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Potential Protective Effects of Strawberry (*Fragaria Ananassa*) Leaves Against Alloxan Induced Type 2 Diabetes in Rats: Molecular, Biological and Biochemical Studies

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Abstract

The present study aimed to clarify the effect of strawberry (*Fragaria ananassa*) leaves powder on some biological, biochemical and molecular parameters of alloxan-induced diabetes in rats. Diabetic rats were fed treated daily with four doses (2.5, 5 , 7.5 and 10%, w/w) of strawberry leaves powder (SLP) for 28 days, and some molecular parameters for diabetic rats were fed treated daily with two doses (7.5 and 10%, w/w) of strawberry leaves powder for 24 hours ,3 days and 10 days after preliminary study. Diabetic rats exhibited significant decreases in BWG, FI, FER values and increases the relative organs (liver, kidney, heart, spleen and lungs) weight as compared to the control. Also, significant increases in blood glucose, liver functions (AST and ALT) and kidney functions (urea and creatinine) parameters in diabetic rats as compared to the control. Supplementation of the rat diets with 2.5% to 10% w/w by SLP decreased the blood glucose level and improved the hyperglycemia complications includes liver and kidneys functions. For molecular data, feeding diabetic rats with SLP caused a significant ($p \leq 0.05$) increased in the values of mean maximal optical density (MODY) of RNA as compared with control diabetic rats. Also, diabetes increase DNA damage (maximal optical density of apoptotic fragments) of liver and pancreas in rats which has been significantly improved by feeding SLP. In conclusion, date of the

present study provide a basis for the use of SLP for the prevention and/or treatment of T2D instead of/beside the synthetic medications which may have unwanted side effects.

Keywords: Strawberry leaves; Organs weight; Liver functions; Kidney functions; Apoptosis; RNA.

1. Introduction

Diabetes mellitus (DM) defined a metabolic disease commonly observed in the clinic and is characterized by hyperglycemia due to impaired insulin secretion, insulin resistance, or both, resulting in impaired metabolism of sugars, lipids, and protein [1]. Diabetes causes long-term microvascular issues in the eyes, kidneys, and nerves, as well as an elevated risk of cardiovascular disease [2, 3]. As a consequence, DM is a huge public health issue around the world and become a global epidemic as its prevalence is steadily increasing everywhere at an alarming rate [4, 5]. It is one of the largest and most popular non-communicable diseases, impacting 422 million people worldwide, and has grown tremendously in the previous three decades. Similarly, 108 million people had DM in 1985, the number increased to 422 million by 2016 which is predicted to be doubled by 2030 [6, 7]. From 2013 to 2035, according to the International Diabetes Federation (IDF), the number of diabetic patients in the Middle East and North Africa region will increase by 96 percent, from 34.6 million to 67.9 million [8]. In Egypt, Diabetes is a rapidly increasing health issue that has a considerable impact on morbidity, mortality, and health-care resources. Diabetes affects 15.56 percent of persons aged 20 to 79, with 86,478 deaths caused by the disease each year. Egypt is one of the top ten countries in the world for diabetes patients, according to the International Diabetes Federation IDF [8]. As a result, diabetes remains a public health issue that has a substantial economic impact in Egypt. In general, there are two main categories of this disease. Type 1 diabetes (T1D) also called insulin-dependent diabetes mellitus (IDDM) and Type 2 (T2D), the noninsulin- dependent diabetes mellitus (NIDDM). T2D patients account for 90-95 percent of all diabetic patients globally, and the disease is on the rise [9]. T2D one of the most common chronic disorders in the world, since changing lifestyles result in less physical activity and more obesit [10]. It's also a leading cause of heart attacks, blindness, kidney failure, strokes, neurological and immunological diseases, and amputations of the lower limb [2]. Early phenomenon of T2D is insulin insensitivity, which not only has negative metabolic consequences but also contributes subsequent pancreas β-cell exhaustion, resulting in the onset of clinical hyperglycemia [11]. Thus, understanding the regulation of the insulin response and identifying the related mechanisms are important to early treatment and prevention of T2D.

The treatment of diabetes is based on insulin and/or oral hypoglycemic medications [12]. These medications act by various mechanisms to control blood glucose levels, but many side effects have been reported [13]. Although, there are several commercially available drugs for treatment of diabetes their long terms may cause unwanted side effects on the kidney, liver and stomach, etc. [14]. On the other hand, diabetes costs are a major concern for patients and their families, as well as health care providers, insurance, and the general public [15]. Therefore, the world's attention began to turn to the method of T2D treatment with Phytotherapy. It is typically characterized as the use of plant extracts, plant parts, and natural preparations as medicines for the prevention and treatment of diseases. Medicinal plants or herbs are the plants that have traditionally been utilized in phytotherapy [16]. Nowadays, there is considerable interest in the field of medicinal plants due to their natural origin and fewer side effects [17]. Many natural plants and components of them have recently been utilized as traditional medicines to treat a variety of human illnesses where these plants are natural sources of antioxidants [18]. Leaves which are rich in antioxidants and bioactive ingredients, can help protect humans from many chronic diseases including T2D [19]. They are known for their therapeutic value, as being a rich source of antioxidants, vitamins and pigments [20, 21].

Strawberry (*Fragaria ananassa L.*) is a bushy, evergreen plant native to the Mediterranean [22]. Traditional phytotherapy has utilised all parts of the plant [23]. Strawberry leaves are a rich source of physiologically active substances and have long been used to treat the symptoms of a variety of illnesses and disorders. They include a variety of phenolic chemicals that have health benefits, two major compounds related to their beneficial activities in animals and humans are arbutin and hydroquinone [24]. Previous research has suggested that arbutin is a powerful antioxidant and cytoprotective agent [25, 26]. Water leaves of strawberry extract has positive effects in the treatment of diabetes, hypertension, and inflammation, chiefly due to its diuretic, uroantiseptic and astringent properties [23, 27]. Furthermore, strawberry leaves are considered to be an excellent source of compounds, with high antioxidant capacity, high biological activities and potential health benefits such as reducing myocardium ischemia, reduction of thrombosis risk and anti-cancer activity [28]. Despite this, various countries of the world including Egypt, still grow strawberries for their fruits only, while the vegetative part, which represents the majority of green leaves, is left as waste thrown into the environment without use causing many harmful environmental effects. Therefore, in an attempt to take advantage of this important plant part (strawberry leaves), which is present in Egypt in huge quantities, the current molecular and biochemical study was conducted in order to explore their potential protective effects against type 2 diabetes caused by alloxan in experimental rats.

