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Effect of Diets Containing Skimmed Milk in the Presence of Green Coffee and Lotus Leaf Aqueous Extracts on Obese Rats Suffering from Diabetes

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Abstract: This study aimed to investigate the effect of three doses from (green coffee and lotus leaf) aqueous extracts on obese rats which were suffering from diabetes. Forty eight male albino rats (Sprague Dawley Strain) used in this study, the rats divided into two main groups. The first main group (6 rats) fed on basal diet (as a control negative group). The second main group (42 rats) was fed eight weeks on high fat diet HFD to induce obesity in rats. The rats in the second main group (obese group) injected with alloxan (150 mg alloxan/kg body weight) to induce diabetes. The second main group divided into seven subgroups, Subgroup (1) fed on HFD as a control positive group, Subgroup (2, 3 and 4) fed on HFD and treated with (2, 3 and 4 ml green coffee aqueous extract/each rat/day). Subgroup (5, 6 and 7) were fed on HFD and treated with (2, 3 and 4 ml lotus leaf aqueous extract/each rat/day), the experimental period lasted six weeks. Results showed that, obese rats which were suffering from diabetes (control positive group) recorded significant increase $p < 0.5$ in body weight gain% (liver & kidney) weights/body weight%, cholesterol, triglycerides, (low and very low density lipoprotein-cholesterol), uric acid, urea nitrogen, creatinine, liver enzymes (AST, ALT and ALP) and glucose, as compared to the rats in the first main group (control negative group). Diabetic obese rats which treated with the three dosage from (green coffee and lotus leaf) aqueous extracts decreased body weight gain%, liver and kidney weights/body weight%, serum cholesterol, triglycerides, LDL-c, VLDL-c, uric acid, urea nitrogen, creatinine, AST, ALT ALP and glucose, while HDL-c increased. The highest improvement in these parameters recorded for the group treated with the high dose from lotus leaf aqueous extract followed by the group treated with high dose from green coffee. Green coffee and lotus leaf aqueous extracts reduce the weight gain and improved lipid profile, kidney functions, liver enzymes and glucose of obese rats which suffer from diabetes.

Key words: Diabetes · Obesity · Rats · Green coffee · Lotus leaf · Glucose · Lipid profile · Liver enzymes · Kidney function

INTRODUCTION

Obesity occurs when the body's energy intake exceeds the body's energy consumption for a prolonged period of time. The degree of obesity is characterized by the volume and number of adipocytes, which is regulated in the so called adipocyte life cycle [1]. Obesity is associated with many metabolic diseases, including cardiovascular disease, diabetes mellitus, high blood pressure, atherosclerosis, various cancers and hyperlipidaemia [2]. Thus, treatments targeting the regulation of adipocyte size and number may provide a therapeutic approach [3]. Several plant extracts and their respective bioactive components are well recognized for their potential to exert anti-obesity effects [1]. Type 2 diabetes mellitus is characterized by abnormalities in carbohydrate, fat and protein metabolism due to insulin resistance [4]. Cardiovascular complications are a major cause of premature mortality in patients with type 2 diabetes [5] and tight control of hyperglycemia and dyslipidemia is crucial for reducing the risk for cardiovascular diabetic complications [6]. Milk intake is widely recommended for healthy diet, not only for bone growth and maintenance, but also as a protein, calcium and magnesium sources as part of an adequate diet. Several lines of evidence suggest that milk and dairy products are associated with a lower risk of type 2 diabetes mellitus (T2DM) and hypertension. On the other

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hand, high calcium intake has been associated with a higher risk of certain types of cancer, mainly prostate cancer [7].

Coffee has recently received scientific attention as current epidemiologic and *in vivo* studies have revealed its health benefits against obesity and metabolic disorders, especially type 2 diabetes [8]. These health advantages are mostly derived from chlorogenic acids contained in coffee beans [9]. Raw coffee beans are rich in chlorogenic acids and caffeine and their contents in coffee beans are significantly decreased during the roasting and decaffeination processes [10]. Scientific studies have revealed that both coffee and caffeine play a preventive role against various degenerative diseases of modern society Van Dam [11] reported that moderate daily consumption of coffee helped to reduce the risk of type 2 diabetes, while Fredholm and Lindgren [12] found that caffeine promotes lipolysis in rat adipocytes. Green coffee extract GCE is present in green or raw coffee [13]. It is also present in roasted coffee, but much of the GCE is destroyed during the roasting process. Some GCE constituents, such as chlorogenic acid (CGA) can also be found in a variety of fruits and vegetables [14]. Evidence is accumulating from animal studies regarding the use of GCE as a weight loss supplement [15, 16]. Lotus (*Nelumbo nucifera* Gaertn.) an aquatic vegetable, is extensively cultivated in eastern Asia, particularly in China. The root, seed and young leaf of lotus are widely favored by Asian people as vegetables [17]. The matured leaf is fibrous and it usually used as a functional food in Asia [18]. Louts leaf has been demonstrated to possess anti-obesity and antioxidant properties [19]. Chronic consumption of lotus leaf reduces fasting blood glucose and improves blood lipid profiles in alloxan-treated mice, suggesting that it could be beneficial for managing type 1 diabetes mellitus [20]. Therefore, this study aimed to investigate the effect of three doses from (green coffee and lotus leaf) aqueous extracts on obese diabetic rats fed on diet contained skimmed milk.

