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Magnification the use of some white grape seed that found in the AL baha area in applications of therapeutic nutrition

Magbolah, S. H. Al-Zahrany[*] and Hala M.A. Wahba[**]

[*]Biology Department, Faculty of Science, Al-baha University, KSA.
[**]Home Economics Department, Faculty of Sciences and Arts (Buljurshi) Al-baha University, KSA.
*Corresponding Author

Abstract

Grape seed extract is a natural plant substance that has a concentrated source of oligomeric proanthocyanidins (OPC). These anti-oxidants help protect cells from free radical damage and also promotes healthy. Also Grape seed extract is a medicinal herb used primarily for it's high proamthocyanidin content. Its a bioflavonoid which has demonstrated anti- oxidant properties and treatment for arthritis and other vascular problems. This study aimed to determine the effect of supplementation white grape seed that found in the baha area in applications of therapeutic nutrition through the preparation of the extract from the White grape seed and feeding rats on this extract and the effect of this extract on biochemical and immunological changes of infected mice with high blood fats and hepatitis. Thirty – six male albino rats weight (150 – 200 g) per each were used in the study. All rats (hyperlipidemic and hepatitis) were feed on casein basal diet with white grape seed extract (WGS) for 4 weeks for acclimatization, the control (-) rats fed on basal diet for four weeks. They hyperlipidemic and hepatitis rats were then divided into two groups. At the end of experiment the weight gain was calculated. Blood samples were used for estimation of serum glucose, liver functions (GOT and GPT) serum lipid profile, kidney functions, cellular and humoral immunity. Data showed that serum lipid profile, GOT and GPT activities decrease significantly ($p<0.05$) in all treated groups that fed on (5%,10% and 15%)WGS when compared with hyperlipidemic and hepatitis control positive. While the increased level of immunity. The best results for lipid profile were 15% WGS, for liver and kidney functions the best results were recorded for 10% WGS preparation, for immunity the best samples were noticed that of 10% group. Conclusively, this study suggests that white grape seed extract may be useful for patients who suffer from cardiovascular disease and hepatitis.

Keywords: hyperlipidemic ;hepatitis;Antioxidant; biochemistry ; immunity ; proamthocyanidin.

1. Introduction

The underlying pathology is atheromatous vascular disease, resulting in coronary artery disease (CAD), cerebrovascular disease, and peripheral vascular disease, and the subsequent development of heart failure and cardiac arrhythmias. The major risk factors for these disorders were recognized over many years, and they include high levels of low-density lipoprotein (LDL) cholesterol, smoking, hypertension, diabetes, abdominal obesity, psychosocial factors, insufficient consumption of fruits and vegetables, excess consumption of alcohol, and lack of regular physical activity ***(Yanni et al.,2015)*** . A wide variety of compounds have been identified in grapes, most of which with health promoting properties. A number of studies have shown that beneficial effects of grape and grape products consumption are related to the presence of polyphenols, mainly flavonoids and phenolic acids, with antioxidant, anti-inflammatory, antimicrobial,

antiviral, and cancer preventive properties ***(Wu, 2009)***. Flavonoids, stilbene, and proanthocyanidins are considered the most abundant class of bioactive compounds in grapes. It has also been demonstrated that grape juices, both white and purple, represent an important source of minerals, which would help explain the antioxidant and antimutagenic properties of grapes ***Dani et al., (2012)*** . We have recently shown that an extract from white grape juice could have a beneficial effect on radiocontrast medium toxicity in human renal proximal tubular cells and exert neuroprotective effect in a mouse model of experimental autoimmune encephalomyelitis ***Giacoppo et al., (2015) .*** the grape seeds extracts (GSE) have been studied or readily used for many diverse purposes: therapy in several cardiovascular disorders ***Afonso et al., (2013)***; reduction of oxidative stress and neuronal apoptosis related with diabetes mellitus ***Yonguc et al., (2015)*** ; protective activity against UVB radiation ***Filip et al., (2011)***; protection against early weaned stress syndrome in piglets ***Haom et al., (2015)***; fortification of yoghurts or even reduction of free formaldehyde at appreciable levels in the retanning process of leather ***(Bayramoglu , 2013)*** .There are also some studies that demonstrate the antioxidant and antibacterial activities of the GSE and also the antiviral effects against a number of viruses ***Joshi et al., (2015) .*** the polyphenolic profile of GSE is characterized by a very important presence of flavanols and some phenolic acids (e.g., gallic, protocatechuic, and caftaric acids) although in much less extent. Flavanol monomers (catechin, epicatechin, galocatechin, and epigallocatechin) are usually the most abundant compounds, followed by procyanidins (consisting of the flavan-3-ol units catechin and epicatechin simply linked by C-C bonds or doubly linked by an additional ether bond) ***Afonso et al., (2013)***. Polyphenols are bioactive food compounds that are primarily present in fruits and vegetables and exert many protective effects against cardiovascular disease ***(Bladé et al., 2010; Quiñones et al., 2013). Ward et al., (2004)*** studied that (grape S.E) provides a concentrated source of polyphenols. Most of which are proanthoeyandins polymeric proanthocy anidins are poorlyabsorbed in the small intesrine of humans. And exposure many result from metabolism to phenolic acid by colonic bacteria. Any biological effects of proanthocyanidin may be due to phenolic acid metanolites.Several phenolic acids have been identified as proanthocyanidin metabolictes. This work was conducted to study the effect of some white grape seed that found in the AL baha area in applications of therapeutic nutrition.

2. Materials and Methods

2.1. Rats

A total of 36 adult male albino rats (Sprague Dawley strain) were used in the investigation. The animal were obtained from the the Faculty of Medicine, Um Al Qura University . Each rats was housed in special cage under controlled condition every day. The animal were observed for external appearance, shape, color and distribution of hair and physical activity. All rats were fed one week on control diet before the beginning of the experiment for adaptation, the rats were weight tow is a week for 4 weeks. The diet was presented to rats in special covered cups to avoid food loss. All rats were provided with water by glass tubes through wire cage.The rats were fed a diet libitum through the period of experiment. The final weight was recorded for organs weight calculation. All the experiment occur in biological manufactory of faculty of Science AL-baha University .