2. Materials and Methods

2.1. Materials

2.1.1. Plant Part

Strawberry (*Fragaria ananassa*) leaves were obtained in February 2020 by special arrangement with some village farmers lived in Badr Center, Menoufia Governorate, Egypt.

2.1.2. Chemicals

Sigma Chemical Co., St. Louis, Mo., provided the alloxan utilized to induce T2D in rats. Morgan Company for Chemicals, Cairo, Egypt, provided casein as the main source of protein for the rat diet preparation. Vitamins and salts mixtures, cellulose, choline chloride, L-methionine, all organic solvents, buffers and other analytical chemicals were acquired from Cairo, Egypt's El-Ghomhorya Company for Trading Drugs, Chemicals, and Medical Requirements.

2.1.3. Kits

Gama Trade Company, Cairo, Egypt, provided the kits used to detect serum glucose, AST, ALT, urea nitrogen, and creatinine.

2.2. Methods

2.2.1. Preparation of Strawberry Leaves Powder (SLP)

In the morning, mature fresh and healthy leaves were harvested and promptly transported to the laboratory. Impurities such as dust were removed from the gathered leaves by cleaning and washing them with distilled water. Leaves were dried at 55 0C in a hot air oven (Horizontal Forced Air Drier, Proctor and Schwartz Inc., Philadelphia, PA) until the moisture content in the final product was about 8%. In a high-speed mixer, the dried leaves were ground into a fine powder (Moulinex Egypt, ElAraby Co., Benha, Egypt). The material that filtered through an 80 mesh sieve was saved and stored in polyethylene pages at 4 0C until needed.

2.2.2. Biological Experimental

2.2.2.1. Ethical Approval

Biological experiments for this study were ethically approved by the Scientific Research Ethics Committee (Animal Care and Use), Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt (Approval no. 05- SREC- 10-2019).

2.2.2.2. Animals

Animals used in this study, eighty-four (84) adult male albino rats of Sprague Dawley Strain weighing (140 ±10) g were obtained from the Medical Insects Research Institute, Doki, Cairo, Egypt.

2.2.2.3. Basal Diet

The basic meal for rats was made according to the following formula, as stated by American Institute of Nutrition AIN [29]: corn oil (10 percent), protein (10 percent), choline chloride (0.2 percent), vitamin mixture (1 percent), mineral mixture (4 percent), methionine (0.3 percent), cellulose (5 percent), and corn starch (69.5 percent). According to American Institute of Nutrition AIN [29], the employed vitamin and salt mixture component was created.

2.2.2.4. Induction of Diabetes

Diabetes was induced in normal healthy rats by injection into operationally with freshly prepared alloxan monohydrate in saline at a dose level of 150 mg/ kg body weight [30]. To overcome drug-induced hypoglycemia, rats were given a 5% glucose solution immediately after injection and kept overnight [31, 32]. After five days, a drop of blood was collected from the tail vein and put to a strip of haemogluco test using a particular kit (AlGomhoryia Company for Trading Drugs, Chemicals, and Medical Instruments, Cairo, Egypt). Diabetic rats were defined as those having an FBG of greater than 126 mg/dl and were included in the study.

2.2.2.5. Experimental Design

The Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council NRC, [33] ruled on all biological studies conducted. The experiments were carried out in two stages.

2.2.2.5.1. First Stage: Biological and Biochemical Experiments

Thirty-six adult male albino rats (Sprague Dawley Strain), weighing (140 ±10) g used in this study. Rats were housed in individual stainless-steel cages under the normal environmental condition of temperature with 12 h light–dark cycle during the experiment, in the animal house of the Faculty of Sciences, Menoufia University, Egypt. For acclimation, all rats were fed a basic diet for one week before beginning the experiment. The rats were divided into two groups after a one-week period, the first group (Group 1, 6 rats) still fed on basal/standard diet (SD) as a negative control group (-ve) and the other main group (30 rats) was used for diabetes induction and classified into five sub groups. Subgroup 1: fed on the basal diet as a positive control group (+ve). Subgroups (2 to 5): fed on the SLP by 2.5, 5, 7.5 and 10 % (w/w) of the basal diet weight, respectively. All the rats had free access to the diet and water as well as the treatments continued for a total duration of 4 weeks. During the experimental period, the body weight and food intake were estimated weekly.

2.2.2.5.2. Second Stage: Molecular Experiments

After biological and biochemical studies on the effect of SLP on diabetic rats, optimum doses that showed the best results were applied for molecular studies. The applied doses were 7.5% and 10% of SLP. Sixty rats weighing (140 ±10g) were used in this study. For acclimation, all rats were fed a basic diet for one week before beginning the experiment. The rats were divided into three groups after a one-week period. Group 1 (n=15): negative control (-ve), normal rats were fed on the basal diet. Group 2 (n=15): positive control (+ve), the diabetic rats were fed on the basal diet. Group 3 (n=30): diabetic rats were divided randomly into two subgroups (15 rats each), Sub-group 1: Diabetic rats fed on the SLP by 7.5% (w/w) of the basal diet weight. Sub-group 2: diabetic rats will be fed on the SLP by 10% (w/w) of the basal diet weight.

2.2.2.6. Biological Evaluation

During the experimental period (28 days), the diet consumed was recorded every day and body weight was recorded every week. The body weight gain (BWG, %), feed intake (FI, g/day/rat) and food efficiency ratio (FER) were determined according to Chapman, *et al.* [34] using the following equations: BWG (%) = (Final weight – Initial weight)/ Initial weight ×100 and FER = Grams gain in body weight (g/28 day)/Grams feed intake (g/28 day).