MATERIALS AND METHODS

Materials: Casein, vitamins, minerals, cellulose, alloxan and choline chloride were purchased from El-Gomhoria Company, Cairo Egypt. Corn starch, saturated fat "beef tallow", soybean oil, sucrose, green coffee and lotus leaf (*Nelumbo nucifera* Gaertn.) were purchased from local market, Cairo, Egypt. Forty eight male albino rats (Sprague Dawley Strain) (160±10g) were obtained from Helwan farm, Egypt.

Methods:

Preparation of Lotus Leaf and Green Coffee Aqueous Extract: Two and a half gram from lotus leaf extracted for 5 min in 100 ml boiled water then filtrate. While green coffee was prepared traditionally by using 2.5 gram green coffee in 100 ml water and kept on heat until boiling.

Biological Part: Male Albino rats (160±10g) were kept in individual stainless steel cages under hygienic conditions and fed one week on basal diet *adlibitum* for adaptation according to Reeves *et al.* [21]. After a period of adaptation on basal diet (7 days), the rats were divided into two main groups. The first main group (6 rats) fed on basal diet, as a control negative group. The second main group (42 rats) was fed eight weeks on high fat diet HFD containing (saturated fat 19%, soybean oil 1% to provide essential fatty acids, sucrose 10%, casein 20%, cellulose 5%, vitamin mixture 1%, salt mixture 3.5%, choline chloride 0.25% and the remainder is corn starch) to induce obesity in rats [22]. The rats in the second main group (obese group) injected with alloxan (150 mg alloxan/kg body weight) to induce diabetes according to the method described by Kumar *et al.* [23]. After four days, body weight gain % was estimated in the first and second main groups and the blood samples were collected from the eye of all rats to determine the levels of glucose, cholesterol and triglycerides to insure the induction of obesity and diabetes. Then the rats in the second main group were divided into seven subgroups (n=6) according to the following. Subgroup (1): fed on HFD (containing half amount of protein from casein and the other from skimmed milk) as a control positive group (obese diabetic group), Subgroup (2, 3 and 4): were fed on HFD (containing half amount of protein from casein and the other from skimmed milk) and treated with (2, 3 and 4 ml green coffee aqueous extract, respectively. Subgroup (5, 6 and 7): were fed on HFD (containing half amount of protein from casein and the other from skimmed milk) and treated with (2, 3 and 4 ml lotus leaf aqueous extract, respectively.

During the experimental period (6 weeks), the diets consumed and body weights were recorded every week. At the end of the experiment, the rats were fasted overnight, then the rats were anaesthetized and sacrificed and blood samples were collected from the aorta. The blood samples were centrifuged and serum was separated to estimate some biochemical parameters, i.e. serum total lipids. [24], cholesterol [25], triglycerids [26], high density lipoprotein HDL-c [27], low density lipoprotein LDL-c and VLDL-c [28], glucose [29], uric acid [30], urea nitrogen [31], creatinine [32], Aspartate Amine Transaminase

(AST) and Alanine Amine Transaminase (ALT) [33] and Alkaline Phosphatase (ALP) [34]. Liver and kidney were separated from each rat and weighted to calculate the liver and kidney to body weight %.

Statistical Analysis: Results of biological evaluation of each group were statistically analyzed (mean ± standard deviation and one way ANOVA test) using SAS package and compared with each other using the suitable test (least significant differences at P< 0.05 [35].