2.2.Preparation of hepatitis rats

Normal healthy adult male albino rats were injection by Carbon tetrachloride (CCL4) twice weekly for two weeks(0.2 mg/100g) body weight according to the method described by ***(Lin and Lin, 2006)*** then investigated level of Got and GPT by random select to any rat to obtained sample blood serum, after positivism form infect rats were divided into 4 sub groups 4 rats in each sub group

2.3.Preparation of hypercholestrolemic rats

Normal healthy adult albino rats were fed on especial diet for inducing hyperlipidemic for 3 weeks according to the method described by ***(Rash wave, 1994)*** the investigation level of HDL, LDL, VLDL. by random select to sample blood serum after positivism from infect rats were divided 4 sub group 4 rats in each sub group.

2.4. Preparation of grape seeds extracts:

Three hundred and (5,10 ,15 grams) of fine powder of grape seeds were soaked in one liter of hot water (to obtain 5%,10 % and 15 % concentration) at 90°C for 2 hours and thereafter kept in a refrigerator with daily shaking for 5 days. The watery infusion was obtained in by filtration with double layers of gauze to get red of grape seeds extracts debris.

2.5. Preparation of basal diet:

Basal diet was prepared according to ***Reeves et al. (1993)***. It consists of 20 % protein, 10 % sucrose, 4.7% corn oil, 2% choline chloride, 1% vitamin mixture, 3.5 % salt mixture and 5% fibers. The remainder was corn starch up to 100 %.

2.6.Experiment Design :

The animal age (45 days) were used Divided into third major groups (hyperlipidemic , hepatits groups addition to negative control group) after that second and third major group were divided into 4 sub groups (6 group treatment by Wight grape seed with 3 concentration 5%, 10%, 15%) and one group positive controls has the diseased without treatment. That means all rats divided into 9 groups 4 rats in each sub group and fed several diets for 4 weeks.

Groups :

The First Group (4 rats) :

Healthy rats fed on basal diet as the control negative: fed on basal diet only.

The Second Main Group (16 rats):

Hepatitis rats were divided into 4 subgroup according to the following scheme 4 rats in each subgroup.

Subgroup (2): fed on basal diet only as the control positive.
Subgroup (2a): fed on basal diet with 5% white grape seeds extract.
Subgroup (2b): fed on basal diet with 10% white grape seeds extract.
Subgroup (2c): fed on basal diet with 15% white grape seeds extract.

The third Main Group (16 rats) :

Hyperlipidemic rats were divided into 4 subgroup according to the following sheme 4 rats in each subgroup.
Subgroup (3): fed on basal diet only as the control positive.
Subgroup (3a): fed on basal diet with 5% white grape seeds extract.
Subgroup (3b): fed on basal diet with 10% white grape seeds extract.
Subgroup (3c): fed on basal diet with 15% white grape seeds extract.

Blood samples were collected after 12 hour fasting at the end of the experiment. Using the retro - orbital method by means of a micro capillary glass tubes, blood was collected into a dry clean centrifuge tube and left to clot in a water bath (37ºC) at room temperature for half an hour. The blood was centrifuged for 10 minutes at 3000 r.p.m. to separate the serum. A pare of Serum was subsected to glucose determination and the reminder was carefully aspirated and transferred into clean quit fit plastic tubes and kept frozen at (20ºC) until analysis. The organs (liver, kidney, heart) were removed and washed in saline solution, weighted and kept in formalin solution (10%). Biochemical analysis were determined (Liver Function - Serum lipids — Kidney function– Immunity Induce – Serum Detoxifying Enzymes). Liver and kidney were studying histopathologically .

2.7. Biochemical analyses:

Concentrations of blood urea nitrogen ***(Patton and Crouch, 1977)***; uric acid **(*Fossati et al., 1980)*** and creatinine ***(Husdan and Rapoport, 1969)*** were estimated using specific diagnostic kits. The activity of aspartate amino transferase ***(Reitman and Frankel, 1957)*** was determined using standard reagent kits. Serum concentration of calcium ***(Gindler and King, 1972)*** was determined colorimetrically using specific diagnostic kit (BioMérieux, France). Magnesium was estimated colorimetrically by dye (methyl thymol blue) method ***(Connerty et al., 1971)***. Measurements were performed using spectrophotometer (Model T80, UV/visible, double beam, UK).

2.8. Assessment of oxidant/antioxidant parameters:

2.8.1. Determination of reduced glutathione (GSH):

GSH content of kidney tissue was determined according to ***Ellman (1959)***. The assay is based on the reduction of 5, 5´dithiobis (2-nitrobenzoic acid) with glutathione producing a yellow compound. The reduced chromogen was directly proportional to GSH concentration and its absorbance was measured at wave length 405 nm.

2.8.2. Determination of superoxide dismutase (SOD):

The renal SOD activity was measured according to ***Nishikimi et al. (1972)***. This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitro blue tetrazolium dye.

2.8.3. Determination of glutathione peroxidase (GPx):

Renal GPx activity was measured by the method of ***Paglia and Valentine (1967)***. This assay is an indirect measurement of the activity of GPx. The oxidized glutathione (GSSG), produced upon reduction of organic peroxide by GPx, was recycled to its reduced state by the enzyme glutathione reductase (GR). The reaction was initiated by the addition of hydrogen peroxide, and the oxidation of NADPH to NADP + is accompanied by a decrease in the absorbance at wave length 340 nm.

2.8.4. Determination of catalase (CAT):

Renal CAT activity was measured in tissue homogenate according to ***Aebi (1984)***. The assay is based on that catalase reacts with a known quantity of hydrogen peroxide. This reaction is stopped after exactly one minute with catalase inhibitor. In the presence of peroxidase, the remaining hydrogen peroxide reacts with 3, 5-Dichloro-2-hydroxybenzenesulfonic acid and 4-aminophenazone to form a chromophore with a colour intensity inversely proportional to the amount of catalase.