2.2.2.7. Blood Sampling and Organs Removal

At the end of experiments, all rats were fasted up to 12 hours and then sacrificed under ether anesthesia. Blood samples were drawn from the portal vein and placed in clean, dry centrifuge tubes. They were allowed to clot for 30 minutes at room temperature before being centrifuged for 15 minutes at 3000 rpm to separate the serum. The serum was carefully extracted and transferred to clean eppendorf tubes, which were then frozen at -200C for further investigation. Internal organs including heart, liver, kidneys and spleen of each rat were removed, washed in saline solution, dried by filter paper and weighed separately according to the method mentioned by Drury and Wallington, [35].

2.2.3. Hematological Analysis

2.2.3.1. Serum Glucose

Enzymatic determination of serum glucose was carried out colorimetrically according to Yound [36].

2.2.3.2. Liver Functions

Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured in serum using the modified kinetic method of Tietz [37].

2.2.3.3. Kidney Functions

Serum levels of urea and creatinine were determined according to Pattn and Croush [38] and Schultz [39], respectively.

2.2.4. Molecular Analysis

2.2.4.1. Electrophoretic Pattern of Nucleic Acids (DNA and RNA) Electrophoresis of Lysate Tissues

Electrophoresis of lysate tissues used to evaluate RNA pattern according to the method described by Hassab, *et al.* [40]. A piece of 10 mg of liver, and pancreas from each mouse was excised immediately after perfusion. Yellow tips were used to gently crush the hepatic and pancreatic tissues in eppendorf tubes before lysing them in 200l lysing buffer (50mMNaCL, 1mMNa2 EDTA and 0.5 percent SDS, pH 8.3). Gel Preparation: Gel was prepared using 1.8% electrophoretic grade agarose. The agarose was boiled with tris borate EDTA (TBE) buffer (1xTBE buffer; 89

mMTris, 89mM boric acid, 2mM EDTA, pH 8.3). The agarose mixture was then treated with 0.5 microgram/ml ethidium bromide at 40°C. Before loading samples, the gel was poured and allowed to harden at room temperature for 1 hour. 20µl of tissue lysate were put into gel wells, along with 5µl of loading buffer, to identify the electrophoretic pattern of nucleic acids in tissue lysate. Electrophoresis was performed on (Biomtra Standard Power Pack P25) for 2h. at 50V in gel buffer where DNA and RNA were visualized using a 312 nm UV trasilluminator (Cole-Parmer; Cole Parmer instrument Co. Chicago, USA). The intensity of RNA was measured by gel pro analyzer program as maximal optical density.

2.2.4.2. DNA Extraction and Apoptosis

Nucleic acids extraction based on salting out extraction method, and modification introduced by Hassab and EL-Nabi [41], where a piece of 10 mg of tissue was squeezed and lysed in 600 µl lysing buffer and was shake gently. The mixture was kept overnight at 37ºC. For protein precipitation, an amount of 200 µl of saturated Nacl was added to the samples. After that, they were gently shaken and centrifuged for 10 minutes at 12000 rpm. A new eppendorf tube was used to transfer the supernatant. The DNA in the supernatant was precipitated by 700 µl cold isopropanol. The mixture was inverted numerous times until fine nucleic acid fibres emerged, then centrifuged at 12000 rpm for 10 minutes. The pellets were rinsed with 500 l of 70% ethyl alcohol and centrifuged at 12000 rpm for 5 minutes after the supernatant was collected. The alcohol was decanted or tipped out after centrifugation, and the tube was blotted with Whatman paper or cleans tissue until the pellets seemed to be dry. For 30 minutes, the pellets were resuspended in 50 l of TE buffer (10 mMtris, 1 mM EDTA; pH 8) supplemented with 5% glycerol and 15 l of loading mixture (5µl of RNAse and10 µl loading buffer). Finally, the samples were loaded into gel-wells for detection of apoptosis.

2.3. Statistical Analysis

Data were expressed as mean ±SD. Differences between groups were examined for statistical significance using the ANOVA. Analyses of differences between the means of the treated and control groups were performed using Dennett's *t*-test. A p-value ≤ 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Effect of Feeding SLP on Body Weight Gain (BWG, %), Feed Intake (FI) and Feed Efficiency Ratio (FER) of Diabetic Rats

BWG, FI and FER of diabetic rats and consumed SLP were shown in Table (1) from such data it could be noticed that the diabetic rats exhibited significantly ($p \leq 0.05$) decreased in BWG (-76.67), FI (-47.37) and FER (-55.46) compared to the normal group. Supplementing the rat diets with SLP (2.5 to 10 g/100g) for 28 days increased the levels of BWG, FI, and FER considerably ($p \leq 0.05$). With SLP ingestion, the rate of increase in all of those metrics increased dosage dependently. Such data are in accordance with that reported by Abdulazeez and Pannusamy [42] and Faid, *et al.* [43] who mentioned SLP increasing the BWG, FI and FER. Also, Moustafa, *et al.* [44] showed SLP and its water extract (as a drug) increasing in BWG, FI and FER nephrotoxic rats. In the same direction, Hamzawy, *et al.* [45] and Abd El-Rahman [46] reported that liver rat's disorders probably induced by diabetes reveal significant reduction of the BWG and FI. Furthermore, several studies showed that diabetes and liver disorders can lead to malnutrition and the major causes of malnutrition in patients with diabetes and liver disease are poor dietary/feed intake, maldigestion, malabsorption and abnormalities in the metabolism and storage of macro and micro nutrients [46-49]. Recently, Elhassaneen *et al.* [50, 51] discovered that consuming plant parts containing bioactive substances like SLP increased the decrease in both FER and BWG in experimental rats.