RESULTS AND DISCUSSION

Effect of Diet Containing Skimmed Milk in the Presence of Green Coffee and Lotus Leaf Aqueous Extracts on Feed Intake, Body Weight Gain% and Some Organs Weight/Body Weight% of Obese Rats Suffering from Diabetes: The effect of diet containing skimmed milk in the presence of three doses of (green coffee and lotus leaf) aqueous extracts on feed intake (g/day/each rat), body weight gain% and (liver & kidney) weights/body weight% of obese rats suffering from diabetes presented in Table 1. The mean value of feed intake (g/day/each rat) of the negative control group increased than that of the positive control group. Feed intake of all treated groups with the three doses of (green coffee and lotus leaf aqueous) decreased than that of the control negative and positive groups. The lowest feed intake recorded for the group which treated with 4ml green coffee aqueous extract/rat/day followed by the group treated with 4ml green coffee aqueous extract/rat/day and 4ml lotus leaf aqueous extract/rat/day, respectively. The mean value of body weight gain % of obese group fed on high fat diet increased significantly p<0.05, as compared to the healthy group fed on basal diet. Feeding obese rats which suffer from diabetes with high fat diet and treated with 2ml, 3ml and 4ml (green coffee and lotus leaf) aqueous extracts led to significant decrease in body weight gain%, as compared to the positive control group. The results in this table indicated that, non-significant changes in body weight gain % between the groups which were treated with low and medium doses from coffee and low dose of lotus leaf aqueous extracts. The highest decrease in body weight gain % recorded for the groups treated with 3 and 4 ml lotus leaf aqueous extract/ each rat/day, followed by the group treated with 4ml green coffee, respectively.

The mean value of liver and kidney weights/body weight% increased significantly P<0.05 in the positive control group (obese rats suffering from diabetes), as compared to the negative control group. Feeding obese group which were suffer from diabetes on high fat diet and treated with three doses from (green coffee and lotus leaf) aqueous extracts induced significant decrease P<0.05 in liver and kidney weight/body weight%, as compared to the positive control group. The high dose of lotus leaf aqueous extract recorded the best results in liver and kidney weights/ body weight %, followed by the group treated with the medium level from this extraction. In this respect, Pittas *et al.* [36] and Zemel *et al.*[37] reported that, the mechanisms underlying the effects of dairy on T2DM development includes the calcium and vitamin D content in dairy foods and the possible positive effect of high milk and calcium intake on weight control. In human subjects, coffee intake has been reported to be inversely associated with weight gain [38]. Consumption of coffee has also been shown to produce changes in several glycaemic markers in older adults [39]. Similarly, other research has indicated that the consumption of caffeinated coffee can lead to some reductions in long-term weight gain, an effect which is likely to be due to the known thermogenic effects of caffeine intake [40]. Reports from animal studies have suggested that green coffee extract GCE mediates its antiobesity effect possibly by suppressing the accumulation of hepatic triglycerides [15]. Some authors have also posited that the antiobesity effect of GCE may be mediated via alteration of plasma adipokine level and body fat distribution and downregulating fatty acid and cholesterol biosynthesis, whereas upregulating fatty acid oxidation and peroxisome proliferator-activated receptor alpha (PPAR*á*) expression in the liver [16].

Recently the discovery of new dietary supplement has become of interest in western countries, especially the green coffee bean extract, from *Coffea arabica.* This was found to contain large amounts of the crucial substance “chlorogenic acid” which is an antioxidant [41]. This can be used as a supplement for weight loss and reduction of body mass index BMI [42]. Human studies show that caffeine enhances energy expenditure and improves the clinical conditions of diabetic patients [43]. Chlorogenic acid is also a dietary polyphenolic compound in the coffee with antioxidative activity. Thus, it is suggested that caffeine, chlorogenic acid and other polyphenolic compounds in GCBE act synergistically to suppress body weight gain and visceral fat accumulation in mice [44]. Lotus leaf extract contains multiple bioactive components such as flavonoids [45], flavonoid glycosides [46] and alkaloids [47]. In obese mice, it has been reported that lotus leaf extract prevented the increase in body weight,

Table 1: Effect of diet containing skimmed milk in the presence of green coffee and lotus leaf aqueous extracts on feed intake, body weight gain% and some organs weight/body weight% of obese rats suffering from diabetes

Parameters			Organs weight/body weight %	
Groups	Feed intake (g/day/each rat)	Body weight gain%	Liver	Kidney
Control (-)	19.880	24.00^{d}±1.510	3.120^{f}±0.088	0.600^{fg}±0.062
Control (+)	19.00	45.700^{a}±1.200	4.131^{a}±0.120	1.431^{a}±0.209
2 ml Green coffee aqueous extract	18.109	40.951^{b}±1.622	3.680^{b}±0.148	1.204^{b}±0.113
3 ml Green coffee aqueous extract	17.421	39.511^{b}±1.631	3.634^{bc}±0.070	1.213^{b}±0.131
4 ml Green coffee aqueous extract	17.000	35.700^{c}±1.800	3.525^{c}±0.107	1.116^{bc}±0.109
2 ml Lotus leaf aqueous extract	18.554	39.653^{b}±1.700	3.332^{d}±0.102	1.027^{cd}±0.064
3 ml Lotus leaf aqueous extract	18.000	33.900^{c}±1.902	3.122^{e}±0.072	0.975^{de}±0.064
4 ml Lotus leaf aqueous extract	17.500	30.421^{c}±1.831	3.108^{ef}±0.094	0.916 ^{e}±0.064

All results are expressed as mean ± SD.

