World Journal of Dairy & Food Sciences 5 (1): 58-66, 2010
ISSN 1817-308X

Potential Effect of Egyptian Anna Apple Pomace (*Malus domestica*, Rosaceae) Supplementation on Kidney Function, Liver Function and Lipid Profile of Diabetic Rats

[1]R.S.A. Ismail and [2]S.H. Abd El-Gawad

[1]Department of Nutrition and Food Science, Faculty of Home Economics, Helwan University, Egypt
[2]Faculty of Family Sciences, Taibah University, KSA

Abstract: Polyphenols responsible for the antioxidant activity in apple are still in the pomace. The apple pomace could provide a cheap and readily available source of dietary antioxidants. The present work carried out to investigate the effect of diets supplemented with different levels of Anna apple pomace powder (AAPP), decoction (AAPD) and their combinations on blood sugar level, daily food intake, organs weight to body weight ratio in alloxan diabetic rats. Serum lipid profile, kidney function and liver function were also studied. Forty eight male albino rats (Spragu Dawley Strain) were divided into two main groups. The first main group (n = 6) was fed on basal diet (B.D) and was considered negative control group. The second main group (42 rats) was injected with (150 mg/kg b.wt) of recrystallized alloxan to induce hyperglycemia. Diabetic rats was randomly assigned to seven equal subgroups: one of them left as positive control and the other six subgroups as a following: groups (1^{st} and 2^{nd}) received basal diet containing two levels from AAPP (2.5 & 5%) groups (3^{rd} and 4^{th}) fed on B.D and received (2.5 and 5%) (AAPD), respectively. Fifth and six groups received (B.D) containing combinations of (1.25% AAPP Plus 1.25% AAPD) and (2.5% (AAPP) Plus 2.5% (AAPD) respectively. Supplemented diet with different levels of (AAPP), (AAPD) and their combinations resulted in improvement of the body weight gain, in addition to the percent of organs weight/ body weight. Results revealed that the mean values of serum albumin increased in treated diabetic groups. While the levels of uric acid, urea nitrogen and creatinine, glucose, aspartate aminotransferase (AST) and alanine amino transferas (ALT) very low density lipoprotein VLDL-c were decreased in all treated groups as compared to the positive control group, while (HDL-c) increased. The conclusion was drawn that Egyptian Anna apple pomace contain considerable number of healthy compounds namely polyphenols and flavonoids. This study demonstrated that diabetic rats were fed on high levels of (AAPP), (AAPD) or their combinations realized the best modulating effects on diabetic rats.

Key words: Anna Apple pomace · Liver function · Liver function · Lipid profiles · Kidney function · Diabetes mellitus rats

INTRODUCTION

Diabetes mellitus is a group of metabolic alterations characterized by hyperglycemia resulting from defects in insulin secretion, action or both. It has already been established that chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and eventually the failure of organs, especially the eyes, kidney, nerves, heart and blood vessels [1]. Increasing evidence from both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of diabetes mellitus. Free radicals are formed in diabetes by glucose oxidation, non-enzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins [2]. Abnormally high level of free radicals and the simultaneous decline of antioxidant defense mechanism can lead to damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance. These consequences of oxidative stress can promote the development of complications of diabetes mellitus [3].

It is now widely accepted that dietary polyphenolics have beneficial effect in protecting the body against chronic disease, such as cancer, cardiovascular disease

Corresponding Author: S. Rasha Ahmed Esmail, Department, Nutrition and Food Science, Faculty of Home Economics, Helwan University, Egypt

and diabetes mellitus [4]. Flavonoids are biologically active, polyphenolic constituents of plant foods and are found in various fruits, vegetables and legumes. Flavonoids are composed of several subclasses that have the ability to scavenge free radicals and chelate metals [5]. Given the hypothesized relation between diabetes and inflammation Ziegler [6] and the potential for flavonoids to protect the body against free radicals and other pro-oxidative compounds. The mechanism of many of the protective actions of flavonoids remains little known. One of common denominators is antioxidant activity. Flavonoids can exert their antioxidant activity by various mechanisms e.g. scavenging or quenching the free radicals, by chelating of metal ions, or by inhibiting enzymatic systems responsible for free radical generation [7].

Apple pomace including seeds has been demonstrated to be rich source of polyphenolics and some phenolic constituents, especially the procyanidins and quercetin glycosides, have shown to exert strong antioxidant [8]. Lu and Foo [9] reported that the polyhenols, which are mainly responsible for the antioxidant activity, are present in apple pomace. These phenolics isolated from apple pomace have been found to have high antioxidant activity suggesting that apple pomace may have dietary health benefits [10]. Ismail and Bayomey [11] found that the Egyptian Anna apple pomace have considerable amount of catchein, ferrulic acid, Chlorogenic, gallic acid, vanillic, syrngic, chatechol and others. The predominant flavonoid compounds were Quercetin, which was found to be the predominant compound and amounted in 271.13 mg/ g, while the mediated compounds were luteolin, apignin, rutin, narengenin and sakurantin. The minor compounds were hypersoid and kampferol. On other side, the biological results showed that diabetic group which were fed on BD. supplemented with different levels of (AAPP), (AAPD) and their combinations recorded significant improvement in feed efficiency ratio.

