) as well as relatively high level of monounsaturated fatty acid (MUSFA) oleic acid (23.465%). These results are in accordance with those of Lee et al.(2000), while lower than those of Vanhanen and Savage (2000).In conclusion, it could be clearly observed that grape seeds oil had high content

of total USFA (80.158%). It is worthy to mention that the percentage of the unsaturated fatty acids of grape seeds oil was high and reached approximately to 80.158% especially, essential fatty acids (Linoleic , Omega 6), which reflect the nutritional value of the oil.

Table (4): Fatty acids composition of grape seeds oil

Fatty acids %	Grape seeds oil
Palmitic acid C16:0	9.458
Stearic acid C18:0	4.046
Oleic acid C18:1	23.465
Linoleic acid C18:2	56.334
Linolenic acid C18:3	0.359
Arachidic acid C20:0	0.388
Others	5.950
Total saturated fatty acids%	13.892
Total unsaturated fatty acids%	80.158
Total Fatty acids	100

Total phenolic compounds content of grape seeds

Total phenolic compounds content of grape seeds were determined and the results are shown in Table (5). From these results, it was found that grape seeds had the high total phenolic compounds content (3681.6mg\100mg). The total phenolic compounds in grape seeds was lower than those reported by Abou Rayan (1998), and close to the value reported by Goni et al., (2005). From aforementioned data, it could be concluded that grape seeds are considered to be rich source of natural antioxidants and had necessary biocomponents that play a great role to protect human body and can be used in food industries.

Fractionation of phenolic compounds

Data in Ttable (5) show the fractions of phenolic compounds of grape seeds. As recorded in this table it can be noticed that the pyrogallic acid was the most abundant phenolic compound in grape seeds (51.407mg/100mg), while hydroquinone, gallic acid and rutin contents were 40.364, 6.844 and 6.041mg / 100mg, respectively.

Table (5) : Fractions of phenolic compounds of grape seeds

Phenolic compounds (mg/ 100g)	Grape seeds
Pyrogallic acid	51.407
Hydroquinone	40.364
Gallic acid	6.844
Rutin	6.041
Resorcinol	--
Protocatechuic	--
Chlorogenic	--
Kaempferol	--
Total phenolic compounds	3681.58

α–tochopherol and total phenolic compounds of grape seeds oil

The obtained results (Table 6) show that content of α–tochopherol is 2.17 mg/100g oil and the concentration of total phenolic compounds content is 2.63 mg/100g oil. The content of α-tocopherol in the present study is in good agreement with those of Baydar *et al.,* (2007) and Wie *et al.,* (2009). *α*-tocopherol reduces the risk of cardiovascular diseases, diabetes, and cancer and prevents sexual impotence Schwartz *et al.,* 2008 ; Charoensiri *et al.,* (2009).

Total concentration of phenolic compounds of grape was about 2178.8, 374.6, 23.8, and 351.6 mg/g GAE (gallic acid equivalent) in seed, skin, flesh, and leaf, respectively (Pastrana-Bonilla *et al.,* 2003). The concentration of total phenolic compounds present in the oils of the varieties showed no significant changes and ranged between 1.23-2.37 mg/100g of grape seeds Agostini *et al.,* (2012).

Table (6): α–tochopherol and total phenolic compounds of grape seeds oil.

α–tochopherol (mg/100g oil extract)	Total phenolic (mg/100g oil extract)
2.17	2.63

Biological assay

Effect of GSP and GSO on growth parameters

The effect of GSP and GSO on growth parameters of rats including body weight gain (BWG), daily food intake (FI), and feed efficiency ratio (FER) of hypercholesterolemic rats were illustrated in Table (7). The results show that rats fed on 5% GSP (G3) has the lowest values of all parameters as compared to all other groups. BWG of rats treated with GSP or GSO (G3-5) was significantly less than G1 (-ve control) and G2 (+ve control). The BWG was significantly increased in G1 compared to G2 (+ve control) with mean values of 0.81±0.145 and 0.65±0.048, respectively.