Values in each column which have different letters are significant different ($p<0.05$)

Table 2: Effect of diet containing skimmed milk in the presence of green coffee and lotus leaf aqueous extracts on serum glucose of obese rats suffering from diabetes

Parameters	
Groups	Glucose (mg/dl)
Control (-)	77.742±4.020^{f}
Control (+)	186.737±6.741^{a}
2 ml Green coffee aqueous extract	176.350±5.591^{b}
3 ml Green coffee aqueous extract	162.657±4.634^{c}
4 ml Green coffee aqueous extract	148.070±2.803^{d}
2 ml Lotus leaf aqueous extract	170.000±4.410^{b}
3 ml Lotus leaf aqueous extract	144.639±2.699^{d}
4 ml Lotus leaf aqueous extract	129.230±5.765^{e}

All results are expressed as mean ± SD.

Values in each column which have different letters are significant different ($p<0.05$)

inhibited absorption of lipids and carbohydrates, accelerated lipid metabolism and up-regulated energy expenditure, suggesting beneficial effects for the suppression of obesity [48]. Potato and lotus leaf extract intake might prevent obesity and improve obesity related syndromes in students of the South Korea [49]. In addition, it has been reported that lotus extract has an effect of improving obesity and hyperlipidemia [50], reducing blood glucose [51], anti-oxidation and protecting neurons [52] in animals with high fat diet-induced obesity. Also, it has been known that long-term use of lotus leaves might exert a suppressive effect on adipose tissue differentiation at the cellular level [53].

Effect of Diet Containing Skimmed Milk in the Presence of Green Coffee and Lotus Leaf Extracts on Serum Glucose of Obese Rats Suffering from Diabetes: Data presented in Table 2 illustrates the effect of three doses of (green coffee and lotus leaf) aqueous extracts on serum glucose of obese rats suffering from diabetes. The mean value of serum glucose increased in the positive control group fed on high fat diet, as compared to the negative control group. Serum glucose increased by about 140.20% than that of the negative control group. Using green coffee and lotus leaf aqueous extracts with doses (2, 3 and 4 ml/rat/day) in treating obese rats which were suffering from diabetes led to a significant decrease in serum glucose, as compared to the positive control group, on the other hand serum glucose decreased gradually with increasing the levels of both extractions. The best results of serum glucose recorded for the group fed on high fat diet and treated daily with 4ml lotus leaf aqueous extract/rat/day, followed by the group fed on the same diet and treated with 4ml green coffee aqueous extract/rat/day. These treatments decreased the mean value of serum glucose by about 30.795% and 38.667%, than that of the positive control group respectively. In this respect, Hoppe [54] indicated that a short-term high milk, but not meat, intake increased insulin secretion and resistance. On the other hand, dairy products have been hypothesized to protect against type 2 diabetes because of their high content of calcium, magnesium, vitamin D and whey proteins, which may reduce body fat and insulin resistance [55]. Green coffee extract GCE is inhibiting the enzymatic activity of hepatic glucose-6-phosphatase, which is involved in the homeostasis of glucose [56]. Frequent consumption of coffee may reduce risk of type 2 diabetes and liver cancer [57].

Coffee has been shown to be a major contributor to the total in vitro antioxidant capacity of the diet which may be relevant as oxidative stress can contribute to the development of type 2 diabetes [58]. Coffee is the major source of the phenol chlorogenic acid [59]. Intake of chlorogenic acid has been shown to reduce glucose concentrations in rats [60]. Coffee also contains substantial amounts of magnesium, which has been linked

to better insulin sensitivity and insulin secretion [61]. Alpha-glucosidase inhibitors are oral hypoglycemic agents for patients with type 2 diabetes that inhibit digestion of dietary carbohydrates and thereby flatten the postprandial glucose response. Although á-glucosidase inhibitors such as acarbose and miglitol effectively alleviate both fasting and postprandial hyperglycemia [62]. Lotus leaves could be helpful in the management of diabetes mellitus, as a lotus leaf extract has α-glucosidase inhibitory activity *in vitro* [63]. The flavonoids from lotus leaf FLL may have beneficial effects as both hypoglycemic and antihyperglycemic agents. Toxicity data have already proved that the FLL did not show any toxic reactions [20].