Table-1. Effect of feeding SLP on body weight gain (BWG, %), feed intake (FI) and feed efficiency ratio (FER) of diabetic rats

Value	Control (-Ve) Std diet	Control (+Ve) Diabetes	Diabetic treated groups (SLP, w/w)				L.S.D
			2.5%	5%	7.5%	10%	
BWG (%)							
Mean	64.54^{a}	15.05^{e}	23.38^{d}	27.13^{d}	34.67^{c}	44.00^{b}	5.33
SD	6.66	3.01	1.46	2.2	3.28	5.33	
% of change	0.00	-76.68	-63.77	-57.96	-46.28	-31.93	
FI (g/day/rat)							
Mean	18.62^{a}	9.80^{e}	13.12^{d}	14.29^{d}	15.58^{c}	16.98^{b}	1.26
SD	1.14	0.68	1.32	0.81	0.92	0.75	
% of change	0.0	-47.37	-29.52	-23.23	-16.2	-8.81	
FER							
Mean	3.48^{a}	1.55^{d}	1.8^{cd}	1.9^{cd}	2.23^{bc}	2.60^{b}	0.412
SD	0.27	.18	0.09	0.12	0.35	0.15	
% of change	0.00	-55.4 6	-48.28	-45.4	-35.92	-25.29	

Means in the same row with different superscript letters are significantly different (p≤0.05).

3.2. Effect of Feeding of SLP on Relative Weight of Organs of Diabetic Rats

Relative weight organs of diabetic rats and consumed SLP were shown in Table (2). From such data it could be noticed that the diabetic rats exhibited significantly ($p \leq 0.05$) increased in relative liver (46.11%), kidney (24.02), heart (18.93%), spleen (18,28%) and lung (32.5%) compared to the normal group. Supplementing the rat diets with SLP (2.5 to 10 g/100g) for 28 days reduced the relative weight of those organs significantly ($p \leq 0.05$). With SLP ingestion, the rate of decrease in all of those metrics increased in a dose-dependent manner. Such data are in accordance with that reported by Abdulazeez and Pannusamy [42] and Faid, *et al.* [43].

Table-2. Effect of feeding SLP on relative weight of liver and kidney of diabetic rats

Value	Control (-Ve) Std diet	Control (+Ve) Diabetes	Diabetic treated groups (SLP, w/w)				L.S.D
			2.5%	5%	7.5%	10%	
Liver (%)							
Mean	3.34[a]	4.88[b]	3.45[a]	3.802[a]	3.48[a]	3.43[a]	0.489
SD	.38	0.383	0.26	0.39	0.36	0.43	
% of change	0.00	46.11	3.29	13.77	4.19	2.69	
Kidney (%)							
Mean	0.766[a]	0.95[b]	0.79[a]	0.787[a]	0.77[a]	0.78[a]	0.113
SD	0.11	0.08	0.08	0.06	0.06	0.09	
% of change	0.00	24.02	3.13	2.74	0.522	1.82	
Heart (%)							
Mean	0.37[a]	0.44[a]	0.394[a]	0.39[a]	0.378[a]	0.396[a]	0.0616
SD	0.09	0.05	0.01	0.013	0.012	0.06	
% of change	0.00	18.93	6.48	5.4	2.16	7.02	
Spleen (%)							
Mean	0.58[a]	0.68[a]	0.61[a]	0.59[a]	0.60[a]	0.592[a]	0.1687
SD	.08	.11	.12	0.03	0.11	0.05	
% of change	0.00	18.28	6.20	1.72	3.44	2.06	
Lung (%)							
Mean	0.72[a]	0.954[b]	0.768[a]	0.766[a]	0.762[a]	0.74[a]	0.1686
SD	0.07	0.07	0.08	0.23	0.12	0.09	
% of change	0.00	32.5	6.66	6.38	5.83	2.77	

Means in the same row with different superscript letters are significantly different ($p \leq 0.05$).

3.3. The Effect of Feeding SLP on Serum Glucose Level of Diabetic Rats

Glucose concentration in diabetic rats consumed SLP was shown in Table (3). From such data it could be noticed that diabetes induced significant ($p \leq 0.05$) increased in serum glucose (60.59%) compared to normal controls. Supplementation of the rat diet with 2.5% ,5% ,7.5% and 10% SLP induced significant ($p \leq 0.05$) decreasing on serum glucose level by the ratio of (43.96% , 14.34% ,19.03% , and 20.24 % (as % change of the control positive), respectively. The rate of amelioration effect in serum glucose rising induced by diabetes in rats was increased with the increasing of SLP. The increase in blood glucose level may be due to low plasma insulin level after using alloxan which destroy β -cells. These results were supported by those of Shaheen [6], Abd El-Moneim, *et al.* [52] and El-Shafey, *et al.* [53]. Also, Hannum [54] reported that the effects of SLP extract on diabetes are regulating the activities of α-amylase and α-glycosidase that lowers the blood glucose content. This is associated with the existence of antioxidant compounds as flavonoids, ellagic, and anthocyanin in SLP extract. Furthermore, Mima [55] found that SLP extract elevated plasma insulin level in diabetic rats. Insulin has been suggested to be an endogenous protective factor that prevents the progression of DM. Additionally; several researches have been done on the effect of plant parts extract including strawberry leaves consumption on diabetic conditions. Such activity may be related to diverse bioactive compounds present in strawberry leaves including phenolics, glycosides, flavonoids, polysaccharides and alkaloids [56, 57]. Results of previous studies suggested that arbutin, glycoside from the strawberry plant, is a potent antioxidative and cytoprotective [25, 26]. Water leaves of strawberry extract has positive effects in the treatment of diabetes, hypertension, and inflammation, chiefly due to its diuretic, uroantiseptic and astringent properties [23, 27].Also, other compounds such phenolics, flavonoids, polysaccharides and alkaloids, are known for their properties in scavenging free radicals, inhibiting lipid oxidation, improve glucose response, alleviating metabolic dysregulation of free fatty acids and insulin resistance associated with type 2 [47, 50, 58-60]. Also, such bioactive compounds have been reported to improve damages/complications caused by many diseases including DM [61, 62]. Furthermore, these compounds inhibit glucose transport across the intestine by inhibiting sodium glucose co-transporter-1 (S-GLUT-1) [63]. Another mechanism for the effect of SLP on serum glucose