Daily feed intake (FI) show non-significant difference between the animals of different control and treated groups (Table7). Meanwhile, the mean values of rats fed on GSP and GSO showed significant decrease compared with positive control. This means that the food consumption was unchanged in G1, G2 rats but decreased in treated group and thus the loss of BWG in these rats (G3-G5) is parallel to decrease food consumption.

On the other hand, G3-G5 showed a significantly decrease in feed efficiency ratio (FER) as compared to G2 (Table 7). Since insignificant changes in food consumption was not parallel to the growth of rats. There was a negative BWG, which means body weight loss, in G3 and G5 with a maximum decrease in BDW in G5 which were supplied by a mixture of GSP and GSO. Similarly, Charradi *et al.*, (2011) have found that a grape seed and skin extract at high doses (500 mg per kg of body weight per day) protected rats against weight gain and dyslipidemia-associated pathologies when the rats were fed a high fat diet for 6 weeks. This poor body weight gain may be due to the overall increased degeneration of lipids and proteins as a result of the direct effects of the Chlorpyrifos CPF (Khalifa *et al.*, 2011)

Table (7) : Effect of GSP , GSO and GSP+GSO on growth parameters of rats.

Groups	Parameters		
	BWG (g/day)	FI	FER
G 1 (– ve)	0.81±0.145[a]	12.85±0.95[a]	0.063±0.011[a]
G2 (+ve)	0.65±0.048[b]	11.60±0.74[a]	0.056±0.007[a]
G3 + (5% GSP)	0.20±0.087[d]	10.15±0.71[b]	0.019±0.007[c]
G4 + (4% GSO)	0.34±0.077[c]	12.30±1.43[a]	0.028±0.008[b]
G5+ (5% GSP + 4% GSO)	0.35±0.125[e]	10.00±0.78[b]	0.036±0.013[d]

(BWG), Body weight gain daily food intake (FI), and feed efficiency ratio (FER) Different superscript letters in the same column show significant mean difference at P ≤0.05. The data represented as mean ± SD.

Effect of GSP and GSO on organs weight

The mean value of heart weight showed a significant decrease in G3 as compared to high fat group while G1, G4 and G5 showed non-significant difference in compared with G2 (Table 8). The liver weight was significantly increased in G2 and G5 when compared with G1. The mean values of kidney weight of G1, G3 and G4 were not significantly changed when compared with G2, meanwhile the mean value of G5 showed significant decrease in compared with G2 (Table 8). Wren *et al.,* (2001) have found a significant increase in food consumption in rats fed on grape seed extract diets as compared to control. This different results may be due to variations in the concentration of grape seed extract (4-5%) versus 2% Wren *et al.*, (2001). On the other hand, Kim *et al.,* (2010) reported that food intake, feeding efficiency, after 32 day experimental period were slightly high in the rats fed GSO (4.2 g/day).

Remarkable decease in heart weight and increase liver weight, with slight decrease in kidney weight following supplementation of GSP and GSO were noticed. The determination of heart weight and body weight in induced hypercholesterolemic animals is considered to be a positive factor to find out the prognosis of CVD.

Table (8): Effect of GSO and GSP on organs weight.

Groups	Organs weight (g)		
	Heart	Liver	Kidney
G 1 (– ve)	0.34±0.016[a]	2.98±0.28[c]	0.83±0.104a
G 2 (+ ve)	0.38±0.015[a]	4.32±0.45[a]	0.88±0.072[a]
G3+(5% GSP)	0.34±0.107[a]	3.54±0.54[bc]	0.77±0.149[ab]
G4+(4% GSO)	0.29±0.013[ab]	3.78±0.63[b]	0.84±0.091[a]
G5+ (5% GSP + 4% GSO)	0.33±0.067[a]	3.62±0.31[b]	0.70±0.126[ac]

Different superscript letters in the same column show significant mean difference at P ≤0.05. The data represented as mean ± SD.