Effect of Diet Containing Skimmed Milk in the Presence of Green Coffee and Lotus Leaf Aqueous Extracts on Lipid Profile of Obese Rats Suffering from Diabetes: The effect of three doses of (green coffee and lotus leaf) aqueous extracts on lipid profile including (total lipids, cholesterol and triglycerides) and serum lipoproteins (high, low and very low density lipoprotein–cholesterol) are presented in Tables 3 and 4 of obese rats suffering from diabetes, respectively. The mean value of serum lipids, cholesterol and triglycerides increased significantly p?0.05 in obese rats which suffering from diabetes, as compared to the negative control group. Total serum lipids, cholesterol and triglycerides increased in the positive control group by about 39.939%, 141.474% and 150.650 %, than that of the negative control group, respectively (Table 3). All treated obese groups which were suffer from diabetes showed significant decrease p?0.05 in serum lipids, cholesterol and triglycerides, as compared to the positive control group. Treating obese rats which suffering from diabetes with 2ml lotus leaf extracts led to significant decrease in serum lipids, as compared to the group which treated with 2 ml green coffee aqueous extract/rat/day, on the other hand total cholesterol and triglycerides did not changed significantly between these groups. Treating obese groups which were suffering from diabetes with 3 and 4 ml lotus leaf extracts decreased the mean values of serum lipids, cholesterol and triglycerides significantly $p<0.05$, as compared to the groups which treated with the same levels from green coffee aqueous extract.

The highest decrease in serum lipids, cholesterol and triglycerides recorded for the group which treated with the high dose of lotus leaf extract, this treatment decreased these parameters by about 13.857%, 25.365% and 37.232%, than that of the positive control group, respectively. The effect of three doses of (green coffee and lotus leaf) aqueous extracts on serum high density lipoprotein-cholesterol (HDL-c), low and very low density lipoprotein-cholesterol (LDL-c & VLDL-c) of obese rats suffering from diabetes are presented in Table 4. The mean value of serum HDL-c decreased significantly ($p<0.05$), while LDL-c and VLDL-c increased significantly ($p<0.05$) in obese rats which suffer from diabetes, as compared to the negative control group. Treating obese groups which were suffering from diabetes with (2ml, 3ml and 4ml) coffee or lotus leaf aqueous extraction led to significant increase in serum HDL-c, while LDL-c and VLDL-c decreased significantly, as compared to the positive control group. Serum HDL-c increased gradually with increasing the doses of coffee or lotus aqueous extracts, while LDL-c and VLDL-c decreased gradually with increasing these doses. The highest improvement of serum lipoproteins recorded for the group which treated with 4 ml lotus leaf aqueous extract/rat/day, followed by the group treated with 4ml green coffee aqueous extract/rat/day.

The data in Tables 3 and 4 revealed that, the highest improvement of lipid profile (total lipids, cholesterol, triglycerides, HDL-c, LDL-c and VLDL-c) recorded for the group which were treated with (4ml lotus leaf /rat/day) followed by the group which treated with (4ml green coffee aqueous extract/rat/day), respectively. These treatments showed significant decrease in serum lipids, cholesterol, triglycerides, LDL-c and VLDL-c & increased HDL-c, as compared to the other treated groups. In this respect, Akiyama *et al.* [64] reported that, obesity induced by high fat intake is usually accompanied by hyperlipidemia which presents as an abnormally high concentration of lipids in blood. Generally, this abnormally high concentration of lipids in blood means elevated blood total cholesterol (TC) and/or triglyceride (TG) levels [65]. Although hyperlipidemia does not cause any symptoms by itself, these abnormally high blood lipids levels can lead to various cardiovascular diseases (CVD) such as atherosclerosis and coronary heart disease (CHD) [66], which together are one of the most common causes of death in modern society [67]. High milk intake is reported to be associated with a decreased ischaemic heart disease risk [68]. These reports suggest that milk and milk products may contain antiatherogenic bioactive substances to negate the effects of saturated fatty acids and cholesterol. Diets rich in polyphenols may help to prevent various kinds of diseases associated with

Table 3: Effect of diet containing skimmed milk in the presence of green coffee and lotus leaf aqueous extracts on serum lipid, cholesterol and triglycerides of obese rats suffering from diabetes