2.9.1: Immunity study: Assessment of both cellular and humoral immunity efficiency was carried out in the Faculty of Science -AL-Baha University . Total serum proteins were determined by using biuret reaction according to ***Weichselbaum (1946)*.**

2.9.2: Quantitative estimation of fractions of serum proteins: according to the technique described by ***Laemmli (1970).***Cellular immune response : Separation of lymphocytes : ***(Boyum, 1968 and Burrels and Wells,(1977).*** Total lymphocyte count ***(Hudson and Hay, 1980).***According to viability cell count ***(Rai-El-Balahaa et al.,*** *1985).* ***(Denise et al., 1992)***Phagocytosis according to *:(****Woldehiwet and Rowan, 1990).***

2.10. Histological procedure:

The testes and liver of rats were taken and fixed in neutral formalin 10 % solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. These specimens were then stained with Hematoxylen and Eosin(H&E).and examined microscopically according to **Bancroft and Stevens (1990).**

2.11. Statistical analysis:

Data were presented as means ± SD. Statistical analysis was performed using computerized Statistical Package of Social Sciences (SPSS) program with one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests according to ***Snedecor and Cochran (1986).***

3. Results

3.1–Body weight gain (BWG) and food efficiency ratio (FER):

Data of tables (1) show BWG and FER of hepatic and hyperlipidemic rats. Rats as affected by feeding on white grape seed extract (WGS) (1), (2), & (3). It could be observed table (1) that due to hepatic and hyperlipidemic rats loss of weight occurred and negative value of BWG was recorded; BWG for control (+) group was 250.0 ± 7.3^{a} this was not due to loss of appetite but was possibly due to physiological disorders causing lower food efficiency ratio which decreased from 1.20 ± 0.04^{a} to 0.45 ± 0.01^{c}

3.2. Immunity Parameters:

The results of ELISA test showed that hepatic and hyperlipidemic rats induced significant ($P < 0.05$) increases in serum tumor necrosis factor alpha (TNF-α) and inflammatory cytokines IL4 and IL8. Experimental diets supplemented with white grape seed extract (WGS) significantly ($P < 0.05$) decreased the elevated levels of serum TNF-α, IL4 and IL8 in hepatic and hyperlipidemic rats when compared to the positive control group2 and 3 (Table 2). Data of tables (3) show Effect of white grape seed extract (WGS) on differential leukocyte count in hepatic and hyperlipidemic rats . It was notice that phagocyites and hymphocyte for control (+) were lower than that of the control (-) group. Also it is evident that all groups fed on (WGS) treatments showed significantly higher differences ($P<0.05$) compared with control (+). As for as neutrophils and eosinophils groups fed on (WGS) treatment showed significantly higher difference ($P<0.05$) levels compared with control positive and Oral administration of watery infusions (WGS) decreased levels of neutrophils and eosinophils count in hepatic and hyperlipidimic rats when compared with control (+) rats. Table (4) show effect of white grape seed extract (WGS) on humoral immunity of hepatic and hyperlipidemic rats (Globulins fractions) . It was revealed that globulins

Table 1: Effect of white grape seed extract (WGS) on body weight gain (BWG) and feed efficiency ratio (FER) in hepatic and hyperlipidemic rats.

Groups	Initial b.wt.	Final b.wt.	BWG	FER
Group 1 Normal control	255.0±7.5[a]	300.0±7.3[a]	17.64	1.20±0.04[a]
Group 2 hepatic control	250.0±7.3[a]	275.0±6.1[c]	10.00	0.45±0.01[c]
Group 2a 5% WGS	254.0±8.6[a]	285.0±4.2[b]	12.20	0.90±0.02[b]
Group 2b 10% WGS	252.5±6.9[a]	288.0±6.3[b]	14.05	0.92±0.03[b]
Group 2c 15% WGS	251.0±7.1[a]	289.0±5.9[b]	15.13	0.90±0.01[b]
Group 3 hyperlipidemic control	250.0±7.1[a]	287.0±5.9[b]	14.8	0.90±0.02[b]
Group 3a 5% WGS	253.5±6.9[a]	294.0±5.9[a]	15.97	0.95±0.01[b]
Group 3b 10% WGS	254.0±6.3[a]	295.0±6.2[a]	16.14	1.11±0.01[a]
Group 3c 15% WGS	255.0±6.2[a]	296.0±6.1[a]	16.07	1.09±0.02[a]

Means ± SD with different superscript letters in the same column are significant at $P < 0.05$ using one way ANOVA test. n=4 rats.

Table 2: Effect of white grape seed extract (WGS) on serum tumor necrosis factor alpha (TNF-α) interleukins IL-4 and IL-8 in hepatic and hyperlipidemic rats.

Groups	Serum concentration of TNF-α (ng/ ml)	IL4 (pg/ml)	IL8 (pg/ml)
Group 1 Normal control	1.95 ± 0.01[e]	855.8±3.5[c]	244.4±4.3[e]
Group 2 hepatic control	4.82 ± 0.07[a]	944.4±5.3[a]	632.1±3.3[a]
Group 2a 5% WGS	2.65 ± 0.01[c]	810.8±5.2[d]	332.2±5.3[c]
Group 2b 10% WGS	2.45 ± 0.02[c]	813.2±4.2[d]	335.2±5.3[c]
Group 2c 15% WGS	3.76 ± 0.02[b]	844.5±2.5[b]	425.1±5.3[b]
Group 3 Hyperlipidemic control	3.32 ± 0.05[b]	846.2±5.2[b]	427.1±3.2[b]
Group 3a 5% WGS	2.95 ± 0.05[c]	820.8±4.2[d]	332.2±5.3[c]
Group 3b 10% WGS	2.00 ± 0.01[d]	830±3.2[c]	312.2±2.3[d]
Group 3c 15% WGS	1.96 ± 0.02[c]	840.8±6.2[d]	332.2±4.3[c]

Means ± SD with different superscript letters in the same column are significant at $P < 0.05$ using one way ANOVA test. n=4 rats.

