Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, Egypt
28(4), 1131-1141, 2020
Website: http://ajs.journals.ekb.eg

Effect of Grape Pomace on Some Biological Assays of Experimental Rats

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Maha A Mahmoud, Hala A Thabet*, El-Shahat G El-Dreny
Special Food and Nutrition Dept, Food Technol Res Inst, Agric Res Center, Giza, Egypt

*Corresponding author: drhala.thabet@gmail.com

Received 9 July, 2020 Accepted 2 October, 2020

Abstract

This study is carried out to reduce the environmental impact of grape proceeding wastes through study the effect of the grape pomace (GP) utilization on some biological parameters which reflect the health status of rats fed on high fat diets. It was carried out via an experimental biological study, where adult healthy male albino rats, weighing 185±5g, were fed on high-fat diets supplemented with 5, 10, and 15% of GP. The proceeding results showed that the protein amount in GP was up to 8.75%, lipids content was 7.38% and the total dietary fiber was 46.63%. K, Mg, Ca, Na, P and S were found in higher concentrations. The lipids of GP contained 85.75% unsaturated fatty acids (19.14% monounsaturated and 66.60% polyunsaturated fatty acid).The predominant fatty acid was linoleic acid (65.29%). Total phenolic (TP) compounds (expressed as mg gallic acid equivalent (GAE) per g sample) were about 50.35mg GAE/g, total flavonoids (expressed as mg rutin equivalent (RE) per g sample) were 22.25 mg RE/g and the DPPH radical scavenging activity was 51.92%. Gallic acid was the most abundant polyphenols, (9.76 mg/100g). Catechin was the most abundant flavonoid compound (52.5 mg/100g). Resveratrol as stilbenes content was 14.11 mg/100g. Supplementation of the high-fat diet with GP resulted in a significant reduction in cholesterol, triglycerides, LDL-C and vLDL-C levels of the tested rat groups. Treated rats had higher mean values of HDL-C than that fed on HFD only. The best result for estimation of specific studied parameters (AST, ALT, urea, uric acid and creatinine) revealed that the rat groups fed on the high fat diet containing 15% GP was the healthiest. It was found that a non-significant alteration in the levels of AST, ALT, uric acid and creatinine, compared with the negative control rat group was detected. Consequently, it could be concluded that GP could be considered as a source of healthy compounds that could be applied in animal feed, pharmaceutical, cosmetic or food industries.

Keywords: Grape pomace (GP), Total phenolic, Total flavonoids, Resveratrol, Biological assay

1 Introduction

Grapes are considered as the most value traditional fruit crops with a great global production. After processing, approximately 2.5 million tons of grape by-products are produced (Food and Agriculture Organization of the United Nations, 2012). Grape pomace (GP) is the main residue of grape processing, composed of skins, seeds and stems. It comprises approximately 25% of harvested grape weight during grape processing. After the manufacture of grape juice and wine, grape pomace represents a high waste problem. Such wastes could offer fundamental economic benefits if found value added tools (Fontana et al 2013).

In Egypt, grapes are considered the second main crop production after citrus, almost 200,000 Ton fruits, in which the by-product pomace represents about 10 to 20 thousand Tons/year (Ministry of Agriculture Egypt, Agricultural Development System Project, ADS. 2006).

In recent years, the possibility of conversion by-product wastes to nutraceutical and value added products have an increased attention for their potential health benefits. These several biological properties are believed to be due to the presence of many healthy components. Dietary fibers are presence in grapes by-product with high concentrations having beneficial physiological effects; including

gastrointestinal function improvement. Dietary fibers, also, caused a reduction in total cholesterol, low density lipoprotein (LDL-C) and moderation of postprandial insulin response (Llobera and Cañellas 2007).

The grape seed pomace oils rich in oleic and linoleic acids. Alpha-tocopherol was the most abundant tocopherol in the oil (Mildner-Szkudlarz and Bajerska 2013).