attenuation has been proposed as follow, hyperglycemia arise because of β-islet cells irreversible destruction in pancreas by alloxan which leads to a reduction of insulin secretion, and also the generated reactive oxygen species and the subsequent increase of local oxidative stress, DNA methylation, and protein modification all are considered as the pathophysiological mechanisms of alloxan induced diabetes [54]. Furthermore, oxidative stress contributes to tissue damage caused by hypoglycemia and diabetes [5]. Different bioactive compounds categories found in SLP counteract the previous diabetes adverse biological effects through their known properties as antioxidant activities (scavenging free radicals and inhibiting lipid oxidation) caused by T2D [47, 50, 51, 58, 64].

Table-3. Effect of feeding SLP on blood glucose level (mg/dl) in diabetic rats

Value	Control (-Ve) Std diet	Control (+Ve) Diabetes	Diabetic treated groups (SLP, w/w)				L.S.D
			2.5%	5%	7.5%	10%	
Mean	149.2[a]	239.6[d]	214.8[c]	170.6[b]	177.6[b]	179.4[b]	19.29
SD	13.89	19.67	9.88	10.06	11.14	20.09	
% of change	0.00	60.59	43.96	14.34	19.03	20.24	

Means in the same row with different superscript letters are significantly different (p≤0.05).

3.4. Effect of Feeding SLP on Liver Functions of Diabetic Rats

Liver functions of diabetic rats consumed SLP was shown in Table (4). From such data it could be noticed that diabetes induced significant (p≤0.05) increased in serum liver functions parameters, ALT , (173.32%) ,and AST ,(33.91%) compared to normal controls. Supplementation of the rat diet with 2.5% ,5% ,7.5% and 10% SLP induced significant (p≤0.05) decreasing on serum liver functions concentration by the ratio of ALT (129.6% , 33.79% ,22.03% , and 30.03 %) and AST (35.75% , 14.03% , 4.80% , 3.57%) as % change of the control positive, respectively. The rate of amelioration effect in serum ALT and AST rising induced by diabetes in rats was increased with the increasing of SLP ratio. The results are in agreement with Shaheen [6] who mention that plasma treatment with SLP extract, especially 200 mg/kg BW, decreases AST and ALT level plasma as compared to the control group. Also, Jurica, *et al.* [24] reported that strawberry tree water leaf extract acceptable biocompatibility with liver tissue both in male and female rats. Furthermore, Shalaby [65] confirmed that strawberry leaves powder greatly reduces high levels of serum AST and ALT.

Table-4. Effect of feeding SLP on liver functions of diabetic rats

Value	Control (-Ve) Std diet	Control (+Ve) Diabetes	Diabetic treated groups (SLP, w/w)				L.S.D
			2.5%	5%	7.5%	10%	
Serum alanine aminotransferase (ALT, IU/L)							
Mean	34.83[a]	95.2[d]	80[c]	46.6[b]	42.5[b]	45.5[b]	7.05
SD	4.46	6.14	8.15	3.59	4.51	4.19	
% of change	0.00	173.32	129.68	33.79	22.03	30.03	
Serum aspartate aminotransferase (AST, (IU/L)							
Mean	76.46[a]	102.4[c]	103.8[c]	87.2[b]	80.14[ab]	79.2[ab]	8.54
SD	5.25	5.89	11.96	4.95	3.92	3.43	
% of change	0.00	33.91	35.75	14.03	4.80	3.57	

Means in the same row with different superscript letters are significantly different (p≤0.05).

In general, aminotransferases are normally intracellular enzymes. Two amino transferases were found in plasma are of particular diagnostic value AST and ALT. As a result, the presence of high amounts of aminotransferase in the plasma shows that cells that produce these enzymes have been damaged. Also, Ohaeri [66] discovered that diabetic rats' livers were necrotized. As a result, the rise in AST and ALT activity in serum could be attributed to leakage of these enzymes from the liver cytosol into the bloodstream. Furthermore, Abd El-Moneim, *et al.* [52] and El-Shafey, *et al.* [53] stated that alloxan-mediated free radicals cause lipid peroxidation and damage the membrane architecture of the liver and kidney, causing membrane phospholipids to leak out. Toxicity to alloxan causes aberrant lipid accumulation in the liver and kidney. Many studies have shown that extracts from plant parts can reduce the activity of serum liver function enzymes [47, 49, 50, 58, 64, 67, 68]. Such effects could be attributable to the high concentration of bioactive chemicals in their composition. Several previous studies reported that SLP are a rich source of different classes of bioactive compounds phenolics, glycosides, flavonoids, polysaccharides and alkaloids [56, 57]. The possible mode of action of liver serum enzymes-lowering activity of the tested SLP could be explained by one or more of the following process. Phenolic compounds found in SLP are known to block the hepatocellular uptake of bile acids. Phenolics such as found in SLP pretreatment improved the antioxidant capacity of the liver, diminished the bilirubin concentration and reduced the elevated levels of the following serum enzymes, AST and

ALT, reduce the damage of hepatocytes, and scavengers of reactive oxygen species (ROS) compared with the groups without treatment [47, 49-51, 69].