The present study was a trail to investigate the effect of supplementation with different levels from Egyptian Anna apple pomace as a potential of natural source of polyphenols and flavonoids responsible for the antioxidant activity on body weight gain percent, organs weight/ body weight ratio. Serum glucose level, liver function, kidney function and lipid profile of diabetic rats.

MATERIALS AND METHODS

Materials: Anna apple (*Malus domestica*), family Rosaceae were purchased from El-Oboor market. Olive section and semi arid zone, Horticultural Research Institute, Agriculture Research Center, Giza, Egypt, authenticated Anna apple (*Malus domestica*). Apple were washed and cut to slices, the slices were pressed under hydraulic system (in the unit of olive oil production, Food Technology Research Institute) to separate the apple juice, the residual, (Anna apple pomace) (AAP) were dried by solar energy at National Research Center and ground to powder to pass through 150μm Sieve. Apple pomace decoction was performed as follow: dried (AAP) were boiling in 1L of water for 10 min. This preparation was filtered and administered to the experimental animals by gastric route according to Carrillo *et al.* [12].

Chemicals: Vitamins, minerals, casein, choline chloride and cellulose were purchased from El-Nasr Pharm. and Chem. Ind. Comp. Cairo, Egypt. Corn starch and corn oil were obtained from local market. Kits used to determine serum biochemical parameters were purchased from Alkan Pharm. Ind. Comp. Cairo, Egypt.

Rats: Forty-eight male albino rats (Spragu Dawley Strain) weighting 100+5g were obtained from the laboratory of animals colony, Ministry of Health, Helwan, Cairo, Egypt.

Experimental Animals Design: Rats were housed in individual cages under hygienic laboratory condition and were fed on basal diet adlibitum for one week for adaptation in the animal house of Faculty of Home Economics, Helwan University. The basal diet in the preliminary experiment consists of 14% casein (protein > 85%), corn oil (4%), Cellulose (5%), vitamin mixtures (1%), salt mixtures (3.5%), choline chloride (0.25%) and cornstarch (72.25%) according to Reeves *et al.* [13]. The salt mixture and vitamin mixture were prepared according to Hegsted *et al.* [14] and Campbell [15]. After this week, rats were divided into two main groups. The first main group (6 rats) fed on basal diet and was considered (negative control group). The second main group: Forty two rats were injected with (150 mg/kg b.wt.) of recrystallized alloxan to induce hyperglycemia according to the method described by Arbeeny and Bergquist [16]. After 4 days, blood samples were obtained from rats eyes to estimate glucose levels. The second main group (diabetic rats) was randomly assigned to seven equal subgroups: one of them left as positive control, fed on basal diet and the other sex groups fed on basal diet containing (1) 2.5% Anna apple pomace powder (AAPP), (2) 5% (AAPP) (3) 2.5% Anna apple pomace decoction (AAPD) (4) 5% % (AAPD) (5) 2.5% combination of

(1.25% "AAPP" plus 1.25 "AAPD") (6) 5% combination of (2.5% "AAPP" plus 2.5% "AAPD").Body weight, food consumption were measured twice a week and total food intake of the experimental period (4 weeks) and body weight gain % were calculated.

Biochemical Analysis of Serum: At the end of the experiment period the rats were starved for 12 h and then sacrificed under ether anaesthetized. Blood samples were received into clean dry centrifuge tube and left to clot at room temperature, then centrifuged for 10 minutes at 3000 r.p.m to separate serum. Serum was carefully separated into dry clean wasserman tubes, using a Pasteur pipette and kept frozen at (-20°C) till estimation of some biochemical parameters, i.e. serum cholesterol (TC) [17], triglycerids (TG) [18], high-density lipoprotein cholesterol (HDL-c) [19]. While serum low-density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c) were calculated according to the equation of Friedwald [20]. Total albumin was carried out according to Dumas *et al.* [21] Uric-acid [22], Urea nitrogen [23]. Serum Creatinine according to Faulkner and King [24], aspartate amino transferase (AST) and alanine amino transferase (ALT) [25] and Serum glucose [26].

Statistical Analysis: Statistical analysis was carried out using SPSS statistical software version 11. The results were expressed as mean ± SD. Data was analyzed by one way analysis of variance (ANOVA). The differences between means were tested for significance using least significant difference (LSD) test at $P<0.05$ and correlation coefficient [27].

RESULTS AND DISCUSSION

Biological Effect of Anna Apple Pomace (*Malus domestica*) Powder. Decoction and Their Combinations on Body Weight Gain % and Organs Weight/ Body Weight Percent in Diabetic Rats: Table 1 illustrated the effect of Anna apple pomace powder (AAPP), Anna apple pomace decoction (AAPD) and their combinations on some nutritional parameter, body weight gain percent and liver, kidney and spleen weight/ body weight %. Results revealed that food intake body weight gain percent of all treated diabetic groups with (BD.) supplemented with different levels of (AAPP) or (AAPD) and their combinations recorded significant increase ($P < 0.05$), as compared to control positive group. In this respect, Farouque and Meredith [28] suggested that this may be explained by possible increased insulin secretion or increased food consumption.