Phenolic compounds represent one of the most numerous, important and widely distributed groups of natural products in plant kingdom. Grape pomace is wealthy in secondary metabolites as phenolic components (Manach et al 2005).The majority of phenolic compounds in grape waste are flavonoids and phenolic acids which have been reported to have beneficial effects on the lipid metabolism (Lee et al 2009). Flavonoid intake has been inversely impact in relation to the coronary heart disease. Resveratrol is a major polyphenol compound in grape that is thought to be a potential contributor of several beneficial properties such as decreasing insulin resistance, preventing the heart failure and hypertension (Hertog 1993).

In addition, Lee et al (2009) reported that supplementation with grape skins was more effective in protecting against oxidative damage, through improving the antioxidant defense system in both rats fed a high-fat diet or fed a low-fat diet.

As is well known, environmental factors (e.g., soil, geographical location, and climate) and agronomic practices play key roles in grape pomace composition and associated properties. So, the current research concerned with studying of the effect of by product Egyptian grape pomace on health status parameters of rats fed on high fat diets. In addition, to investigate its antioxidant activity to assess its capacity as a source of natural antioxidants, thereby reducing the environmental effect of grape-producing waste.

2 Materials and Methods

2.1 Materials

The grape pomace (GP), in this study was collected from El-Kroom Company, Alexandria Governorate, Egypt, which consist of skins, seeds and stems. Fresh GP was spread into thin layers in plastic trays and dried at (20-22°C) in a well-ventilated room. Then, it was grounded into powder that was passed through the 40mesh sieve and was kept at -18°C in a deep freezer until used.

All solvents, standards and chemicals were manufactured by Sigma-Aldrich Chemicals Co., USA and were purchased from Cornell lab company, Egypt. Analytical kits were manufactured by Randox Laboratories Ltd., Diamond Road, Crumlin, Co., Antrim BT29 4QY, United Kingdom and obtained from Biodiagnostic company, Egypt.

2.2 Methods

2.2.1 Determination of proximate chemical compositions of GP

Moisture, protein, total fats, crude fibers, ash, soluble dietary fiber and insoluble dietary fiber contents of GP were determined according to AOAC (2006). Total dietary fiber was obtained by adding soluble dietary fiber and insoluble dietary fiber. Available carbohydrates based on dry weight were calculated by the differences as: 100 – (ash +protein + total fats + crude fibers) in 100 g of GP.

2.2.2 Determination of minerals contents in GP

Mineral contents were determined by using Flame Atomic Absorption Spectrophotometer instrument, AAS (Model 3300, Perkin-Elmer, Beacons field, UK) by wet digestion as the procedure of the AOAC (2006) method.

2.2.3 Determination of fatty acids in GP

Fatty acid methyl esters were prepared from total lipid and injected into Gas chromatography system (GC), Agilent 6790N series according to ISO-12966-2 (2011) which was described in International Organization for Standardization (ISO).

2.2.4 Determination of total phenolic compounds (TP), total flavonoids (TF) compounds and radical scavenging activity (RSA) in GP

The same procedure of sample extraction as described by Yilmaz and Toledo (2006) was used to determine TP, TF and RSA. The total phenolic contents of GP were determined using the Folin-Ciocalteu method as described by Arnous et al (2001). The total flavonoids were determined by using Joyeux et al (1995) method.

The antioxidant activity of GP was estimated by determining the radical scavenging activity (RSA%) of GP extract by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay as described by Brand-Williams et al (1995). The radical scavenging activity was calculated using the formula:

$$\% \text{ Inhibition} = [(A_b - A_s) / A_b)] \times 100.$$

Where: A_b is absorbance of blank,
A_s is the absorbance of the GP extract

2.2.5 Identification of phenolic acids, flavonoids and stilbenes in GP

A High Performance Liquid Chromatography (HPLC), Agilent, Germany 1200 system equipped with a variable wavelength detector was used to determine phenolic acids, flavonoids and resveratrol as stilbenes. Samples preparation and chromatographic conditions were similar to those described by Sagdic et al (2011).