3.5. Effect of Feeding SLP on Kidney Function in Serum of Diabetic Rats

Table (5) was shown the kidney functions (urea and creatinine levels) in diabetic rats consumed SLP. From such data it could be noticed that diabetes induced significant ($p \leq 0.05$) increased in serum urea (29.42%) and creatinine (20.19%) compared to normal controls. Supplementation of the rat diet with 2.5% ,5% ,7.5% and 10% of SLP induced significant ($p \leq 0.05$) decreasing on serum kidney functions concentration by the ratio of 14.71% , 9.60% ,9% , and 2.25 % for urea and 15.02% , 12.34% , 16.04% , 19.75% for creatinine as % change of the control positive, respectively. The rate of ameliorations effect in serum urea and creatinine rising induced by diabetes in rats was increased with the increasing of SLP ratio. In similar study, Moustafa, *et al.* [44] reported that reduce in serum creatinine, urea, for all groups treated with strawberry leaves powder and its water extract at a concentration 10% compared to positive control group. Also, Mohamed and Ashour [70] confirmed that both doses of strawberry leaves extract caused the decrease in urea level, creatinine level. Also, Sato, *et al.* [71] speculated that strawberry leaf extract could have a powerful hypouricemic impact.

The effect of plant parts extracts on decreasing the serum kidney function parameters have been reported by many studies [49, 58, 68, 72-75]. Such as reviewed in these studies the decreasing in serum uric acid and creatinine as the result of feeding phyto by-products including SLP could be attributed to their higher content of phytochemicals such phenolics, anthocyanins, alkaloids, carotenoids, phytosterols and glycosides, flavonoids, polysaccharides compounds. The possible mode of action of kidney serum parameters-lowering level of the phyto SLP could be explained by one or more of the following process. Polyphenols found in such plant parts improved the kidney weight and serum levels of urea nitrogen, creatinine and creatinine clearance as well as increased the activity of superoxide dismutase in the kidney [58, 73, 76]. While, many authors such Mohamed and Ashour [70] found that significant reductions in serum urea and creatinine concentrations, decreased polyuria and body weight loss, and marked reductions in urinary fractional sodium excretion all contributed to significant protection of renal function as well as protected kidney tissues. Additionally, natural phenolic, alkaloids, coumarins and flavonoids such as hesperidin, rutin, silymarin and carotenoids were shown to ameliorate *cis*-mediated nephrotoxicity [77].

Table-5. Effect of feeding SLP on kidney functions of diabetic rats

Value	Control (-Ve) Std diet	Control (+Ve) Diabetes	Diabetic treated groups (SLP, w/w)				L.S.D
			2.5%	5%	7.5%	10%	
Serum urea concentration (mg/dl)							
Mean	66.6^{a}	86.2^{b}	76.4^{ab}	73.0^{ab}	72.6^{ab}	68.1^{a}	14.20
SD	4.03	18.01	12.3	8.3	10.4	4.8	
% of change	0.00	29.42	14.71	9.61	9.01	2.25	
Serum creatinine concentration (g/dl)							
Mean	0.812^{a}	0.980^{b}	0.934^{ab}	0.914^{ab}	0.948^{ab}	0.97^{b}	0.1303
SD	0.093	0.032	0.104	0.177	0.065	0.06	
% of change	0.00	20.6	15.024	12.34	16.74	19.75	

Means in the same row with different superscript letters are significantly different ($p \leq 0.05$).

3.6. Electrophoretic Pattern of Nucleic Acids (RNA and DNA)

3.6.1. Ribonucleic Acid (RNA)

Table (6) and Fig 1 the mean values of optical density of pancreatic and hepatic RNA on diabetic rats after 24 hours, 3 days and 7 days of treated with SLP . It could be noticed that feeding diabetic rats with SLP caused a significant ($p \leq 0.05$) increased in mean values of maximal optical density of RNA as compared with diabetic rats. These results are in agreement with Kaddis, *et al.* [78] and Denis, *et al.* [79].

3.6.2. Pancreas DNA of Diabetic Rats Treated with SLP

As shown in Table (7) and Fig 2 results recorded a significantly decreased in mean of maximal optical density of intact DNA in diabetic rats pancreas as compared with normal rats pancreas. While, mean values of maximal optical density of diabetic rats apoptotic bands at 800bp ,600 bp, 400bp and 200bp illustrated a significant increase as compared with normal rats pancreas. In accordance with Hunt, *et al.* [80] which indicated that the major free radicals that play a role in diabetic type 2 (T2DM) are superoxide, hydroxyl and proxy radicals. These free radicals lead to DNA damage, protein modification, glycation reaction and oxidative modification of lipids. Our results are in parallel with Baynes and Thorpe [81] who reported that ROS may cause selective damage of the pancreatic islet β-

cells**.** According to our preliminary findings, quercetin interacts with alloxan and reduces its oxidative potential. When combined with alloxan, this greatly reduces pancreatic β-cell destruction and thereby slows the progression of diabetes. Many researchers have shown that oxidative stress leads to DNA damage as a result of experimental diabetes and in *vitro* studies. As an indicator of oxidative damage, 8OHdG increases *in* tissues and body fluids in diabetic subjects [82, 83]. Another study discovered that lycopene, a clorofel with anti-oxidant properties, protects DNA by preventing comet formation and lowering the 8OHdG level. SLP, on the other hand, is effective in suppressing superoxide radicals, which cause a variety of disorders in addition to protecting DNA from oxidative damage [84]. Alloxan-induced degeneration in pancreatic beta-cells was determined immune-histochemically. It was concluded that SLP increased the free radical amount and could protect the beta cells against beta cell damage in the (slp) + Diabetes group. This result is similar to the study of Karaca, *et al.* [85].