Concerning the effect of Anna apple pomace powder (AAPP), (AAPD) and their combinations on the weight of some organs such as liver, kidney, heart and spleen to body weight of diabetic rats. Results revealed that diabetic groups which fed on (BD.) only and those fed on (BD.) supplemented with different levels of (AAPP), (AAPD) and their combinations have higher organ weight to body weight ratio as compared to non diabetic rats.

In this respect Alluru *et al.* [29] reported that, in general the kidney weight, 24 hour food intake, water intake, urine volume and albumin urea were significantly higher in diabetic than in normal rats. Increased kidney weight may indicate the progression to renal failure worsened by oxidative stress in diabetic nephropathy. Our results revealed that BD. supplemented with different levels of (AAPP), (AAPD) and their combinations induced significant decrease in organs weight/ body weight% as compared to the positive control group. Diabetic group which fed on BD. supplemented with 5% combination of (2.5% AAPP plus 2.5% AAPD), recorded the best improvement in organs weight/body weight %. In addition results showed, decrease in the liver weight as compared to the positive control group. While the kidney and spleen were recorded non significant different ($P < 0.05$) as compared to non diabetic control group.

In this concern, Thuyaisingham *et al.* [30] reported that antioxidant treatment may have a potential role for the prevention of diabetic nephropathy.

Effect of Anna Apple Pomace Powder, Anna Apple Pomace Decoction and Their Combination on Kidney Functions of Hyperglycemic Rats: Table 2 shows the effect of different levels of (AAPP), (AAPD) and their combinations on serum uric acid, urea nitrogen, creatinine and albumin of diabetic rats. Results revealed that the uric acid, urea nitrogen, creatinine (Mg/dl) for positive control group, significantly increased, while the mean value of serum albumin (g/dl) decreased as compared to control negative group. The mean values ± SD were (2.284 ± 0.108, 58.193 ± 3.754 and 0.875 ± 0.067 vs. 1.572 ± 0.077, 31.074 ± 2.712 and 0.492 ± 0.028, respectively), while the mean value of albumin of the positive control group recorded significant decrease ($P<0.05$) as compared to negative control group (2.447 ± 0.050 vs 3.112 ± 0.236, respectively). This finding was in agreement with the results of Alluru *et al.* [29]. Our results revealed that feeding diabetic rats on supplemented diet with different levels from (AAPP), (AAPD) and their combinations induced a significant ($P < 0.05$) decrease in serum uric acid, urea nitrogen and

Table 1: Effect of Feeding Supplemented diet with Anna Apple Pomace Powder (AAPP), Anna Apple Pomace Decoction (AAPD) and their Combinations on Daily Food Intake Body Weight Gain % and Organs Weight/Body Weight % in Diabetic Rats.

	Parameters		Organs Weight/ Body Weight %		
Groups	Food Intake(g/day rat)	Body weight gain %	Liver	Kidney	Spleen
Control (-)	14.393 [a] ± 0.241	51.696 [a] ± 2.602	2.827 [g] ± 0.088	0.631 [e] ± 0.010	0.284 [e] ± 0.006
Control (+)	12.935 [c] ± 0.351	25.751 [e] ± 2.343	3.439 [a] ± 0.055	0.762 [a] ± 0.021	0.375 [a] ± 0.009
2.5% (AAPP):	13.235 [bc] ± 0.152	33.085 [d] ± 2.233	3.311 [b] ± 0.024	0.719 [b] ± 0.091	0.351 [b] ± 0.007
5% (AAPP)	13.550 [b] ± 0.483	39.953 [c] ± 2.283	3.109 [d] ± 0.077	0.688 [c] ± 0.011	0.327 [d] ± 0.012
2.5% (AAPD)	13.373 [b] ± 0.182	31.460 [d] ± 1.557	3.268 [b c] ± 0.047	0.723 [b] ± 0.008	0.364 [a] ± 0.009
5% (AAPD)	13.662 [b] ± 0.422	38.213 [c] ± 3.135	3.208 [c] ± 0.033	0.685 [c] ± 0.007	0.340 [c] ± 0.009
2.5% Combination of 1.25% (AAPP) + 1.25% (AAPD)	13.572 [b] ± 0.511	38.102 [c] ± 2.452	2.979 [e] ± 0.076	0.669 [d] ± 0.012	0.319 [d] ± 0.008
5% Combination of 2.5% (AAPP) + 2.5% (AAPD)	13.683 [b] ± 0.277	43.770 [b] ± 1.500	2.911 [f] ± 0.064	0.642 [e] ± 0.012	0.292 [e] ± 0.014

Values are expressed as means ± SD Significance differences $P < 0.05$

Values which don't share the same letter in each column are significantly different.