Effect of GSP and GSO on lipid profile of rats

The mean values of total cholesterol (TC) were markedly elevated in G2, and significantly decreased in G1, G3 and G5 (Table 9). However, administration of GSP/GSO (in G3-G5) resulted in reduction in the rate of cholesterol absorption as reflected by alterations in serum and tissue cholesterol levels. Similarly, triglyceride (TG) demonstrated a significant decrease in G1, G3 and 5 in comparing with G2. Serum HDL-cholesterol values were increased significantly in G1 and G3-G5 when compared to G2 (Table 9). The mean values of LDL-C and VLDL-C showed significant decrease in G1-G5 as compared with G2. Therefore, the experiment was expended to monitor any changes in heart weight. An abnormal uptake of cholesterol from the high fat diet provided to diabetic animals and this led to increased levels of serum cholesterol Yasuda *et al.*, (2008). The obtained results are consistent with those of Ganjali *et al.*, (2012); Pilehvar *et al.,* (2013) and indicate that GSP and GSO are good replacers. GSP and GSO were shown to decrease the total and LDL-C cholesterol in serum and tissues as well as increasing the antioxidant status (Khor *et al.*, 1998). Furthermore, Natella *et al.*, (2002) reported that GSO improved resistance of LDL-C to oxidation in volunteers consuming a lipid-rich test meal. Thus, GSO and GSP would be considered as effective agents for lipid lowering purposes.

Table (9): Effect of GSP and GSO on lipid profile of rats

Groups	Parameters				
	TG(mg/dl)	TC(mg/dl)	HDL-C(mg/dl)	LDL-C(mg/dl)	VLDL-C(mg/dl)
G 1 (– ve)	114.06±6.38^{c}	162.86±13.^{b}	48.18±5.90^{bc}	33.31±9.13^{b}	32.57±2.73^{c}
G 2 (+ ve)	161.58±28.01^{a}	231.94±30.0^{a}	40.60±6.31^{c}	74.59±29.47^{a}	46.38±6.14^{a}
G3+(4%GSO)	153.46±21.73^{b}	200.10±15.3^{a}	70.48±11.85^{a}	42.96±17.10^{b}	40.02±3.12^{b}
G4+(5%GSP)	105.40±11.26^{c}	152.12±183^{b}	52.32±2.24^{b}	25.86±8.76^{b}	30.42±3.62^{c}
G5+(4% GSO + 5% GSP)	111.58±12.57^{c}	154.40±166^{b}	56.10±5.52^{b}	24.58±12.14^{b}	30.88±3.21^{c}

Different superscript letters in the same column show significant mean difference at P ≤0.05. The data represented as mean ± SD.

Effect of GSP and GSO on liver function

The serum levels of aspartate aminotransferases (AST), alanine aminotransferases (ALT), Alkaline Phosphatase (ALP) were significantly increased in rats fed only on high cholesterol diet (G2). The means of these enzyme were a significantly decreased in G1 and G3-G5 compared to G2 (Table 10). Moreover, the supplementation GSP and GSO showed marked hepatoprotective effect. This decrease in the level of ALT and AST could be a good indicator for the anti-inflammatory effects of GSP/GSO on liver. These results agree with those of Khalifa *et al.,* (2011), Uma Maheswari and Rao, (2005)

Increased lipid peroxidation is generally believed to be an important cause of the inhibition of oxidative stress related of various tissue injury, cell death and further progression of many acute and chronic diseases (Halliwell and Gutteridge, 1999). The increase in MDA and hydroperoxide levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms. Treatment with GSO could be

prevented significantly these changes. Hence, the mechanism of hepatoprotection of GSO may be due to its antioxidant effect. Since GSO has significantly increased the glutathione, SOD and CAT contents of the liver, it may also be useful in hepatotoxicity induced by other agents (Uma Maheswari and Rao, 2005)

The supplementation of GSO decrease the atherogenic factors, which indicates that GSO has cardio protective action, probably by reducing the factors activating apoptosis and improving the antioxidant status. On treatment with GSO, the apoptosis was reduced, proving the apoptosis reducing property of GSO on cholesterol and cholic acid-induced hypercholesterolemia model (Thiruchenduran *et al.*, 2011).