2.2.6 Biological assay

Adult healthy male albino rats (30 rats), weighing 185 ± 5g, were obtained and housed at the animal house of Faculty of Veterinary Medicine Kafar El Shiekh University. Rats were randomly divided into five groups of six rats each. Rats were fed on a basal diet (BD) which was prepared according to Reeves et al (1993). Animals were acclimatized to the laboratory conditions for one week before starting the experiment.

The tested rats were isolated into two primary groups. The first one (six rats) was benefited from (BD) and kept as a negative control group (NC). The second (24 rats) were fed on a high-fat diet (HFD) for two weeks according to Wang et al (2007). After that the last group was divided into four subgroups, each one consists of six rats. The first subgroup was fed on the high-fat diet only until the end of experiment and kept as a positive control group (PC), while the other three subgroups were fed on the high-fat diet supplemented with 5, 10, and 15% of GP and known as 5% GP, 10% GP and 15% GP, respectively.

At the end of the experiment (60 days), the tested rats were overnight fasted and then were sacrificed under anesthesia. Rat's blood were drawn into tubes and centrifuged at 3000g for 15 min. at 4^{o}C to obtain serum that were kept frozen at -18^{o} C until analysis.

Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and Triglyceride (TG) Serum contents were determined according to the methods of Allain et al (1974), Lopes-Virella et al (1977) and Fossati and Prencipe (1982), respectively. Very low density lipoprotein cholesterol (vLDL-C) and Low density lipoprotein cholesterol (LDL-C) were calculated using Friedewald equations (Friedewald et al 1972) as follows:

vLDL-C (mg/dl) = Triglycerides/5.
LDL-C (mg/dl) = (Total cholesterol –HDL-C) –vLDL-C.

Serum creatinine, urea and uric acid were estimated according to Bonsens and Taussky (1984), Patton and Crouch (1977) and Fossati et al (1980), respectively. The activities of Serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were determined using the methods of Bergmeyer and Harder (1986).

2.2.7 Statistical Assessment

Statistically, the results were analyzed using a one-way variance analysis (ANOVA). The Duncan test was used to assess variations between treatments. All p values of < 0.05 have been deemed significant (Bouveresse et al 2011)

3 Results and Dissction

3.1 Chemical composition of GP

The proximate chemical composition of the GP is shown in **Table 1**. Moisture content is 6.61%. This ratio is in agreement with Tangolar et al (2009) who reported that moisture content in seeds of seven grape varieties is between 4.95 to 6.54%. But Ziarati et al (2017) found that it is ranged from 3.40 to 3.87%. The amount of protein is corresponded to 8.75 % of sample analyzed. It was closely with Sousa et al (2014), meanwhile, Guemour et al (2010) who reported that it was 14%.

With regard to the lipid content, its value was 7.38% and nearly with findings of Gülcü et al (2018) who mentioned that the oil content in wine by products (pomace) was 8.9%.

With respect to the amount of total dietary fiber, its value was 46.63%. Such result is agreed with Sousa et al (2014) who found that it is 46.17%. While, Bampi et al (2010) found that total dietary fiber was by 31.66%. GP fibers are associated with polyphenols with antioxidant activity. So, GP fibers are structurally different from those found in other cereals and other fruits.

Table 1. Proximate chemical composition of GP

Parameters	(%)
Moisture	6.61±0.03
Ash*	7.20±0.10
Protein*	8.75 ±0.16
Total lipids*	7.38±0.04
Crude fiber*	24.07±0.21
Available carbohydrate*	52.60±0.36
Total dietary fiber	46.63
Soluble dietary fiber	4.37
Insoluble dietary fiber	42.26

*On dry weight basis. Each value represents the mean ± standard deviation

The study of Deng et al (2011) on five varieties of GP indicated that insoluble DF weighed more than 95.5% of total DF in all samples. In the present study the insoluble DF weighted more than 90 % of total DF. Although data about the soluble and insoluble fiber contents of GP vary from study to other, there is no doubt that GP fiber is low in solubility.