(AAPP) Anna Apple Pomace Powder

(AAPD) Anna Apple Pomace Decoction

Table 2: Effect of Anna Apple Pomace Powder (AAPP), Anna Apple Pomace Decoction (AAPD) and their Combinations on Serum Uric Acid, Urea nitrogen, Creatinine and Albumin in Diabetic Rats

	Parameters (Mg/ dl)			g/dl
Groups	Uric acid	Urea nitrogen	Creatinine	Albumen
Control (-)	1.572 [g] ± 0.077	31.074 [d] ± 2.712	0.492 [e] ± 0.028	3.112 [a] ± 0.236
Control (+)	2.284 [a] ± 0.108	58.193 [a] ± 3.754	0.875 [a] ± 0.067	2.447 [e] ± 0.050
2.5% (AAPP)	1.891 [c] ± 0.148	46.913 [b] ± 3.457	0.688 [b] ± 0.069	2.801 [c d] ± 0.158
5% (AAPP)	1.655 [e f] ± 0.071	42.791 [c] ± 2.956	0.556 [d] ± 0.038	3.004 [a b] ± 0.159
2.5% (AAPD)	1.994 [b] ± 0.099	49.273 [b] ± 3.455	0.697 [b] ± 0.057	2.718 [d] ± 0.087
5% (AAPD)	1.708 [d e] ± 0.979	46.053 [b] ± 1.833	0.631 [c] ± 0.028	2.879 [b c] ± 0.031
2.5% Combination of 1.25% (AAPP) + 1.25% (AAPD)	1.813 [c d] ± 0.068	47.476 [b] ± 2.077	0.594 [c d] ± 0.019	2.829 [c d] ± 0.095
5% Combination of 2.5% (AAPP) + 2.5% (AAPD)	1.743 [d e] ± 0.044	42.418 [c] ± 2.042	0.569 [d] ± 0.032	2.885 [b c] ± 0.053

Values are expressed as means ± SD Significance differences $P < 0.05$

Values which don't share the same letter in each column are significantly different

(AAPP) Anna Apple Pomace Powder

(AAPD) Anna Apple Pomace Decoction

Table 3: Effect of Anna Apple Pomace Powder (AAPP), Anna Apple Pomace Decoction (AAPD) and their Combinations on Serum Glucose and Liver Functions of Diabetic Rats

	Parameters		
		Liver Functions (U/L)	
Groups	Glucose (mg/dl)	AST	ALT
Control (-)	84.257 [f] ± 4.034	75.207 [f] ± 4.215	29.532 [f] ± 3.246
Control (+)	198.057 [a] ± 8.052	103.609 [a] ± 5.144	62.533 [a] ± 4.842
2.5% (AAPP)	169.950 [c] ± 4.216	92.098 [b c] ± 4.675	50.365 [c] ± 3.346
5% (AAPP)	142.625 [e] ± 4.975	82.769 [e] ± 3.701	41.975 [d] ± 2.496
2.5% (AAPD)	176.227 [b] ± 4.208	96.489 [b] ± 3.627	56.176 [b] ± 3.664
5% (AAPD)	149.971 [d] ± 5.543	85.039 [de] ± 1.642	44.128 [d] ± 1.911
2.5% Combination of 1.25% (AAPP) + 1.25% (AAPD)	170.518 [c] ± 3.233	89.497 [cd] ± 4.807	48.572 [c] ± 2.280
5% Combination of 2.5% (AAPP) + 2.5% (AAPD)	144.992 [de] ± 3.391	85.614 [de] ± 5.031	37.739 [e] ± 2.189

Values are expressed as means ± SD Significance differences P < 0.05
Values which don't share the same letter in each column are significantly different.
AST : Aspartate amino transferase (AAPP) Anna Apple Pomace Powder
ALT : Alanine amino transferase (AAPD) Anna Apple Pomace Decoction

creatinine, while results showed a significant (P < 0.05) increase in the serum albumin level. The best results were noticed in the group of rat which received (5% AAPP).

In this respect, Mongensen and Christensen [31] reported that the natural history of diabetic nephropathy is well known. i.e., dipstick-positive proteinuria and the development of renal failure follow the appearance of microalbuminuria. The production of peroxynitrite increases in the proximal tubules of patients with diabetic nephropathy, suggesting that oxidant injury of the proximal tubles plays an important parting the pathogenesis of diabetic nephropathy.

In another study we could identify flavonoids compounds extracted from Egyptian Anna apple pomace which were eight components. Quercetin was found to be the predominant compound and amounted 271.13 mg/g concerning polyphenols, we could identify twelve phenolic compound in dry Egyptian Anna apple pomace. The predominant phenolic compounds were catchein, which amounted 47.16 mg/g. Our results revealed that Egyptian Anna apple pomace have considerable number of healthy compounds namely polyphenols and flavonoid [11].

In this respect, Jerums *et al.* [32] reported that oxidative stress has been though to be a potential factor in the progression of diabetic complications thus far attenuation of diabetic nephropathy has been demonstrated by using many antioxidants such as vitamin E, α-lipoic acid and flavonoids. Antioxidant treatment may have a potential role for the prevention of diabetic nephropathy Anjaneyulu and Chopra [33].The major finding of the present study were that polyphenol containing Anna apple pomace powder, Anna apple pomace decoction and its combinations induced significant improvement in kidney functions of diabetic rats. In this concern, Kanter and Oter [34] reported that Quercetin led to reduce oxidative stress and nephropathy in diabetic rats.