Table 3: Effect of white grape seed extract (WGS) on differential leukocyte count in hepatic and hyperlipidemic rats

Groups	Neutrophils (%)	Eosinophils (%)	Lymphocytes (%)	Monocytes (%)
Group 1 Normal control	58.4±3.2[b]	6.6±0.5	25.6±0.5[a]	8.4±0.8[b]
Group 2 hepatic control	62.2±2.4[a]	6.8±0.3	20.4±0.6[b]	11.8±0.2[a]
Group 2a 5% WGS	60.8±4.2[b]	6.3±0.2	19.8±0.6[b]	10.60±0.2[a]
Group 2b 10% WGS	59.2±4.4[b]	6.3±0.4	19.9±0.5[b]	10.30±0.2[a]
Group 2c 15% WGS	58.8±2.6[b]	6.9± 0.2	19.9±0.9[b]	9.70±0.3[a]
Group 3 hyperlipidemic control	79.3±2.9[b]	7.8± 0.3	20.6±0.6[b]	9.90±0.2[a]
Group 3a 5% WGS	75.3±3.6[b]	6.9± 0.3	19.6±0.7[b]	11.80±0.2[a]
Group 3b 10% WGS	58.5.±3.6[a]	5.2 ±0.4	19,3 ±0.8[b]	10.40±0.4[a]
Group 3c 15% WGS	57.5.±3.6[b]	5.8 ±0.4	9,3 ±0.8[b]	10.40±0.4[a]

Means ± SD with different superscript letters in the same column are significant at $P < 0.05$ using one way ANOVA test. n=4 rats.

Table 4: Effect of white grape seed extract (WGS) on humoral immunity of hepatic and hyperlipidemic rats (Globulins fractions)

GLOBULINS Groups	ALPHA (g / dl)	BETA (g / dl)	GAMMA (g / dl)
Group 1 Normal control	1.27±.14[a]	1.41 ±0.1 [a]	1.85±0.04[a]
Group 2 hepatic control	1.15±.27[b]	1.21±0.1 [b]	1.67±0.11[b]
Group 2a 5% WGS	1.02±.009[c]	1.33±0.12 [b]	1.95±0.04[b]
Group 2 b 10% WGS	1.33±.8[a]	1.29±0.1 [a]	2 .02±0.06[b]
Group c 15% WGS	1.26±.3[b]	1.43± 0.7	2.41±0.1[b]
Group 3 hyperlipidemic control	1.092±.07[b]	1.23± 0.8	1.3±0.1[b]
Group 3 a 5% WGS	1.25±.1[b]	1.16± 0.13	1.6±0.05[b]
Group 3b 10% WGS	1.5.±.4[c]	1.11 ±0.14	1,84 ±0.09[b]
Group 3c 15% WGS	1.31.±.6[b]	1.09 ±0.04	1,75 ±0.9[b]

Means ± SD with different superscript letters in the same column are significant at $P < 0.05$ using one way ANOVA test. n=4 rats.

fractions for control (+) were less than that of control (-). Also it is clear that group 2c fed on 15% (for alpha ,beta & gamma globulins) treatments showed significantly differences (P<0.005) higher levels compared with control (+) being ($1.15\pm.27^{b}$, 1.21 ± 0.1^{b} and 1.67 ± 0.11^{b}) and ($1.26\pm.3^{b}$, 1.43 ± 0.7 and 2.41 ± 0.1^{b}) respectively for globulins (alpha, beta and gamma).

3.3. Biochemical analyses:

Data in Table (5) showed that hepatic and hyperlipidemic rats significantly increased blood glucose and decreased insulin levels when compared to the normal control . Diets supplemented with white grape seed extract significantly decreased blood glucose and increased insulin levels as compared with the positive (diabetic) control group. Tables (6 and 7) showed that serum lipid fraction for control positive and different groups of hepatic and hyperlipidemic rats. fed on different (WGS) . It's clear that, cholesterol, triglyceride LDL and VLDL for control (+) rats were higher than that of the control negative group. It was noticed that best group for TC, HDL and LDL was that of 15% (WGS) . TG & VLDL best group was that fed 10 % and15 % oil, treatment showed significant difference (P<0.05) lower compared with control positive being. Table (8) show serum blood urea nitrogen (BUN), uric acid (UA) and creatinine (Cr.) for control positive and difference groups of hepatic and hyperlipidemic rats. feed on white grape seed extract. It could be noticed that uric acid and urea for control positive were higher than that of the control (-). Also, it's clear that all groups fed on white grape seed extract (showed significantly differences (P<0.05) lower levels compared with control (+), in particular the 15 % group 2 and 3.

Table 5: Effect of white grape seed extract (WGS)) on blood glucose (BG) and insulin hormone levels in hepatic and hyperlipidemic rats

Groups	BG (mg/dL)	Insulin (µU/ml)
Group 1 Normal control	112.7 ± 1.9^{d}	45.5 ± 0.1^{a}
Group 2 hepatic control	355.0 ± 2.1^{a}	15.3 ± 0.1^{d}
Group 2a 5% WGS	322.0 ± 1.5^{b}	18.1 ± 0.1^{c}
Group 2b 10% WGS	310.5 ± 2.3^{bc}	19.2 ± 0.2^{c}
Group 2c 15% WGS	215.0 ± 2.1^{b}	30.8 ± 0.2^{b}
Group 3 hyperlipidemic control	210.5 ± 3.1^{b}	39.8 ± 0.1^{b}
Group 3a 5% WGS	219.0 ± 2.1^{b}	36.8 ± 0.2^{b}
Group 3b 10% WGS	215.0 ± 3.1^{b}	37.8 ± 0.2^{b}
Group 3c 15% WGS	195.0 ± 1.3^{c}	43.3 ± 0.1^{a}

Means ± SD with different superscript letters in the same column are significant at $P < 0.05$ using one way ANOVA test. n=4 rats.

Table 6: Effect of white grape seed extract (WGS) on serum total cholesterol (TC) and triglycerides (TG) levels in hepatic and hyperlipidemic rats.

Groups	TC (mg/dL)	TG (mg/dL)
Group 1 Normal control	99.50 ± 3.1^{c}	79.00 ± 3.4^{d}
Group 2 hepatic control	135.70 ± 4.1^{a}	105.00 ± 5.2^{a}
Group 2a 5% WGS	122.80 ± 4.8^{b}	100.00 ± 3.1^{b}
Group 2b 10% WGS	120.60 ± 3.7^{b}	98.50 ± 4.5^{b}
Group 2 c 15% SS WGS	118.50 ± 3.6^{b}	95.50 ± 4.3^{b}
Group 3 hyperlipidemic control	250.5±3.1^{b}	170.8±6.1^{b}
Group 3a 5% WGS	220.70 ± 3.6^{c}	111.50 ± 5.6^{c}
Group 3b 10% WGS	215.80 ± 3.8^{c}	120.50 ± 7.1^{c}
Group 3c 15% WGS	195.0±1.3^{c}	130.3±0.1^{a}

Means ± SD with different superscript letters in the same column are significant at $P < 0.05$ using one way ANOVA test. n=4 rats.