3.2 Determination of fatty acids in GP

The analysis of the fatty acid composition of the GP oil is described in **Table 2**. Palmitic, oleic and linoleic acids were the abundant fatty acids in GP oil. The highest fatty acid content was linoleic acid (65.29%), followed by oleic (18.7%), palmitic (9.24%) and stearic acids (4.6%). These findings were agreed with Anđelković et al (2015) who found that the highest fatty acid in the grape pomace oil was linoleic (72.4%). Gülcü et al (2018), also, mentioned that, in general, the highest fatty acid was linoleic acid, followed by oleic, palmitic, and stearic acids.

It could be noticed, in the present study, that the lipids of GP represented about 85.74% unsaturated fatty acids (19.14% monounsaturated and 66.60% polyunsaturated fatty acids). On the other hand, Fernandes et al (2013), in a previous study, reported that the content of monounsaturated fatty acids reached to 21.29% in some varieties of grape seeds.

The beneficial properties of unsaturated fatty acids, particularly with respect to the cardiovascular system are known. Grape seed oil is rich in unsaturated fatty acids, such as oleic and linoleic acids. The poly-unsaturated fatty acids, such as linoleic and linolenic acids, are essential for the human metabolism because of the lack of enzymes responsible for synthesis of these fatty acids. For this reason, they could be used as foods and so its oil (Diab et al 2017).

Table 2. Relative abundance (%) of fatty acids in grape pomace

Fatty acid		Relative abundance (%)	Type
Palmitic acid	C16:0	9.24	Saturated fatty acid 14.26%
Stearic acid	C18:0	4.60	
Arachidic acid	C20:0	0.25	
Behenic acid	C22:0	0.17	
Palmitoleic acid	C16:1	0.20	Monounsaturated fatty acid 19.14%
Oleic acid	C18:1	18.73	
Gadoleic acid	C20:1	0.21	
Linoleic acid	C18:2	65.29	Polyunsaturated fatty acid 66.60%
γ Linolenic acid	C18:3 n6	0.49	
α Linolenic acid	C18:3 n3	0.82	

3.3 Content of mineral in GP

According to the present results of the minerals content (mg/100g) shown in **Table 3** K, Mg, Ca, Na, P and S, with descending order, were the major elements in GP. While, Fe, Zn, Cu and Mn were presented in small quantities. These results are in accordance with those of Ziarati et al (2017) who reported that K had the highest content in GP followed by Ca.

While, Gülcü et al (2018) showed that Fe was the main element in grape juice pomace (10.6 mg/100g) followed by P, Mn and Zn (2.1, 1.6 and 1.2 mg/100g, respectively). The current results showed some small differences compared to literature. These differences can be probably due to the parts of grape, processing equipment contamination, soil structure and fertilizer in growing stage.

Cetin et al (2011) mentioned that K levels were higher than those of Na and led to a mineral balance that favors hypertension control. A diet rich in K lowers blood pressure and consequently the risk of morbidity and mortality due to cardiovascular diseases. In addition, K intake can decrease urinary Ca excretion and consequently reduce the risk of develop in osteoporosis.

Table 3. Minerals content of GP

Minerals	Amount (mg/100g*)
Potassium (K)	3668.73
Magnesium (Mg)	1243.58
Calcium (Ca)	1053.14
Sodium (Na)	323.29
Phosphorus (P)	219.57
Sulfur (S)	159.50
Iron (Fe)	10.63
Zinc (Zn)	2.30
Copper (Cu)	2.04
Manganese (Mg)	1.42

***** as basis

3.4 Total phenolic compounds, total flavonoids and DPPH in GP

As observed in **Table 4** the content of total phenolic compounds in GP, which expressed as mg gallic acid equivalent (GAE) per gram weight of sample, was 50.35 mg GAE/g. This is in agreement with Rockenbach et al (2011) who tested TP content in four grape pomace varieties and reported that it was ranged from 33 to 75 mg GAE/g. While, Butkhup et al (2010) found that TP content in GP was 480 mg GAE/g. Several factors including environment, degree of maturity, berry size, and variety of grapevines identify phenolic composition in GP (Pourali et al 2014).