Effect of Anna Apple Pomace Powder, Decoction and Their Combinations on Serum Blood Glucose and Liver Functions of Diabetic Rats: Table 3 illustrate the effect of different levels of (AAPP), (AAPD) and their combinations on glucose, aspartate amine transferase (AST) and alanine aminotransferase (ALT) levels in serum of diabetic rats. The results revealed that, glucose level for the diabetic control group highly significant increased (P<0.05) as compared to the negative control group. The values were 198.057±8.052vs 84.257±4.034mg/dl. Treating diabetic rats with different levels of (AAPP), (AAPD) and their combinations led to significant (P < 0.05) decrease in serum glucose level as compared to control positive group. The best results for glucose level were noticed in the groups of rats, which received. BD. supplemented with 5% (AAPP) followed by diabetic group which received combination (2.5% AAPP plus 2.5% AAPD) followed by diabetic group which received BD. plus 5% (AAPD). The lowest decrease in serum glucose level recorded in group that treated with combination of (1.25 AAPP plus 1.25 AAPD).In this respect, Szkudelski [35] reported that Alloxan, a chemical diabetogen, in the presence of glutathione is reduced via the alloxan radical into dialuric acid. During this redox cycling process, reactive oxygen species are formed that destroy β-cells in islets of langerhans. In this concern Maghrani *et al.* [36] suggested that flavonoids may cause potent inhibition of renal glucose reabsorption through inhibition of the sodium glucose symporters located in the proximal renal tubule.

In this respect, Burguieres *et al.* [37] found that phenolics have high antioxidant activity, angiotensin-converting enzyme I inhibitory activity and also good inhibitory activity on carbohydrate - modulating enzyme such as α-glucosidase related to glucose absorption in the intestine. The potential for managing both glucose absorption and cellular redox dysfunction for preventing postprandial hyperglycemia linked to type 2 diabetes and hyperglycemia-induced vascular complications. Machha *et al.* [38] reported that beneficial actions of quercetin were mediated by its antioxidant protection of endothelium-derived nitricoxide, coupled to reduction in blood glucose level and oxidative stress. Lukacinova *et al.* [39] cleared the beneficial effect of flavonoids on glycosuria and antioxidant status, suggested that the protective effect of flavonoids is partly related to their antioxidative chelatory properties and partly to the alteration of renal glucose absorption.

Concerning of AST and ALT, it could be observed that the levels of AST and ALT increased significantly ($P < 0.05$) in the positive control group, as compared with the negative control group (103.609±5.144 and 62.533±4.842) vs (75.207±4.215 and 29.532±3.246), respectively.

In this respect, Sallie *et al.* [40] reported that the rise in levels of serum AST and ALT has been attributed to the damaged structural integrity of the liver, because these enzymes are cytoplasmic in location and released into circulation after cellular damages. Feeding supplemented basal diet with different levels of (AAPP), (AAPD) and their combinations induced a significant decrease ($P<0.05$) in the level of AST and ALT as compared to the control positive group (diabetic group). The highest decrease in AST value was observed in the group which treated with (5% AAPP) followed by (5% AAPD) and 5% of their combinations, while the lowest decrease was recorded in the group that treated with (2.5% AAPD).

In this concern Molina *et al.* [41] found that, in mice liver treated with ethanol, quercetin decreased lipid oxidation and increased glutathione, protecting the liver from oxidative damage.

Effect of Anna Apple Pomace Powder (Aapp), Anna Apple Pomace Decoction (Aapd) and Their Combinations on Lipid Profile of Diabetic Rats: Table 4 shows the effect of (AAPP), (AAPD) and their combinations on serum total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VLDL-c) and high density lipoprotein cholesterol (HDL-c) in diabetic rats. The mean values of serum total cholesterol and triglycerides significantly ($P<0.05$) increased for positive control group while the mean values of HDL-c significantly ($P<0.05$) decreased the mean values of LDL and VLDL-c recorded significantly ($P<0.05$) increased of the control positive group (diabetic group) as compared to the control negative group (normal group).

In this respect, Mehta *et al.* [2] and Ghosh and Konishi [3] reported that oxidative stress plays a major role in the pathogenesis of diabetes mellitus. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance. These consequences of oxidative stress can promote the development of complications of diabetes mellitus. Recent experimental and clinical studies have uncovered new insights into the role of oxidative stress in diabetic complications, suggesting a different and innovative approach to possible "causal" antioxidant therapy e.g. flavonoids [42].

Our results revealed that feeding diabetic rats on BD. supplemented with different levels of (AAPP), (AAPD) and their combinations, induced a significant ($P<0.05$) increase in the mean value of HDL-c and at the same time shows a significant ($P<0.05$) decrease in serum levels of total cholesterol, triglycerides, LDL-c and VLDL-c values of diabetic groups as compared to the positive control group. The highest decrease in total cholesterol, triglycerides, LDL-c and VLDL-c values were observed in

Table 4: Effect of Anna Apple Pomace, Anna Apple Pomace Decoction and their Combinations on Lipid Profile of Diabetic Rats.