Table (10): Effect of GSP and GSO on liver functions of rats.

Groups	Parameters		
	ALT (U/L)	AST (U/L)	ALP (U/L)
G 1 (– ve)	139.62±11.95^{b}	15.18±1.79^{b}	338.04±32.98^{c}
G 2 (+ve)	165.32±30.83^{a}	28.96±7.44^{a}	690.04±58.08^{a}
G3+(5% GSP)	96.32±17.44^{cd}	17.74±2.73^{b}	518.18±143.90^{b}
G4+(4% GSO)	115.62±13.91^{bd}	18.50±2.00^{b}	488.20±107.89^{b}
G5+(5% GSP + 4%G GSO)	89.96±15.40^{c}	19.50±4.56^{b}	461.52±87.74^{bc}

Different superscript letters in the same column show significant mean difference at P ≤0.05. The data represented as mean ± SD.

Effect of GSP and GSO on oxidative stress

Malondialdehyde (MDA) was increased significantly in G2 as compared to G1 and G5. Meanwhile, the animals of G3 and G4 were decreased compared with G2. In addition, the antioxidant activity as measured by serum glutathione-S-transferase (GST) was significantly increased in G1 and G3-G5 compared with G2 (Table 11). From these obtained results, it can be concluded that GSP and GSO exhibited potential hypercholesterolemic antioxidants as they were able singly or in combination to lower cholesterol and protect liver against the oxidative stress. The histopathological examination reveals a potent protective action of GSO, rather than GSP, on heart, aorta, and liver.

Therefore, GSP and GSO mixture could be used as natural antioxidants to enhance the antioxidant properties of functional food and to lower hypercholesterolemia.

Table (11): Effect of GSP and GSO on oxidative stress of rats.

Groups	Parameters	
	Malondialdehyde (NMOL/ml)	Glutathione-S-Transferase (mg/dl)
G 1 (– ve)	3.39±0.099^{b}	195.38±11.25^{b}
G 2 (+ ve)	4.64±0.779^{a}	111.25±14.40^{c}
G3+(5% GSP)	4.57±0.550^{a}	220.12±14.40^{a}
G4+(4% GSO)	4.42±0.601^{ac}	187.62±11.43^{b}
G5+(5% GSP + 4% GSO)	3.82±0.474^{bc}	235.25±7.74^{a}

Different superscript letters in the same column show significant mean difference at P ≤0.05. The data represented as mean ± SD.

Histological examination

The heart of animals fed on hyperchloresterimic diet showed marked myocardial vacuolation, degeneration and subintimal mononuclear infiltration. While, animals fed on diet supplemented with GSP or combined with GSO showed normal separated myocardial fibers without any degenerative and inflammatory changes. The histological observation of heart section of GSP-treated rats showed myocardial degeneration associated with slight fibroblastic cells proliferation and hypertrophic cardiac muscle bundles (Table 12 and Fig.1). In addition, severe vacuolization in tunica media of the aorta of group G2 was noticed while, the animals of G3, G4 and G5 showed mild to moderate vacuolization (Table 12, Fig.1).

The livers of rats of G1 showed normal histopathological structure of the central vein and surrounding hepatocytes (Table 12 and Fig.1). Fatty changes in hepatocytes were observed in rats of G2 (severe), and those of G3 (mild), while no changes were occurred in rats of G4 and G5.

Table (12) : Effect of GSP and GSO on the histopathological alteration of the heart, aorta and liver of rats.