Table 4. Total phenolic compounds, total flavonoids and DPPH in GP

Item	Value
Total phenolic compounds	50.35±0.18*
Total flavonoids	22.25±0.13**
RSA % using DPPH	51.92±0.07***

* mg gallic acid equivalent per g sample as basis (mg GAE/g)

** mg rutin equivalent per g sample as basis (mg RE /g)

*** %

Moreover, **Table 4**, showed that the content of total flavonoids in GP, which expressed as mg rutin equivalent (RE) per gram weight of sample, was 22.25 mg RE /g and the DPPH radical scavenging activity was 51. 92%. These results are in same line of Hogan et al (2010). But, according to the study of Xu et al (2016) TF in GP was reached to more than four times of the present result. The difference may be due to the type of extraction method employed.

3.5 Identification of phenolic compounds

Classification of polyphenols (phenolic compounds) according to the number of hydroxyl groups and the way of bonding of aromatic ring is based on four main groups. Phenolic acids, flavonoids, lignans and stilbenes (Fabjanowicz et al 2018).

Fourteen phenolic acids in GP were identified using HPLC as presented in **Table 5** gallic, protocatchoic, catechol, 4-aminobenzoic, chlorogenic, P-OH- benzoic, caffeic, caffeic, vanillic, p-coumaric, caffeine, ferulic, Iso-ferulic, α coumaric and ellagic As can be seen, gallic acid had the highest value (9.76 mg/100g).

In addition, through HPLC analysis, 11 flavonoid compounds, catechin, naringin, rosmarinic, hesperdin, rutin, quercetrin, hespertin, naringenin, quercetin, kampferol and apigenin were found. Catechin was the most abundant flavonoid compound. These findings are in agreement with Gülcü et al (2018) who reported that catechin and gallic are the most phenolic compounds in GP. Catechins are incorporated into the LDL particles (Suzuki-Sugihara et al 2016), resulting in a reduced oxidization ability of LDL. Moreover, from the same Table, resveratrol as stilbenes content was14.11mg/100g.This is a natural polyphenolic compound which shows beneficial health effects such as, anticancer, antimicrobial, antioxidant activity and decrement in levels of TC and TG (Simental-Mendía and Guerrero-Romero 2019).

3.6 Biological analysis of GP

As shown in **Table 6** there were no significant differences in all tested groups in feed intake. On the other hand, PC group significantly different comparing with other groups in weight gain. This increment was may be due to feeding this group on high fat diet only. These findings seem to be in agreement with Cho et al (2013) who reported that supplementation mice diet with grape pomace extract did not significantly change feed intake among groups.

Data presented in **Table 7** demonstrate that the mean values of serum cholesterol, triglycerides, LDL-C and vLDL-C (mg/dl) were significantly

increased (P< 0.05) in the PC in comparison with the NC group. This observation was in agreement with Diab et al (2017). The increment percentage in total cholesterol value in PC in relative to NC was about 85.5 %. While in case of HDL-C this ratio decreased to be about 33.82 %. It could be noticed that supplementation high-fat diet with 5, 10 and 15% of GP resulted in a significant reduction in total cholesterol (TC) values. Rat groups which received high fat diets with all the previous concentrations of GP had lower mean values of triglycerides (TG), LDL-C and vLDL-C. On the other hand, the same treated rats had higher mean values of HDL-C than that fed on HFD only. The best result for lipid fractions compared with PC was noticed in 15% GP followed by 10% GP and the lowest decrement was in 5% GP.