Table 7: Effect of white grape seed extract (WGS) on serum Lipids profile in hepatic and hyperlipidemic rats.

Groups	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Group 1 Normal control	55.7 ± 2. 52^{c}	28.2 ± 2.2^{b}	15.8 ± 2^{a}
Group 2 hepatic control	36.70 ± 3.51^{b}	77.6 ± 4.3^{a}	21.4 ± 3^{a}
Group 2a 5% WGS	39.70 ± 7.2^{b}	63.1 ± 2.1^{b}	20 ± 2.2^{a}
Group 2b 10% WGS	45.7 ± 6.02^{b}	55.2 ± 4.3^{d}	19.7 ± 3.3^{c}
Group 2 c 15% SS WGS	45.30 ± 2.52^{b}	54.1 ± 1.1^{c}	19.1 ± 3.21^{b}
Group 3 hyperlipidemic control	43.3±7.51^{b}	173.04 ± 2^{a}	34.16 ± 3^{a}
Group 3a 5% WGS	42.3 ± 2.52^{c}	155.4± 4.1^{c}	22.3 ± 5.3^{b}
Group 3b 10% WGS	44.3 ± 4.3^{c}	147.4 ± 2.1^{a}	24.1 ± 2.1^{a}
Group 3c 15% WGS	44.51±4.1^{c}	124.43 ± 2^{a}	26.06 ± 2^{a}

Means ± SD with different superscript letters in the same column are significant at $P < 0.05$ using one way ANOVA test. n=4 rats.

Table 8: Effect Effect of white grape seed extract (WGS) on Blood urea nitrogen (BUN), uric acid (UA) and creatinine (Cr.) in hepatic and hyperlipidemic rats.

Groups	Parameters BUN (mg/dL)	UA (mg/dL)	Cr. (mg/dL)
Group 1 Normal control	36.3 ± 1.4[d]	1.50 ± 0.01[a]	0.75 ± 0.01[d]
Group 2 hepatic control	56.0 ± 2.4 [a]	1.49 ± 0.06[a]	1.64 ± 0.04[a]
Group 2a 5% WGS	54.1 ± 2.6 [b]	1.48 ± 0.02[a]	1.63 ± 0.02[b]
Group 2b 10% WGS	52.8 ± 2.3 [b]	1.49 ± 0.04[a]	1.62 ± 0.02[b]
Group 2 c 15% WGS	51.5 ± 2.1[b]	1.50 ± 0.01[a]	1.60 ± 0.03[b]
Group 3 hyperlipidemic control	45.3 ± 3.1	2.6 ± 0.02[a]	2.70 ± 0.03[b]
Group 3a 5% WGS	41.4 ± 2.5[c]	1.47 ± 0.05[a]	0.94 ± 0.02[c]
Group 3b 10% WGS	40.6 ± 1.8[c]	1.48 ± 0.01[a]	0.92 ± 0.03[c]
Group 3 c 15% WGS	42.7 ± 1.9[c]	2.5 ± 0.01[a]	0.91 ± 0.02[c]

Means ± SD with different superscript letters in the same column are significant at $P < 0.05$ using one way ANOVA test. n=7 rats.

Table 9: Effect of white grape seed extract (WGS) on liver function of in hepatic and hyperlipidemic rats.

Groups	Liver Enzymes GOT (U / L)	GPT (U / L)
Group 1 Normal control	62.3 ± 2.52[d]	26 ± 2[b]
Group 2 hepatic control	150.3 ± 2.52[a]	83.3 ± 4.51[a]
Group 2a 5% WGS	65.3 ± 4.51[d]	26 ± 4[bc]
Group 2b 10% WGS	73.6 ± 3.51[c]	24.3 ± 5.51[bc]
Group 2c 15% SS WGS	75.3 ± 3.51[c]	35.3 ± 4.51[b]
Group 3 hyperlipidemic control	87.6 ± 3.52[d]	22.67 ± 7.51[c]
Group 3a 5% WGS	62.33 ± 2.5[d]	25.3 ± 3.51[bc]
Group 3b 10% WGS	65.6 ± 8.02	35.6 ± 3.51[b]
Group 3c 15% WGS	72.33 ± 7.5[e]	23.3 ± 6.5[c]

Means ± SD with different superscript letters in the same column are significant at $P < 0.05$ using one way ANOVA test. n=4

Table (9) show serum GPT and GOT activies for control (+) and different group of hepatic and hyperlipidemic rats. fed on different concentration of white grape seed extract (WGS). It's clear that GPT and GOT for control positive were higher than control (-). Also it could be noticed that all group fed on (WGS) treatments showed significantly differences ($P<0.05$) lower level compared to that of the control (+) groups provided that lowest GPT and GOT levels recorded to 5%(WGS) group3 (62.33 ± 2.5, 25.3 ± 3.51), 5% group 2 (65.3 ± 4.51, 26 ± 4) respectively

3.2. Oxidant/antioxidant parameters:

It is clear from Table (10) that injection by carbon tetra chloride twice weekly for two weeks consecutive days caused significant decreases in the activity of tissue superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) enzymes when compared with the normal control group. Oral administration of white grape seed extract (WGS) increased the activity of SOD, GPx and CAT enzymes when compared with hepatic and hyperlipidemic rats.

Table 10: Effect of white grape seed extract (WGS) on activity of antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) in liver tissue of hepatic and hyperlipidemic rats.