Parameters			
Groups	Lipids (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Control (-)	411.250±6.238^{h}	78.390±4.320^{f}	37.607±0.951^{f}
Control (+)	575.500±4.203^{a}	189.292±4.765^{a}	94.262±3.029^{a}
2 ml Green coffee aqueous extract	557.500±5.066^{b}	178.227±3.675^{b}	86.801±3.075^{b}
3 ml Green coffee aqueous extract	537.000±5.715^{d}	166.750±4.814^{c}	75.801±4.926^{c}
4 ml Green coffee aqueous extract	508.750±2.986^{f}	151.019±4.057^{d}	66.241±3.159^{d}
2 ml Lotus leaf aqueous extract	548.000±4.320^{c}	171.722±3.450^{bc}	82.226±2.068^{b}
3 ml Lotus leaf aqueous extract	517.750±8.845^{e}	156.125±6.609^{d}	67.782±4.234^{d}
4 ml Lotus leaf aqueous extract	495.750±5.315^{g}	141.277±6.423^{e}	59.166±3.771^{e}

All results are expressed as mean ± SD.

Values in each column which have different letters are significant different ($p<0.05$)

Table 4: Effect of diet containing skimmed milk in the presence of green coffee and lotus leaf aqueous extracts on serum lipoproteins of obese rats suffering from diabetes

Parameters			
Groups	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
Control (-)	45.330±2.445^{a}	25.588±1.777^{f}	7.471±0.232^{f}
Control (+)	20.267±2.081^{f}	150.172±5.885^{a}	18.852±0.605^{a}
2 ml Green coffee aqueous extract	23.220±1.398^{e}	137.647±3.315^{b}	17.360±0.615^{b}
3 ml Green coffee aqueous extract	29.399±1.309^{d}	122.190± 4.920^{c}	15.160±0.985^{c}
4 ml Green coffee aqueous extract	32.751±2.227^{c}	105.020±1.749^{d}	13.248±0.631^{d}
2 ml Lotus leaf aqueous extract	21.936±1.631^{ef}	133.340±2.667^{b}	16.445±0.413^{b}
3 ml Lotus leaf aqueous extract	35.640±1.484^{b}	106.928±4.858^{d}	13.556±0.847^{d}
4 ml Lotus leaf aqueous extract	37.989±1.364^{b}	91.454±5.344^{e}	11.833±0.754^{e}

All results are expressed as mean ± SD.

Values in each column which have different letters are significant different ($p<0.05$)

oxidative stress, including coronary heart disease and some forms of cancer [69]. GCE has been reported to have antioxidant activity, demonstrated by its ability to scavenge free radicals *in vitro* and to increase the antioxidant capacity of plasma *in vivo* [70].

Serum and hepatic TG levels were lowered with intravenous administration of chlorogenic acid in Zucker fa/fa rats. However, the TG level in the adipose tissue was not lowered. Therefore, chlorogenic acid is suspected to be effective on hepatic TG and not adipose TG [60]. Further studies were prompted to examine the anti-obesity effect of GCBE on dietary fat absorption using olive oil-loaded mice. The elevated serum TG level was lowered by GCBE and caffeine in olive oil-loaded mice. Coffee has been reported to delay gastric emptying [71]. The concentrations of serum TG, TC and LDL-C were significantly lower in high fat diet group and treated with lotus leaf hot water extract compared to high fat diet group [72]. Lotus leaf hot water extract alone or with taurine supplementation has effects of decreasing the concentration of serum TG, TC and LDL-C and of increasing the ratio of HDL-C/TC. On the other hand, combined supplementation of lotus leaf hot water extract and taurine showed better blood lipid profiles compared to lotus leaf hot water extract alone[73]. *Nelumbo nucifera*, known as the sacred lotus, has many medicinal uses in traditional cultures. Previous studies showed that various pharmacologically active substances were separated from different parts of lotus mainly including alkaloids, flavonoids, triterpenoids, polyphenols, steroids and glycosides [74]. Among the different parts, lotus leaf showed a concentration-dependent inhibition of the activities of á-amylase and lipase and up-regulated lipid metabolism [48]. Improved insulin sensitivity due to administration of a lotus leaf extract could contribute to controlling dyslipidemia, which is important in reducing the risk of micro and macrovascular complications in patients with diabetes [75]. Pharmacological action of lotus leafs has been investigated nationally and internationally. Especially, its anti-obesity action, effect on the endocrine system and effect on the lipid metabolism have been reported in previous studies. Lotus extract has been reported to have an effect of reducing fasting blood glucose, total cholesterol and triglyceride in

diabetic animals, thus showing anti-diabetic and antilipid effects [76]. Lotus leaf was effective in controlling postprandial hyperglycemia in STZ-induced diabetic rats and fasting hyperglycemia in db/db mice. Lotus leaf also alleviated hypertriglyceridemia and hypercholesterolemia and increased HDL-CHOL in db/db mice. These results suggest that lotus leaf could play a beneficial role in management of hyperglycemia and dyslipidemia in animal model of diabetes mellitus [51].