Table 5. Chromatographic compounds analysis of phenolic acids, flavonoids and stilbenes

Phenolic acids	Amount (mg/100g)	Flavonoids	Amount (mg/100g)	Stilbenes	Amount (mg/100g)
Gallic	9.76	Catechin	52.50	Resveratrol	14.11
Protocatchoic	5.27	Naringin	10.70		
Catechol	2.82	Rosmarinic	16.14		
4-aminobenzoic	0.41	Hesperdin	5.23		
Chlorogenic	7.25	Rutin	2.23		
P-OH- benzoic	5.92	Quercetrin	2.00		
Caffeic	1.35	Naringenin	3.1		
Vanillic	6.72	Quercetin	2.56		
ρ-Coumaric	0.10	Hespertin	2.08		
Caffeine	6.23	Kampferol	0.16		
Ferulic	0.12	Apigenin	0.18		
Iso-ferulic	0.76				
α coumaric	0.03				
Ellagic	0.13				

Table 6. Effect of supplementation with GP on weight gain, feed intake and feed efficiency ratio in tested rats

Rat groups	Initial weight (g)	Final weight (g)	Weight gain (g)	Feed intake (g)	Feed efficiency ratio
NC	185.33^{a}±1.55	215.51^{b}±1.64	30.18^{b}±1.52	825.78^{a}±1.04	0.037^{b}±1.11
PC	183.00^{a}±2.51	231.67^{a}±2.51	48.67^{a}±2.7	820.63^{a}±1.63	0.059^{a}±1.24
5% GP	184.67^{a}±1.51	217.11^{b}±1.52	32.44^{b}±2.51	826.68^{a}±1.08	0.039^{b}±1.51
10% GP	188.54^{a}±2.51	219.24^{b}±1.57	30.70^{b}±2.64	819.25^{a}±1.05	0.037^{b}±1.84
15% GP	190.00^{a}±1.55	221.33^{b}±1.15	31.33^{b}±1.70	824.32^{a}±1.02	0.038^{b}±1.37

Each value represents the mean ± standard deviation (SD) NC = Negative control PC=Positive control
GP = Grape pomace 5% GP= HFD+5% GP 10% GP= HFD+10% GP 15% GP= HFD +1 5%GP
The mean values within the same column with different superscript alphabets indicate significant differences (P<0.05)
Feed efficiency ratio was calculated as gain of weight (g) / total feed intake (g)

Table 7. Effect of supplementation with GP on lipid profile of the tested rat groups serum

Rat groups	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
NC	89.66 ^{e}±5.03	103.00^{d} ±4.9	45.33^{a} ±1.5	23.73^{e} ±4.2	20.60^{d} ±0.9
PC	166.33^{a} ±1.5	178.00^{a} ±4.0	30.00^{d} ±2.0	100.73^{a} ±2.2	35.60^{a} ±0.8
5% GP	131.33^{b} ±3.5	140.00^{b} ±3.0	38.00^{c} ±2.0	65.33^{b} ±4.3	28.00^{b} ±0.6
10% GP	121.33^{c} ±3.5	125.66^{c} ±2.5	41.00^{b} ±1.0	55.20^{c} ±3.3	25.13^{c} ±0.5
15% GP	98.33^{d} ±2.5	108.66^{d} ±2.5	41.69^{b} ±0.6	34.90^{d} ±1.9	21.73^{d} ±0.5

Each value represents the mean ± standard deviation (SD) NC = Negative control PC=Positive control GP = Grape pomace 5% GP= HFD+5% GP 10% GP= HFD+10% GP 15% GP= HFD+15%GP

The mean values within the same column with different superscript alphabets indicate significant differences (P <0.05)

These findings are in good agreement with a study by Smith et al (2017) who investigated the effect of rats' diets containing different amounts of GP (6.9%-20.7%) on blood lipid profile and found that as GP increased, the blood triglycerides and very low-density lipoproteins (vLDL-C) decreased, while the high-density lipoprotein (HDL-C) increased. On the other hand, the increment in low-density lipoprotein (LDL-C) and total cholesterol remained constant.

In the same direction, El Ayed et al (2017) mentioned that grape seed and skin extracts successfully backed triglycerides, cholesterol, and LDL cholesterol to near the control level, while the HDL-C level was maintained higher than the control level in rats that fed on a high fat diet with grape seed and skin extracts.