Parameters Groups	SOD (U/mg protein)	GPx (nmol/min/mg protein)	CAT (nmol/min/mg protein)
Group 1 Normal control	59.70 ± 2.22^{a}	0.69 ± 0.01^{a}	0.199 ± 0.01^{a}
Group (2) hepatic control	39.50 ± 2.68^{5}	0.28 ± 0.04^{d}	0.148 ± 0.02^{d}
Group 2a 5% WGS	47.74 ± 3.26^{c}	0.35 ± 0.03^{b}	0.155 ± 0.01^{b}
Group 2b 10% WGS	48.95 ± 2.38^{a}	0.45 ± 0.01^{b}	0.168 ± 0.01^{b}
Group 2c 15% WGS	50.25 ± 2.73^{b}	0.55 ± 0.01^{c}	0.185 ± 0.02^{c}
Group 3 hyperlipidemic control	49.31 ± 3.24^{b}	0.56 ± 0.01^{c}	0.198 ± 0.02^{c}
Group 3a 5% WGS	56.25 ± 3.23^{b}	0.42 ± 0.04^{c}	0.190 ± 0.02^{c}
Group 3b 10% WGS	57.30 ± 7.53^{b}	0.45 ± 0.03^{c}	0.193 ± 0.01^{c}
Group 3c 15% WGS	57.30 ± 3.53^{b}	0.55 ± 0.02^{c}	0.195 ± 0.03^{c}

Means ± SD with different letters superscripts (a, b, c, d) in the same column are significant at $P < 0.05$ using one way ANOVA test.
Unit of GPx = nmol of GSH utilized/min/mg protein.
Unit of CAT = nmol of H_2O_2 utilized/min/mg protein. n= 4 rats.

4. Discussion

The present study aimed to evaluate the effect t of white grape seed extract (WGS) on body weight gain (BWG) , feed efficiency ratio (FER), immune status, blood glucose (BG), insulin, total cholesterol (TC), triglycerides (TG), blood urea nitrogen (BUN), uric acid (UA) and creatinine (Cr) levels as well as activities of liver tissue antioxidant enzymes in hepatic and hyperlipidemic rats.

Results of the present study showed that diets fortified with white grape seed extract (WGS), increased body weight gain and improved feed efficiency ratio in hepatic and hyperlipidemic rats. These finding were in agreement with those reported by ***Choi et al.,(2010)*** there were no significant differences in the weight gain among the groups, but liver and kidney weights were significantly increased in cholesterol supplement groups compared to the normal diet group .

Free radicals are chemically unstable atoms that cause damage to cell lipids, proteins and DNA due to imbalance between the generation of reactive oxygen species(ROS) and the antioxidant enzymes. Free radicals are known to be the main cause of oxidative stress which is grossly implicated in the pathogenesis of various diseases such as cancer, diabetes and cardiovascular diseases. Natural antioxidants have been gained much attention from consumers because they are considered safer than synthetic antioxidants. Natural antioxidants derived from fruits, vegetables, spices, and cereal grains are very effective and can protect the human body from oxidative stress caused by ROS ***(Sreeramulu and Raghunath, 2010).*** A Previous study showed that the elevated glucose , Lipids profile and liver function concentrations significantly affected production of cytokines **.** In accordance, the results presented herein showed changes in the level of the pro-inflammatory cytokine, TNF- α, the anti-inflammatory cytokine, IL-4, and the chemoattractant cytokine, IL-8, between hepatic , hyperlipidemic rats and the other groups. The changes in the TNF-α level could be due to the changes in insulin secretion, where insulin modulates the development of the inflammatory reaction to allergen challenge by its ability to modulate the production/release of TNF-α. ***(Martins et al., 2010; and Abdel-Salam,2012).*** The later author reported that white grape seed extract (WGS) produced an immunomodulatory effect in hepatic and hyperlipidemic rats. In gamma-irradiated rats, ***Assayed (2010)*** found that the anti-inflammatory and antioxidant effects of anthocyanins are of relevance to potential cardioprotective effects of bilberry and other berries. Antihypertensive, lipid-lowering, hypoglycemic, and antiobesity effects would also be cardioprotective ***(Zafra-Stone et al., 2007; Erlund et al. 2008).*** Table (4) Effect of white grape seed extract (WGS) on differential leukocyte count in hepatic and hyperlipidemic rats . It was revealed that globulins fractions for control (+) were less than that of control (-). Also it is clear that group fed on white grape seed extract (WGS) treatments showed significantly differences (P<0.05) higher levels compared with control (+) .This study agree with ***Andreucci et al., (2015)*** they found extract from white grape juice could have a beneficial effect on radiocontrast medium toxicity in human renal proximal tubular cells and exert neuroprotective effect in a mouse model of experimental autoimmune encephalomyelitis ***Giacoppo et al., (2015)***. The present results revealed that diet supplemented with white grape seed extract (WGS) significantly increased serum insulin and decreased blood glucose levels in hepatic and hyperlipidemic. These results were in agreement with those reported by ***Montagut et al., (2010)*** they found GSPE treatments are at improving glucose metabolism in hyperinsulinemic animals. The present results showed that white grape seed extract (WGS) on serum Lipids profile in hepatic and hyperlipidemic rats produced Lipids profile effects. These effects were previously reported by ***Kim et al.,(2014)*** they found that dietary supplementation of chardonnay grape seed flour reduces plasma cholesterol concentration, hepatic steatosis, and abdominal fat content in high-fat diet-induced obese hamsters. These polyphenols mainly originate from the skins and seeds of grapes and, because of differences in vinification, their variety and concentration is higher in red wine than in white wine. In vitro and ex vivo studies have shown that some of these polyphenols are able to slow down LDL-cholesterol oxidation, stimulate NO production, influence prostaglandin synthesis and inhibit platelet aggregation ***(van de Wie,2002).*** Table (9) show white grape seed extract (WGS) on liver function of hepatic and hyperlipidemic rats. It's clear that (GOT) and (GPT) for control positive were higher than control (-). Also it could be noticed that all group fed on white grape seed extract showed significantly differences (P<0.05) lower level compared to that of the control (+) groups provided that lowest (GOT) and (GPT) levels study and agree with ***Ahn, (2002)*** who found the favorable effects of tannin substances in persimmon and grape seeds which well scavenge DPPH radical, and are effective in reducing TBARS and phosphatidlycholine hydroperoxide formation within the body and liver . In the present work, oral administration of white grape seed extract (WGS) caused protective effect as they reversed biochemical and histological alterations induced by carbon tetrachloride in rats. In addition, these white grape seed extract (WGS) produced an antioxidant activity as evident by increasing content of reduced glutathione and restoring activities of antioxidant (SOD, GPx and CAT) enzymes in liver tissue. This study agree with ***Choi et al., (2010)*** The supplementation of grape seed extract caused a 52% and 56% increase, respectively, in the GPx and CAT activities compared to the high cholesterol diet group (CHOL). Finally oral administration of white grape seed extract (WGS) caused protective and raising immunity of hepatic and hyperlipidimic rats. These treatments may promising to avoid the health problems facing patients inflicted with hepatic and hyperlipidemia rat.