Histolost Alterations	G1 (-ve)	G2 (+ve)	G3 (5% GSP)	G4 (4% GSO)	G5 (5% GSP +4% GSO)
Myocardial degeneration and inflammation	–	+++	++	–	+
Vacuolization in tunica media of Aorta	–	+++	+++	++	++
Fatty change in hepatocytes	–	+++	+	–	–

– = nil + = mild ++ = moderate +++ = severe

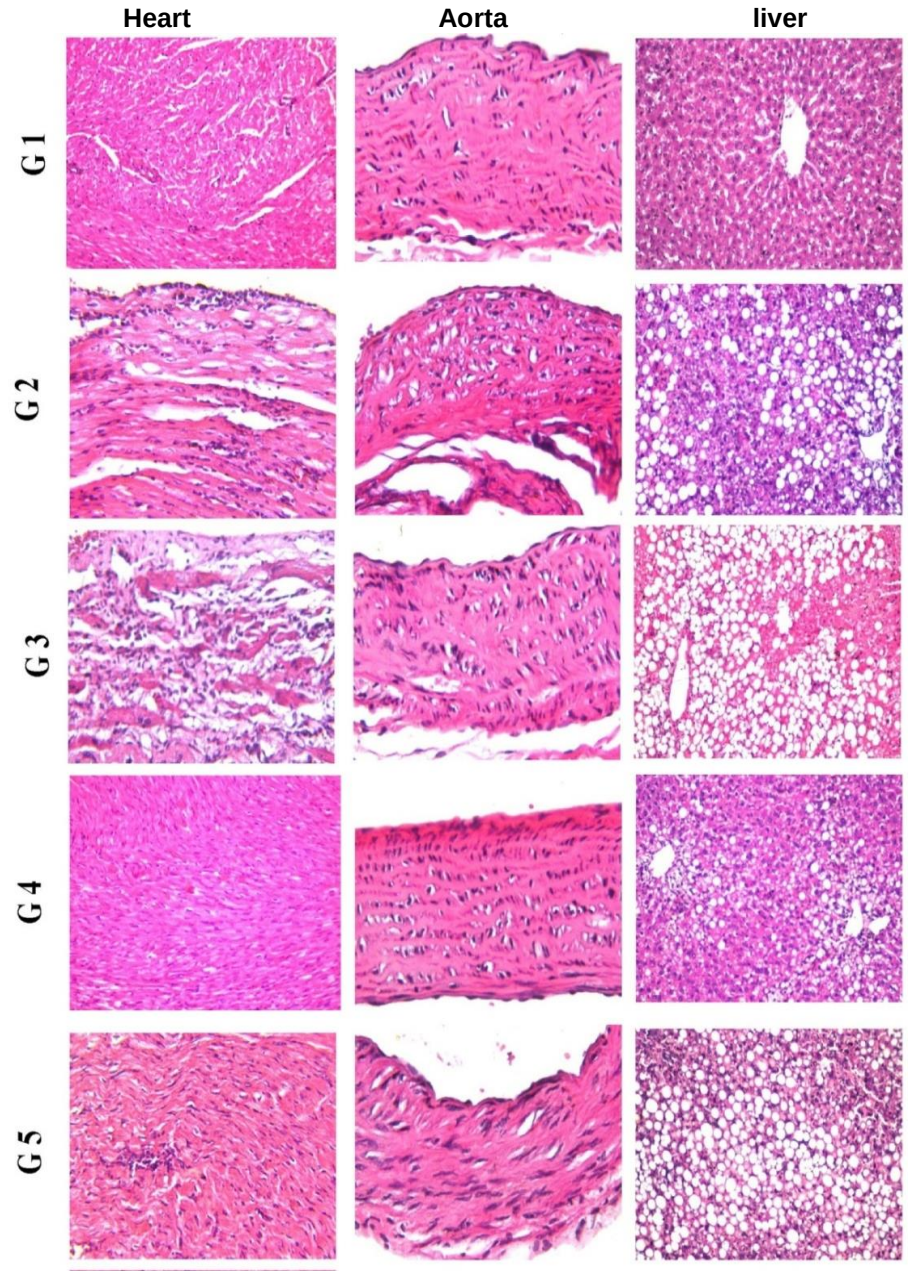

Figure 1 Histological alterations of the heart, aorta and liver of rats.