Martin-Carron et al (2000) stated that the assayed polyphenols rich grape product was successful in reducing total cholesterol and LDL-C in serum by improving HDL: LDL ratio and the atherogenic index in hypercholesterolemic rats.

The improvement in lipid profile of the studied rats according to the current research as a result of supplementing the HFD diet with GP may be due to the dietary fiber content (DF) in GP. Since, soluble dietary fiber can decrease blood cholesterol levels and glycemic responses. Whereas, in the large intestine, DF is fermented, fiber strengthens the colonic environment. In addition, it slows and interferes with cholesterol and bile acid absorption. Insoluble dietary fiber has vital effects by increasing fecal bulk, having benefits in intestinal motility, lowering gastric emptying, and promoting satiety (García-Lomillo and González-SanJosé 2017).

As seen in **Table 8** it could be noticed that rats' serum of PC had a significant higher mean values of AST, ALT, urea, uric acid and creatinine than that of NC. When GP levels (5, 10 and 15%), were added to the high fat diet of rats, the levels of these parameters were decreased in comparison to the PC group. Furthermore, by increment of GP levels in rats' diets, the decrement in these parameters was observed.

Thus, the best result for these studied parameters (AST, ALT, urea, uric acid and creatinine) were noticed in the group of rats fed on the high fat diet containing 15% GP. It could be detected that there was a non-significant changes in the levels of AST, ALT, uric acid and creatinine compared with (NC).

In the present study, the HFD stimulate an increase in AST and ALT serum levels, which is in agreement with Lacerda et al (2018). The raise in urea, uric acid and creatinine levels in PC is in the line with Gara et al (2017) and Diab et al (2017) who found that serum uric acid, urea, and creatinine were significantly increased in HFD groups for male and female rats.

The present study revealed a significant reduction in AST and ALT values in the studied rats when GP was added to HFD. This observation is agreed with Al-Attar (2015) who found that the grape seed oil is able to reduce the activity of AST and ALT in rats as well as, decrease urea, uric acid and creatinine levels in groups fed on high fat diet and GP.

Moreover, Amin et al (2018) studied the Renoprotective effect of grape seed extract in diabetic rats and found a significant improvement in liver and kidney functions'.

The improvement in both liver and kidney vital functions is attributed to the presence of phenolic compounds (Spacil et al 2008).

Table 8. The levels of AST, ALT, urea, uric acid and creatinine in the tested rat groups serum

Rat groups	AST U/L	ALT U/L	Urea mg/dl	Uric acid mg/dl	Creatinine mg/dl
NC	40.00^{d}±2.08	28.83^{c} ±1.58	36.32^{d} ±1.66	2.80^{c} ±0.10	0.73^{d} ±0.06
PC	63.66^{a} ±4.33	43.33^{a} ±1.70	71.00^{a} ±3.05	6.56^{a} ±0.44	2.72^{a} ±0.16
5% GP	57.00^{ab} ±0.57	42.22^{a} ±0.17	60.66^{b} ±2.84	6.10^{a} ±0.06	2.05^{b} ±0.03
10% GP	52.00^{bc} ±0.57	36.62^{b} ±1.70	53.00^{c} ±0.57	5.19^{b} ±0.03	1.50^{c} ±0.09
15% GP	45.33^{cd} ±2.72	30.41^{c} ±0.61	46.00^{c} ±2.08	3.20^{c} ±0.03	0.88^{d} ±0.06

Each value represents the mean ± standard deviation (SD) NC = Negative control PC=Positive control
GP = Grape pomace 5% GP= HFD+5% GP 10% GP= HFD+10% GP 15% GP= HFD+15%GP
The mean values with different superscript alphabets indicate significant differences (P <0.05)

4 Conclusion

Grape pomace is rich in mineral and dietary fibers. It is, also, a good source of total phenolic and total flavonoids which provide its antioxidant activity potential. Supplementation high fat diet with GP exhibited beneficial effects on blood lipid profile. As well as, on liver and kidney functions.GP could be as a source of healthy and technological compounds that should be applied in animal feed, pharmaceutical, cosmetic or food industry to improve stability and nutritional characteristics in the future.