	Parameters				
	Lipid profile		(Mg/dl)		
Groups	Cholesterol	Triglycerides	HDL-c	LDL-c	VLDL-
Control (-)	81.377	43.513 [f]	47.203 [a]	25.472 [g]	8.703 [f]
	± 3.339	± 3.080	± 2.853	± 0.706	± 0.616
Control (+)	157.825 [a]	73.197 [a]	33.933 [e]	106.253 [a]	14.639 [a]
	± 3.785	± 3.949	± 1.744	± 3.519	± 0.788
2.5% (AAPP)	130.555 [c]	59.702 [b c]	39.398 [c]	79.217 [c]	11.940 [bc]
	± 4.009	± 4.584	± 2.916	± 1.519	± 0. 917
5% (AAPP)	123.702 [d]	50.476 [e]	42.439 [b]	71.168 [e]	71.168 [e]
	± 3.393	± 2.637	± 1.549	± 1.546	± 0.527
2.5% (AAPD)	136.878 [b]	61.821 [b]	35.934 [de]	88.590 [b]	12.364 [b]
	± 3.877	± 4.485	± 2.276	± 3.248	± 0.897
5% (AAPD)	128.592 [c]	52.564 [de]	42.187 [b]	75.891 [d]	10.513
	± 4.383	± 2.206	± 2.475	± 2.885	± 0.441
2.5% Combination of 1.25% (AAPP) + 1.25% (AAPD)	130.156 [c]	56.881 [c d]	37.346 [c d]	81.434 [c]	11.376 [c d]
	± 3.511	± 5.605	± 1.601	± 2.068	± 1.121
5% Combination of 2.5% (AAPP) + 2.5% (AAPD)	119.974 [d]	48.288 [e]	42.772 [b]	67.545 [f]	9.657 [e]
	± 4.156	± 4.992	± 2.216	± 2.628	± 0.998

Values are expressed as means ± SD Significance differences $P < 0.05$

Values which don't share the same letter in each column are significantly different.

Mg/dl : Milligram/ deciliter

HDL-c : High-density lipoprotein cholesterol. (AAPP) Anna Apple Pomace Powder

LDL-c : Low-density lipoprotein cholesterol. (AAPD) Anna Apple Pomace Decoction

VLDL-c : Very low-density lipoprotein cholesterol.

the diabetic group which treated with BD. supplemented with 5% combination of 2.5% (AAPP) Plus 2.5% (AAPD), followed by 5% (AAPP) and 5% (AAPD), while the lowest decrease was recorded in the group that fed on BD. supplemented with (2.5% AAPD).

In this respect, Peng and Kuo [43] reported that combined apple pectin and apple phenolic fractions lowered plasma and liver cholesterol, triglycerides and apparent cholesterol absorption to a much greater extent than either apple pectin alone or apple-phenolics alone. Phloridzin, chlorogenc acid, epicatechin and quercetin glucosides have all been isolated from apple pomace. These phenolics isolated from apple pomace have been found to have high antioxidant activity decrease lipid oxidation and lower cholesterol, potentially explaining their role in reducing risk of chronic disease. In this trial treating diabetic rats with Anna apple pomace powder, decoction and its combinations (high dose) recorded the best results of TG, TC, HDL-c, LDL-c and VLDL-c levels, which indicated that this administration improves the metabolic condition with diabetic disorder and that this could help realized the best modulating effects on diabetic rats. In this respect Mayer *et al.* [44] demonstrated that addition of apple phenolics to human serum decreased diphenylhexatriene-labeled phosphatidylcholine (DPHPC) oxidation in a dose dependent manner. (DPHPC) is incorporated into low - density lipoprotein (LDL), high density lipoprotein and very low density lipoprotein (VLDL) fractions and is an indicator of oxidation reflecting the apples antioxidant activity *in vivo*.In this concern, Breinholt *et al.* [45] reported that LDL oxidation inhibition varied greatly between brands of fruit juice, ranging from 9 to 34% inhibition and whole apples inhibited LDL oxidation by 34%. Apple peels inhibited LDL oxidation by 34%, while the flash alone showed significantly less inhibition (21%). Apples significantly lowered lipid oxidation both in humans and rats and lowered cholesterol in human. These effects, which may be attributed to both the phenolics and the dietary fiber found in apples. In this respect, Sanchez-Moreno *et al.* [46] reported that dietary antioxidants that are incorporated in LDL are themselves oxidized when these LDL are exposed to prooxidative conditions before any extensive oxidation can occur in the sterol or polyunsaturated fatty acids. In addition, phytochemicals have been shown to have roles in the modulation of cholesterol synthesis and absorption [47].