Table-6. Mean of maximal optical density (MODY) of total RNA in tissue lysate of diabetic rats in pancreas and liver after 24 hours, 3 days and 7 days from treated with SLP

Parameters		Normal	Diabetic	SLP 7.5%	SLP10%
Pancreas	24H	$133.2^{b} \pm 7.75$	$102^{d} \pm 4.47$	$124.4^{b} \pm 6.05$	$122.02^{b} \pm 5.71$
	3 days	$151.2^{a} \pm 5.5$	$109.6^{d} \pm 5.21$	$176.4^{a} \pm 3.43$	$121.8^{b} \pm 2.5$
	7 days	$181.6^{a} \pm 2.45$	$110.6^{d} \pm 2.56$	$139.5^{a} \pm 5.51$	$166.8^{a} \pm 2.89$
Liver	24 H	$123.4^{c} \pm 7.61$	$100^{d} \pm 3.34$	$120.8^{c} \pm 1.89$	$118.6^{c} \pm 2.54$
	3 days	$217^{a} \pm 4.47$	$116.8^{d} \pm 5.51$	$198.8^{a} \pm 3.45$	$190.8^{a} \pm 2.56$
	7 days	$128.6^{b} \pm 5.45$	$105.4^{d} \pm 7.17$	$120.6^{c} \pm 4.25$	$130.2^{b} \pm 4.64$

Means in the same column with different superscript letters are significantly different at $p \leq 0.05$

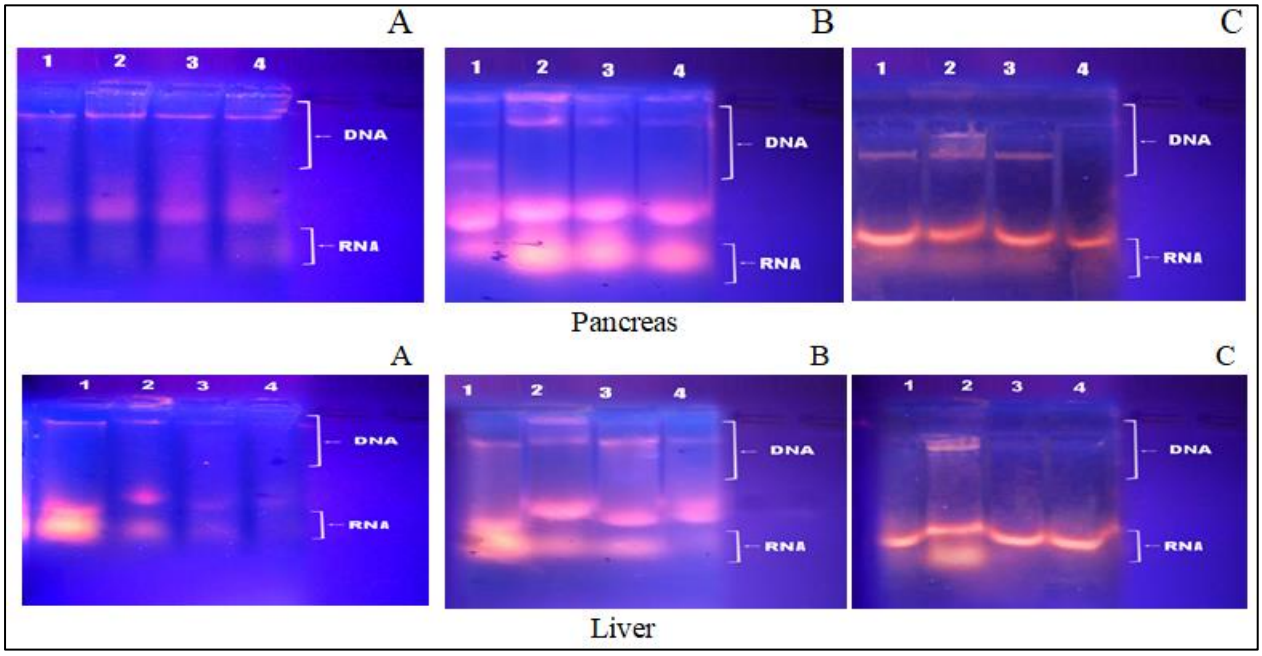

Fig-1. Mean of maximal optical density (MODY) of total RNA in tissue lysate of diabetic rats in pancreas and liver after, A) 24 hours; B) 3 days and C) 7 days from treated with SLP. Lane1: Normal control; Lane 2: diabetic; Lane 3:diabetic treated with 7.5% SLP; Lane 4:diabetic treated with 10% SLP. Deoxyribonucleic acid (DNA)

Table-7. Mean of maximal optical density of apoptotic fragments of DNA of diabetic rats in pancreas after 24 hour,3 days and 7 days from treated with SLP

Groups	After 24 hours				
	200 bp (mean ±SD)	**400 bp (mean ±SD)**	**600 bp (mean ±SD)**	**800 bp (mean ±SD)**	**Intact DNA**
Normal	129.8^{b}±3.5	120.8^{b}±6.8	120^{c}±1.8	123^{c}±2.06	176.8^{a} ±4.3
Diabetic	171.8^{a}±3.1	150.2^{b}±5.1	142^{b}±2.3	150.2^{b}±2.5	151.4^{b}±4.6
7.5%(SLP)	170.4^{a}±4.8	144.2^{c}±3.8	150.4^{b}±4.03	148.4^{b}±6.2	163.4^{a}±2.7
10% (SLP)	168^{a}±7.7	141.4^{c}±10.9	147.2^{c}±1.9	142.6^{c}±3.3	170^{a}±1.8
After 3 days					
Normal	118.4^{d}±2.07	116.6^{c}±4.63	147.4^{a}±2.71	155.8^{a}±2.77	231.8^{a}±5.85
Diabetic	152.4^{a}±3.09	159^{a}±2.911	159.2^{c}±2.77	151.4^{c}±2.07	143.3^{d}±9.4
7.5%(SLP)	127^{c}±3.10	122^{bc}±3.24	139.8^{b}±6.45	134.6^{c}±4.13	184.2^{b}±3.04
10% (SLP)	138^{b}±4.84	128^{a}±6.23	143.6^{b}±7.71	147.8^{b}±4.44	174.2^{c}±3.02
After 7 days					
Normal	118^{b}±5.42	133^{b}± 2.51	138.8^{a}±4.30	147.2^{a} ± 6.11	221.6^{a}±12.31
Diabetic	147^{a} ±4.51	151.8^{a}±6.02	143.6^{a} ±10.01	131.4^{b} ±5.05	150.4^{c}±9.91
7.5%(SLP)	120.2^{b} ± 9.11	110.4^{c}± 9.02	116.6^{b} ±8.06	118.8^{c} ±5.21	161^{b}± 4.81
10% (SLP)	115.8^{b} ±8.31	110.2^{c}± 6.71	110.6^{b} ±5.91	120.6^{c} ±5.52	168.6^{b}± 4.32