Therefore, GSP and GSO mixture could be used as natural antioxidants to enhance the antioxidant properties of functional food and to lower hypercholesterolemia. The heart of normal rats show normal myocardium (H&E, X100), Heart of rat from G 2 (+ve control) showing focal myocarditis (H&E, X200), heart of rat from G3 (5% GSP) increase of myocardial fibrosis (H&E, X200), heart of rat from G4 (4% GSO) showing myocardial fibers within the normal limits (H&E, X100), heart of rat from G5 (5% GSP and 4% GSO) showing mild inflammatory cells in myocardium (H&E, X100). Aorta of rat from G 1 (–ve control) showing normal histopathological structure of the tunica media and intima adventitia (H&E, X200), aorta of rat from G 2 showing vacuolization in the tunica media (H&E, X200), aorta of rat from G٣ showing mild vacuolization in media associated with swelling in the lining endothelium of the intima (H&E, X200), aorta of rat from G4 showing mild vacuolization in tunica media (H&E, X200), aorta of rat from G 5 showing no histopathological alteration (H&E, X200). Liver of rat from G 1 showing normal hepatocytes surrounding the central vein (H&E, X100), liver of rat from G 2 showing fatty change in diffuse manner (H&E, X100), liver of rat from G3 (5% GSP) showing fatty change in diffuse manner (H&E, X100), liver of rat from G٤ showing fatty change in the hepatocytes (H&E, X100), liver of rat from G5 (5% GSP and 4% GSO) showing fatty change in the hepatocytes (H&E, X100).

CONCLUSION

Finally, from all abovementioned obtained results in this study, it could be concluded that GSO can be used as edible oil. Also, GSO and/or GSP can be used as supplements and may provide health benefits in hyperlipidemia and related complications, which increase TG and TC and increase HDLc. GSP and/or GSO showed an obvious hypocholestrolemic effect that may have important pharmaceutical applications in the prevention and treatment of cardiovascular disease and atherosclerosis.

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التأثير الواقي لمسحوق بذور العنب والزيت المستخلص منه ضد ارتفاع مستوي الكوليسترول في فئران التجارب

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تهدف هذة الدراسة الي تقييم التأثير المضاد للكوليسترول لمسحوق وزيت بذور العنب علي دهون الدم في فئران تغذت علي غذاء مرتفع في محتواه من الكوليسترول.

تم تقدير الخواص الكيميائية لكل من الزيت والمسحوق وتقدير محتواهم من المركبات الفينولية. أوضحت النتائج ارتفاع محتوي البذور من المركبات الفينولية ومضادات الأكسدة الطبيعية ذات النشاط الحيوي والتي تلعب دورا هاما في الحفاظ علي صحة الأنسان.أظهر التقييم البيولوجي لكل من المسحوق والزيت بتركيزات ٤ و ٥% من غذاء الفئران المرتفع في محتواه من الكوليسترول انخفاض ملحوظ لكل من الكوليسترول الكلي و الجلسريدات الكلية والكوليسترول منخفض الكثافة . وتميز الزيت بخفض تركيز انزيمات الكبد و ارتفاع محتوي الكبد من الجلوتاثيون. أظهر الفحص الهستولوجي فعالية أعلي للزيت عن المسحوق في وقاية أنسجة الكبد والقلب و الشريان الأورطي. لذا فان استخدام مسحوق بذور العنب وكذلك الزيت المستخلص منه كمدعمات سيؤدي الي حدوث تحسن ملحوظ في المؤشرات الحيوية للفئران المغذاة علي غذاءمحتواه مرتفع من الكوليسترول وكذلك يمكن استخدامها كمضادات أكسدة طبيعة

الكلمات الدالة : غذاء مرتفع الكوليسترول – سحوق بذور العنب – زيت بذور العنب – لبيدات الدم – انزيمات الكبد – الفحص الهستولوجي للكبد.