Effect of Diet Containing Skimmed Milk in the Presence of Green Coffee and Lotus Leaf Aqueous Extracts on Liver Enzymes of Obese Rats Suffering from Diabetes: The effect of the three doses of (green coffee and lotus leaf) aqueous extractions on serum AST, ALT and ALP of obese rats suffering from diabetes are presented in Table 5. Feeding obese rats which suffer from diabetes on high fat diet increased the mean values of serum AST, ALT and ALT significantly $p<0.05$, as compared to the negative control group. Feeding obese groups which suffer from diabetes on high fat diet and treated with the three doses of green coffee or lotus leaf aqueous extracts decreased the mean value of serum AST, ALT and ALP significantly $p<0.05$, as compared to the positive control group. The data in Table 5 showed that, treating obese group which suffering from diabetes with high dose of lotus leaf aqueous extract decreased the mean values of serum AST, ALT and ALP significantly $p<0.05$, followed by the group treated with 4ml green coffee aqueous extract, as compared to other treated groups. These treatments decreased the mean values of AST, ALT and ALP by about (38.245%, 43.577% and 38.080%) and (30.305%, 36.446% and 38.080%), respectively. In this respect, Mcavoy and Hayes [77] reported that, Coffee drinking has an inverse relationship to Gamma Glutamyl Transferase "GGT" production in the liver. GGT that occurs with alcohol is inhibited by coffee and thus may protect the liver against damage from alcohol excess. Increased coffee consumption was strongly and independently associated with decreased GGT activity amongst males ($p<0.0001$), especially amongst those with documented alcohol excess. However, only a weak association between coffee intake and lower GGT levels was demonstrated in females. A similar effect on the serum transaminases was also identified.

Coffee intake may have beneficial effects on the liver. Increasing coffee consumption has been inversely associated with liver enzyme concentrations, including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltransferase [78]. In population studies, among persons with unknown diagnosis of liver disease, greater coffee intake has been associated with lower risk of cirrhosis [79] and chronic liver disease [80]. Lotus leaf extracts LLE possessed strong hepatoprotective and antioxidant activity in a rat model of CCl_4-induced. The hepatoprotective activity of LLE may be due to its free radical-scavenging and antioxidant activity, resulting from the presence of some flavonoids and phenolic compounds in the extracts [81].

Effect of Diet Containing Skimmed Milk in the Presence of Green Coffee and Lotus Leaf Aqueous Extracts on Kidney Functions of Obese Rats Suffering from Diabetes: The data in Table 6 illustrated the effect of three doses of aqueous extracts from green coffee and lotus leaf on kidney functions including (uric acid, urea nitrogen and creatinine) of obese rats suffering from diabetes. Feeding obese rats which suffering from diabetes on high fat diet led to significant increase $p<0.05$ in serum uric acid, urea nitrogen and creatinine, as compared to the negative control group. These treatments increased the mean values of these parameters by about (86.245%, 175.195% and 245.551%), respectively, than that of the negative control group. All treated groups improving kidney functions by reducing the mean values of serum uric acid, urea nitrogen and creatinine significantly, as compared to the positive control group. All kidney parameters decreased gradually with increasing the dosage on (green coffee and lotus leaf) aqueous extracts. The best results in serum uric acid, urea nitrogen and creatinine recorded for the group treated with 4ml lotus leaf aqueous extract, followed by the group which treated with 4ml green coffee aqueous extract, respectively. In this respect, Michael-Clifford [82] reported that, Chlorogenic acid (CGA) is a phenolic compound, a family of naturally occurring organic compounds found in plants. It is present in high quantity in coffee (*Coffea canephora*). It is an ester formed from cinnamic acid and quinic acid and is also known as 5-ocaffeoylquinnic acid (5-CQA). Pharmacologically, CGA has been reported to delay glucose absorption in the intestine through inhibition of glucose-6-phosphate translocase [83]. It has also been reported to possess antioxidant activity and antihyperlipidemic activity [84]. Serum and urinary creatinine and BUN measurement is taken as an index of altered glomerular filtration rate GFR in diabetic nephropathy [85]. The level of serum creatinine and blood urea nitrogen BUN was significantly elevated whereas creatinine clearance was significantly reduced in diabetic untreated rats. However, administration of CGA