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مجلة اتحاد الجامعات العربية للعلوم الزراعية، جامعة عين شمس، القاهرة، مصر
مجلد(28)، عدد(4)، 1131-1141، 2020
Website: http://ajs.journals.ekb.eg

تأثير مخلف تصنيع العنب علي بعض الإختبارات البيولوجية لفئران التجارب

[80]

مها عبدالله محمود – هاله على ثابت* – الشحات جمعه الدرينى

قسم الأغذية الخاصة والتغذية – معهد بحوث تكنولوجيا الأغذية – مركز البحوث الزراعية – الجيزة – مصر

*Corresponding author: drhala.thabet@gmail.com

Received 9 July, 2020 Accepted 2 October, 2020

الموجـــــز

أجريت هذه الدراســـة بغرض تقليل تأثيرالمخلفات التصـنيعيه للعنب علي البيئه. وذلك عن طريق دراسـة تأثيرها علي بعض المعايير البيولوجية التي تدل علي الحاله الصحيه للفئران المغذاه علي وجبه عاليه الدهون. أجريت تجربـه بيولوجيـه حيث تم تغـذيـه ذكور فئران الألبينو (وزن 185 ± 5 جرام)علي وجبه عالية الدهون ومدعمة بمخلف تصـنيع العنب بنسـبه 5%، 10%، 15%. أظهرت النتائج ان نســبة البروتين في مخلف العنـب 8.75% والـدهون 7.38 والألـيـاف الغـذائيـة 46.63%. بالنســبه العناصــر المعدنيه تواجد كل من البوتاســيوم، الماغنســيوم، الكالســيوم، الصــوديوم، الفســفور، الكبريت بتركيزات عاليه. يحتوى الزيت في مخلف العنب علي 85.75% أحماض غير مشــبعه منهـا 19.14% أحمـاض أحـاديـه عـدم التشــبع, 66.60% منها أحماض عديدة عدم التشــبع. يوجد حمض اللينوليك بنســبه 65.29%. كميه الفينولات الكليه (معبرا عنها بملليجرام مكافئ حمض الجاليك لكل جرام من العينـه) هي 50.35ملليجرام مكـافئ حمض الجـاليك لكل جرام. كميه الفلافونويدات الكليه (معبرا عنها بملليجرام مكافئ روتين لكل جرام من العينه) هي 22 ملليجرام مكافئ روتين لكل 100 جرام من العينة. نسبه نشـاط نزع الشـقوق الحرة هي 51.92%. جاليك اســيد أعلي مركب من المركبات الفينولات (9.76 ملليجرام/100جرام)، الكاتشين أعلي مركب من مركبات الفلافونويـد (52.5 ملليجرام/100جرام). في حين ان كميه مركب reversatrol كـــــــ stilbenes هي 14.11 ملليجرام /100جرام. نتج عن تـدعيم الوجبـة العــاليــة الــدهن بمخلف العنــب انخفــاض في مســـتوي cholesterol, triglycerides, LDL–C and vLDL–C. وجد ان تركيز HDL–C في الفئران المغذاه علي الوجبه المدعمه بمخلف العنب أعلي مقارنه بالمغذاه علي وجبه عاليه الدهن فقط. وكانت احســن نتيجه في حاله انزيمات الكبد، اليوريا، حمض اليوريك، الكرياتنين في الفئران المغذاه علي وجبه عاليه الدهن ومدعمه بـ 15% من مخلف العنب. وكانت القيم بدون وجود اختلافات معنويه في حاله انزيمات الكبد وحمض اليوريك والكرياتنين مقارنة بالمجموعه الضابطه السالبه. يمكن اعتبار مخلف العنب مصـدرا للمركبات الصـحية والتكنولوجيه. كذلك يمكن اسـتخدامه في تغذيه الحيوان, الصناعات الدوائيه, مستحضرات التجميل أو الصناعات الغذائيه لتحسين خواص الثبات والخصائص الغذائية.