Histopathological findings:

Liver: Microscopically, liver of rat from group 1 (control -) showed the normal histology of hepatic lobule (Fig. 11). Conversely, liver of rat from group 2 (hepatic control) revealed vacuolar degeneration of hepatocytes (Fig. 12), local area of hepatic necrosis associated with mononuclear cells infiltration (Fig.13). Examined sections of rat from group 2 (5%) showed vacuolar degeneration of hepatocytes (Fig. 14). Moreover, liver of rat from group 2 (10%) revealed hyperplasia of epithelial lining bile duct, few mononuclear cells infiltration and granularity of hepatocytes (Fig. 15).Apparent normal hepatocytes was noticed in liver of rat from group 2 (15 %) (Fig. 16). Meanwhile, liver of rat from group 3 **(hyperlipidemic control)** showed vacuolar degeneration of hepatocytes (Fig. 17). However, liver sections of rat from group 3 (5% diet) revealed slight granularity of hepatocytes (Fig. 18) while, Some examined sections from rat in group 3 (10% diet) showed no histopathological changes (Fig. 19). while, other sections revealed atrophy of hepatocytes (Fig. 19).

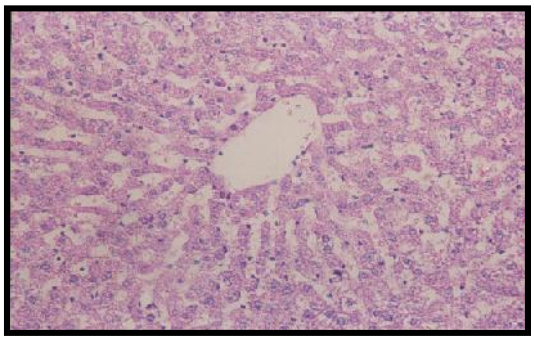

Fig.(11): Liver of rat from group1(control -) showing normal histology of hepatic laubule.(H and E X 200).

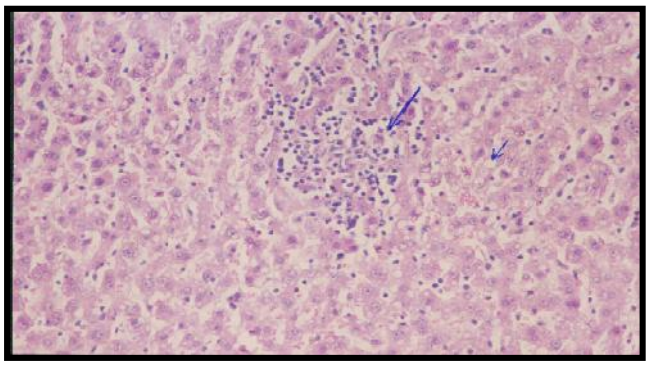

Fig.(12): Liver of rat from group2 (control +) showing vacuolar degeneration of hepatocytes local area of hepatic necrosis associated with mononuclear cells infiltration. (H and E X 200).

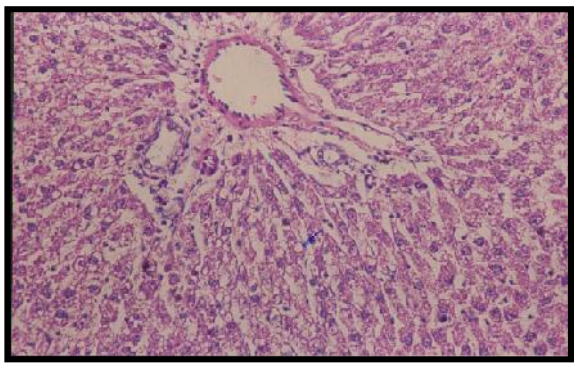

Fig.(13): Liver of rat from group2 (5%) showing vacuolar degeneratio of hepatocytes. (H and E X 200).

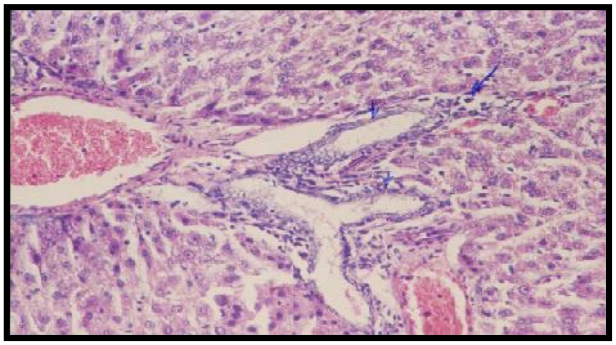

Fig.(14): Liver of rat from group 2 (10% extract) showing hyperplasia of epithelial lining bile duct few mononuclear cells infiltration and granularity of hepatocytes. (H and E X 200).

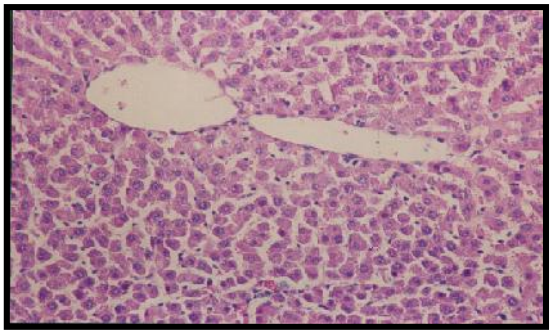

Fig.(15): Liver of rat from group 2 (15 %) showing apparent normal hepatocytes. (H and E X 200).

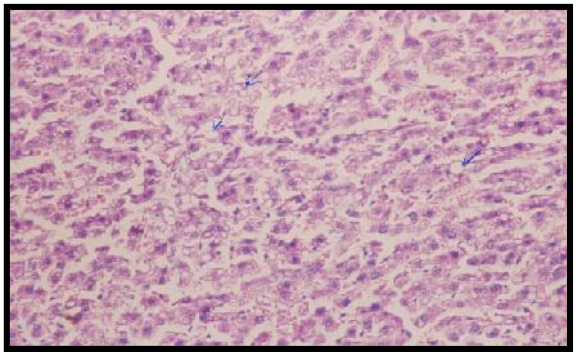

Fig.(16): Liver of rat from group 3 (control +) showing vacuolar degeneration of hepatocytes.