In conclusion the results taken together indicated that Egyptian Anna apple pomace (*Malus domestica*, Rosaceae) as a good source of polyphenols and flavonoids which has antioxidant properties, when incorporated into diet could contribute to potential management of hyperglycemia and disturbed of lipid pattern. This also helps to alleviate complications such as organs functions.

REFERENCES

1. Huang, T.H., G. Peng, B.P. Kota, Q.G. Li, J. Yamahara, B.D. Roufogalis and Y. Li, 2005. Anti-diabetic action of *Punica granatum* flower extract: activation of PPAR-γand identification of active component. Toxical. Pharm., 207: 160-169.
2. Mehta, J.L., N. Rasouli, A.K. Sinha and B. Molavi, 2006. Oxidative stress in diabetes: A mechanistic overview of its effects on atherogenesis and myocardial dysfunction. Int. J. Biochem. Cell. Biol., 38: 794-803.
3. Ghosh, D. and T. Konishi, 2007. Anthocyanins and anthocyanin-rich extracts: role in diabetes and eye function. Asia Pac. J. Clin. Nutr. 16: 200-208.
4. Knekt, P., J. Kumpulainen, R. Jarvinen, H. Rissanen, A. Heliovaara, T. Hakulinen and A. Aromaa, 2002. Flavonoid intake and risk of chronic diseases. Am. J. Clin. Nutr., 76: 560-568.
5. Arts, I.C. and P.C. Hollman, 2005. Polypenols and disease risk in epidemiologic studies. Am. J. Clin. Nutr., 81: 317s-25s.
6. Ziegler, D., 2005. Diabetes as an inflammatory cardiovascular disorder. Curr. Mol. Med., 5: 309-22.
7. Firuzi, O., A. Lacanna, R. Petrucci, G. Marrosu and L. Saso, 2005. Evaluation of the antioxidant activity of flavonoids by "ferric reducing antioxidant power" assay and cyclic voltammetry. Biochim. Biophys. Acta., 1721: 174-184.
8. Keller, P., P. Streker, G. Arnold, A. Schieber and R. Carle, 2001. Bestimmung phenolischer verbindungen in Tafelund Mostapfeln mittel HPLC. Flussiges Obsterics., 68: 480-483.
9. Lu, Y. and Y.L. Foo, 2000. Antioxidant and radical scavenging activities of polyphenols from apple pomace. Food Chemis., 68: 81-85.
10. Leontowicz, M., S. Gorinstein, H. Leontowicz, R. Krezeminski, A. Lojek, E. Katrich, M.C.R. Martin-Belloso, R. Solivafortuny, R. Haruenkit and S. Trakhtenberg, 2003. Apple and pear peel and pulp and their influences on plasma lipids and antioxidant potential in rats fed cholesterol containing diets. J. Agric. Food Chem., 51: 5780-5785.
11. Ismail, R.S.A. and A.M. Bayomey, 2010. The Functional Food Properties of Egyptian Anna apple pomace (*Malus domestica*, Rosaceae) and its effect on feed efficiency ratio in diabetic rats. Egyptian J. Nutrition, 25(2): 29-48.
12. Carrillo, B.T., A.W. Saenz and R.R. Ramos, 2003. Study of the Anti-Hyperglycemic effect of anti-diabetic plants in Rabbits with impaired glucose tolerance. Proc. West Pharma. So., 46: 148-152.
13. Reeves, P.G., F.H. Nielsen and G.C. Fahey, 1993. AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition Ad Hoc writing Committee on the Refor mulation of the AIN-76. A Rodent diet. J. Nutr., 123: 939-1951.
14. Hegsted, D.M, C.R. Mills, C.A. Elvehjin and B.E. Hart, 1941. Salt mixture. J. Biol. Chem., 138: 149.
15. Campbell, J.A., 1963. Methodology of Protein evaluation PG. Nutr. Document R., 101 Add. June Meeting, New York.
16. Arbeeny, C. and K. Bergquist, 1991. The effect of paravastation on serum cholesterol in hypercholesterolemic diabetic rabbits. Biochem. Biophys. Acta., 1096(3): 238-244.
17. Allain, C.C., L.S. Poon, C.S. Chan, W. Richmond and P.C. Fu, 1974. Enzymatic determination of total serum cholesterol. Clin. Chem., 20(4): 470-475.
18. Foster, L.B. and T.T. Dumns, 1973. Determination of triglycerides. J. Clin. Chem., 19: 338-353.
19. Lopes-Virelia, M.F., S. Stone, S. Ellis and J.A. Collewellm, 1977. Cholesterol determination in high-density lipoproteins separated by three different methods. Clin. Chem., 23(5): 882-893.
20. Friedwald, W.T., R.I. Levy and D.S. Fredricksor, 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin. Chem., 18: 499-502.
21. Dumas, B.T., A.W. Watson and H.G. Biggs, 1971. Quantitative Colorimetric determination of albumin in serum or plasma. Clin. Chem. Acta., 31: 87-91.
22. Fossati, P. and L. Prencipe and G. Berti, 1980. Enzymatic colorimetric method of determination of uric acid in serum. Clin. Chem., 26(2): 227-273.
23. Patton, C. and S.R. Crouch, 1977. Enzymatic calorimetric method to determine urea in serum. Anal. Chem., 49: 464-468.
24. Faulkner, W.R. and J.W. King, 1978. In: Fundamentals of Clinical Chemistry, 2nd (ed.,) Philadelphia, pp: 994-998.

25. Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum oxaloacetic and glutamic pyruvic transaminase. Am. J. Clin. Path., pp: 28-56.
26. Trinder, P., 1959. Determination of blood glucose using 4-aminoantipyrine. J. Clin. Path., 22: 246.
27. SAS. 2004. Statistical analysis system, SAS users Guide: Statistics. SAS institute Inc. Editors, Cary, NC.
28. Farouque, H.M.O. and I.T. Meredith, 2003. Relative contribution of vasodilator prostanoids, no. and katp channels to human forearm metabolic vasodilation. Am. J. Physiol., 284: 711-718.
29. Alluru, S., I. Redd and J.S. Bollineni, 2001. Selenium-deficient diet induces renal oxidative stress and injury via TGF-BI in normal and diabetic rats. Kidney International, 59: 1342-1353.
30. Thuyaisingham, R.C., C.A. Hott, S.M. Dodd and M.M. Yaquoob, 2000. Increased nitrotyrosine staining in kidneys from patients with diabetic nephropathy. Kidney Int., 57: 1986-2972.
31. Mongensen, C.E. and C.K. Christensen, 1984. Predicting diabetic nephropathy in insulin dependent diabetic patients. N. Engl. J. Med., 311: 89-93.
32. Jerums, G., S. Panagiotopoulo, J. Forbes., T. Osicka and M. Cooper, 2003. Evolving concepts in advanced glycation, diabetic nephiopathy and diabetic vascular disease. Arch Biochem Biophys., 419: 55-62.
33. Anjaneyulu, M. and K. Chopra, 2004. Quercetin, anti-oxidant bioflavonoids attenuates diabetic nephropathy in rats. J. Clin Exp. Pharmacol Physiol., 31: 244-8.
34. Kanter, M.O. and S.K.A. Oter, 2005. Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and beta-cell damage in rat pancreas. Pharmacol. Res., 51: 117-123.
35. Szkudelski, T., 2001. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol. Res., 50: 536-546.
36. Maghrani, M., J.B. Michel and M. Eddouks, 2005. Hypoglycaemic activity of Retama raetm in rats. Phytother. Res., 19: 125-128.
37. Burguieres, E., P. Mccue, Y. Kwon and K. Shetty, 2006. Health related functionality of phenolic-enriched pea sprouts in relation to diabetes and hypertention management. J. of Food Biochemistry., 32: 3-14.
38. Machha, A., I.F. Achike, I.F. Mustafa and R.M. Mustafa, 2007. Quercetin, a flavonoid antioxidant, modulates endothelium derived nitric oxide bioavailability in diabetic rat aortas. Nitric oxide Biology and Chemis., 16: 442-447.
39. Lukacinova, J., J. Mojzis, R. Benacka, P. Racz and F. Nistiar, 2008. Structure-activity relationship of preventive effects of flavonoids in alloxan-induced diabetes mellitus in rats. Journal of Animal and Food Sci., 17: 411-421.
40. Sallie, R., J.M. Tredger and R. William, 1991. Drugs and the liver. Part I. Testing liver function. Biopharm Drug Disp., 12: 251-259.
41. Molina, M., R.I. Sanchez, I. Iglesias and J. Benedi, 2003. Quercetin, a flavonoid antioxidant, prevents and protects against ethanol-induced oxidative stress in mouse liver. Biolpharm Bull., 26: 1398-1402.
42. Aprikian, O., V. Duclos, S., Guyot, C. Besson, C. Manach, A. Berna, A. Morand, C. Remesy and C. Demigne, 2003. Apple pectin and a polyphenol rich apple concentrate are more effective together than separately on cecal ferementation and plasma lipid in rats. J. Nutr., 133: 1860-1865.
43. Peng, I. and S. Kuo, 2003. Flavonoid structure affects inhibition of lipid conditions. J. Nutr., 2184-2187.
44. Mayer, B., M. Schumacher, H. Branstatter, F. Wager and A. Hermetter, 2001. High-through put fluorescence screening of antioxidative capacity in human serum. Analyt. Biochem., 297: 144-153.
45. Breinholt, V., S. Nielson, P. Knuthsen, S. Lauridsen, B. Daneshvar and A. Sorensen, 2003. Effects of commonly consumed fruit juices and carbohydrate on redox status and anticancer biomarkers in female rats. Nutr. Cancer, 45: 46-52.
46. Sanchez-Moreno, C., A. Jimenez-Escring and F. Sauracalixto, 2000. Study of low-density lipoprotein oxidizability indexes to measure the antioxidant activity of dietary polyphenols. Nutr. Res., 20(7): 941-53.
47. Ridker, P.M., N. Rifai, L. Rose, J.E. Buring and N.R. Cook, 2002. Comparison of C-reactive protein and Low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N. Eng. J. Med., 347: 1557-65.