Table 5: Effect of diet containing skimmed milk in the presence of green coffee and lotus leaf aqueous extracts on serum liver enzymes of diabetic rats suffering from obesity

Parameters			
Groups	AST (U/L)	ALT(U/L)	ALP(U/L)
Control (-)	50.527±3.619^{f}	14.830±1.730^{g}	87.500±4.203^{f}
Control (+)	165.720±4.074^{a}	68.517±3.072^{a}	166.250±4.787^{a}
2 ml Green coffee aqueous extract	149.714±2.924^{b}	60.562±2.097^{b}	149.500±4.654^{b}
3 ml Green coffee aqueous extract	133.583±1.983^{c}	50.767±2.602^{d}	129.365±2.891^{c}
4 ml Green coffee aqueous extract	115.497±3.723^{d}	43.545±2.077^{e}	118.715±3.787^{d}
2 ml Lotus leaf aqueous extract	142.943±3.581^{b}	56.127±3.890^{c}	145.250±4.113^{b}
3 ml Lotus leaf aqueous extract	119.372±10.184^{d}	44.355±2.787^{e}	114.990±2.715^{d}
4 ml Lotus leaf aqueous extract	102.339±2.866^{e}	38.659±2.296^{f}	102.941±.191^{e}

All results are expressed as mean ± SD.

Values in each column which have different letters are significant different ($p<0.05$)

Table 6: Effect of diet containing skimmed milk in the presence of green coffee and lotus leaf extracts on kidney functions of rats and suffering from obesity.

Parameters			
Groups	Uric acid (mg/dl)	Urea nitrogen(mg/dl)	Creatinine(mg/dl)
Control (-)	1.345±0.102^{f}	24.072±1.640^{e}	0.562±0.074^{g}
Control (+)	2.505±0.130^{a}	66.245±3.010^{a}	1.942±0.131^{a}
2 ml Green coffee extract	2.212±0.139^{b}	52.820±5.043^{b}	1.697±0.085^{b}
3 ml Green coffee extract	1.977±0.098^{c}	43.322±5.602^{c}	1.297±0.102^{d}
4 ml Green coffee extract	1.804±0.082^{d}	37.275±4.457^{d}	1.117± 0.102^{e}
2 ml Lotus leaf extract	2.012±0.094^{c}	48.053±4.339^{bc}	1.520± 0.060^{c}
3 ml Lotus leaf extract	1.747±0.133^{d}	36.362±3.211^{d}	1.016± 0.076^{e}
4 ml Lotus leaf extract	1.562±0.056^{e}	31.334±2.624^{d}	0.817±0.072^{f}

All results are expressed as mean ± SD.

Values in each column which have different letters are significant different ($p<0.05$)

for 10 weeks improved the GFR in diabetic rats significantly, implicating its nephroprotective action. We suggested that CGA improved GFR by downregulating TGF-β induced expression of extracellular matrix proteins in the glomerular matrix [86]. Proteinuria is an important indication of diabetic nephropathy which occurs as a result of decreased tubular reabsorption of plasma proteins [87]. The total protein excreted in urine was significantly elevated in diabetic control rats. On the other hand, the total proteins excreted in urine were significantly decreased in chlorogenic acid CGA treated diabetic rats as compared to diabetic control rats. The authors suggested that CGA improved proteinuria by preventing hyperglycemia and TGF-â induced tubular injury that can induce glomerular hyper-filtration leading to protein infusion into Bowman's space [86]. Lotus leaf extracts LLE possessed strong hepatoprotective and antioxidant activity in a rat model of CCl_4-induced [81]. The hepatoprotective activity of LLE may be due to its free radical-scavenging and antioxidant activity, resulting from the presence of some flavonoids and phenolic compounds in the extracts. On the other hand, Rafieian-kopaei [88] reported that, Oxidative stress is an important factor contributing to kidney damage by increasing production of oxidants, particularly insufficiency of endogenous antioxidant defense system. Medicinal plants antioxidants have been shown to ameliorate oxidative induced kidney damage by reduction of lipid peroxidation and enhancement of scavenging ability of antioxidant defense system. Supplementation of medicinal plants antioxidants might be considered important remedies to abrogate pathology of oxidative stress induced kidney damage; however, single antioxidants do not act the same and might not be beneficial.

Recommendations: According to our results, we can recommend the following: Encouragement of nutrition education programmers investigating the importance of green coffee and lotus leaf aqueous extract in weight loss and the complications resulting from obesity and diabetes.

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