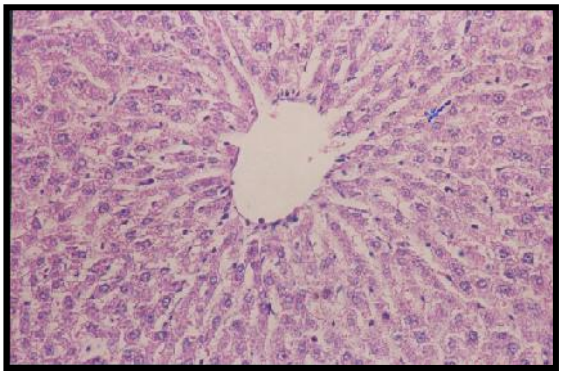

Fig.(17) : Liver of rat from group 3 (5%) showing slight granularity of hepatocytes.(H and E X 200).

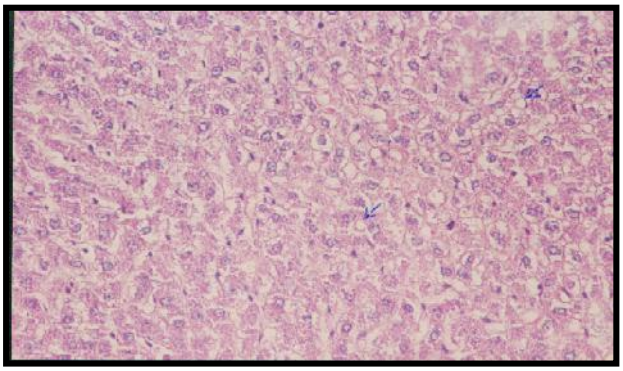

Fig.(18): Liver of rat from group 3 (10% vacuolations of some (H and E X 200).

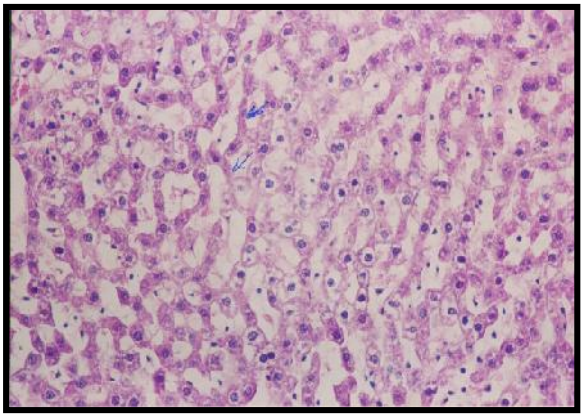

Fig.(19): Liver of rat from group 3 (15%) showing no histopathological (H and E X 200)

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العلاجية التغذية تطبيقات في الباحة في منطقة الموجودة الأبيض العنب بذور بعض من الاستفادة تعظيم "
"

مقبولة سالم هلال الزهرانى[1] هالة محمد على وهبة[2]

1- قسم الأحياء – كليه العلوم – جامعة الباحة – المملكة العربية السعودية0

2 – قسم الاقتصاد المنزلي – كلية العلوم والآداب ببالجرشى – جامعة الباحة – المملكة العربية السعودية 0

الشقوق نفسها من حماية على الحية الخلية تساعد أكسدة مضادات على تحتوى الق و الطبيعية المصادر من الأبيض العنب بذور مستخلصات تعتبر النواحي من عديدة النباتات الطبية التي لها استخدامات من الأبيض العنب بذور مستخلصات و العموم وجه على الصحة تحمى بالتالي و الضارة الحرة التغذية تطبيقات فى الباحة منطقة في الموجودة الأبيض العنب بذور بعض من الاستفادة يهدف البحث الى تعظيم.بأمان استخدامها يمكن التي و الطبية و تأثير هذا المستخلص على التغيرات البيوكيميائيه المستخلص هذا على الفئران تغذية و الأبيض العنب بذور مستخلص من إعداد خلال من وذلك العلاجية لذلك تم استخدام 36 فار البينو وزن (150 – 200 جم) قسمت هذه الفئران إلى ثلاث .والمناعية للفئران المصابة بارتفاع دهون الدم والتهاب الكبدي0
مجموعات رئيسيه ، المجموعة الأولى مكونةمن (4 فئران) تتغذى على الغذاء الاساسى والمجموعة الثانية (16 فار) مصابة بارتفاع مستوى الكوليسترول والمجموعة الثالثة مصابه بالتهاب الكبد وتم تغذيتهم على الغذاء الاساسى بالاضافه الى مستخلص بذور العنب الواقعة تحت الدراسة بنسبة (5% و10% و15%)وقد استمرت التجربة 4 أسابيع وتم وزن الفئران أسبوعيا للوقوف على الوزن المكتسب ونسبة كفاءة الغذاء كذلك تم جمع عينات كما تم الحصول على سيرم الدم الذي تم فية تقدير نسب الجلوكوز والكوليسترول والدهون الثلاثية والليبوبروتينات .الدم فى بداية وبعد انتهاء فترة التجربة المرتفعة والمنخفضة الكثافة ومنخفضة الكثافة جدا وإنزيمات الكبد ووظائف الكلى و كذلك تقدير قيمة الخلايا البلعمية والخلايا الليمفاوية وتقدير الجلوبيولين ولقد أوضحت الدراسة أن الفئران التي . والالبيومين والبروتينات الكلية بالدم الكامل علاوة على بعض القياسات الأخرى مثل وزن الأعضاء الداخلية تغذت على الوجبات الأساسية والمضاف اليها مستخلص بذور العنب قد أظهرت مستوى اقل فى دهون الدم وارتفاع المناعة بالمقارنة بالمجموعة الضابطة كما ظهر انخفاض معنوي في مستوى إنزيمات الكبد ووظائف الكلى بالمقارنة بالمجموعة الضابطة المصابة. وكان أفضل تأثير على دهون الدم هو عند استخدام 15%. وكانت أفضل المعاملات لتحسين وظائف الكبد والكلى هي مجموعة 10% مستخلص بذور العنب أما أفضل تأثير على المناعة 10% مستخلص – وخلصت الدراسة إلى أن إضافة مستخلص بذور العنب يؤدي الى تحسين حالة المرضى الذين يعانوا من أمراض القلب والكبد 0

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