Means in the same column with different superscript letters are significantly different at p≤0.05

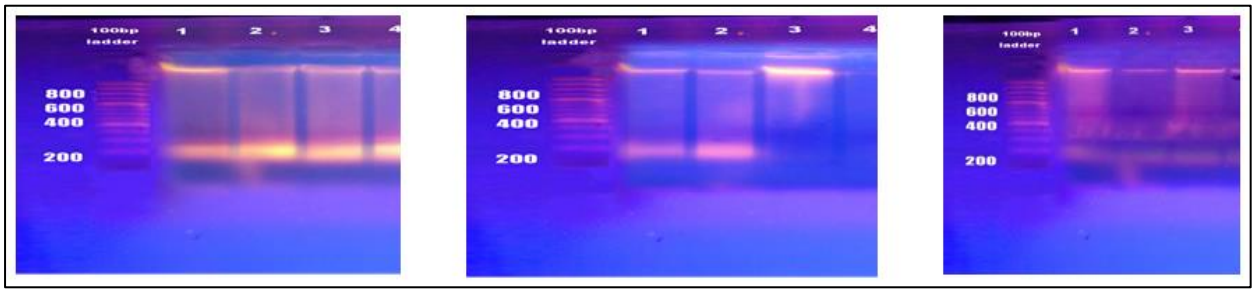

Fig-2. Mean of maximal optical density (MODY) of total RNA in tissue lysate of diabetic rats in pancreas after, A) 24 hours; B) 3 days and C)7 days from treated with SLP. **Lane1:** Normal control; **Lane 2:** diabetic; **Lane 3:** diabetic treated with 7.5% SLP; **Lane 4:** diabetic treated with 10% SLP

3.6.3. Hepatic DNA of Diabetic Rats Treated with SLP

As shown in Table (8) and Fig 3 results recorded a significantly decreased in mean of maximal optical density of intact DNA in diabetic rats liver as compared with normal rats liver . When compared to normal rat liver, mean values of maximal optical density of diabetic rats apoptotic bands at 800bp, 600bp, 400bp, and 200bp showed a substantial increase. In a similar vein, Jain, *et al.* [86] stated that the liver plays a critical role in maintaining blood glucose concentration by being able to both supply glucose to the circulation via glycogenolysis and gluconeogenesis and remove glucose from circulation in the post-absorptive state after meal ingestion, resulting in increased DNA damage in the liver in diabetes. This affects the liver leading to the impairment in the organ damage. Previous Studies have shown that compounds with strong antioxidant property can potentially be effective in delaying diabetes related complications. One such class of compounds is plant-derived polyphenols, which can be divided into several groups like flavonols, flavones and iso flavones (e.g. genistein**)** [87]**.** Also, Vehbi , *et al.* [88] showed that antioxidant and anthocyanin regulates body weight, blood sugar, glycated hemoglobin (HbA1c),insulin, lipid profile levels and glucose homeostasis, suggesting that it may reduce DNA damage by inhibiting oxidative stress. Those anti-oxidants which found in SLP prevent cell damage by neutralizing free radicals. Atherosclerosis and heart disorders are thought to be caused primarily by free radicals. The nucleic acids of free radicals are likewise affected by free radicals. Excessive cell death accelerates the ageing process and creates cell sequences that can lead to cancer and other illnesses [89]. The importance of anti-oxidant substances that operate against free radical production, which causes several life-threatening chronic diseases as a result of oxidation, has increased.

Table-8. Mean of maximal optical density of apoptotic fragments of DNA of diabetic rats in Liver after 24 hour 3 days and 7 days from treated with SLP

Groups	After 24 hours				
	200 bp (mean ±SD)	400 bp (mean ±SD)	600 bp (mean ±SD)	800 bp (mean ±SD)	Intact DNA
Normal	110.4^{d} ±5.51	126.4^{c}±3.04	128.2^{c}±3.03	123^{d} ±3.71	208^{a}±12.01
Diabetic	114.2^{d} ±7.71	158.2^{d}±4.11	158.1^{b}±2.91	128.2^{d}±12.6	150.4^{c}±7.41
7.5%(SLP)	107.5^{d}±5.2 107.8 ±5.02	143.4^{b}±2.81	147.2^{c}±2.11	131.6^{d}±5.81	171.2^{b}±3.44
10% (SLP)	113.4^{d} ±2.81	151.8^{c}±1.31	142.3^{c}±2.31	151.8^{c}±5.71	179.6^{b}±7.5
After 3 days					
Normal	97.41^{c}±3.1	143.61^{a}±5.1	144^{a}±4.2	145.8^{a}±3.71	205.4^{a} ±9.60
Diabetic	151.21^{a}±3.71	151.03^{c}±1.51	151^{b} ±6.21	157.8^{c}±3.92	148^{c}±7.14
7.5%(SLP)	101.6^{bc}±1.81	132.8^{b} ±3.21	138^{a}±5.11	139.6^{b}±4.32	177.6^{b}±4.32
10% (SLP)	104.4^{b}±4.02	134.6^{b}±3.91	143^{a}±2.71	145.6^{a}±2.97	161.4^{b}±7.52
After 7 days					
Normal	95^{c} ±4.62	126^{a}±3.61	128.6^{a}±3.74	133^{a}± 3.09	180.33^{a}±4.1
Diabetic	120.6^{a}±5.71	128^{c}±4.06	90^{c}±3.35	121.6^{c}±1.81	132.2^{c}±3.08
7.5%(SLP)	107.8^{bc}±2.24	109^{c}±3.51	106.4^{b}±5.02	109.8^{bc}± 5.62	153.6^{b}±3.91
10% (SLP)	108.2^{b}±7.31	115^{b}±5.51	101^{b}±6.41	109.2^{b}± 9.31	133.6^{c}±3.7

Means in the same column with different superscript letters are significantly different at $p \leq 0.05$

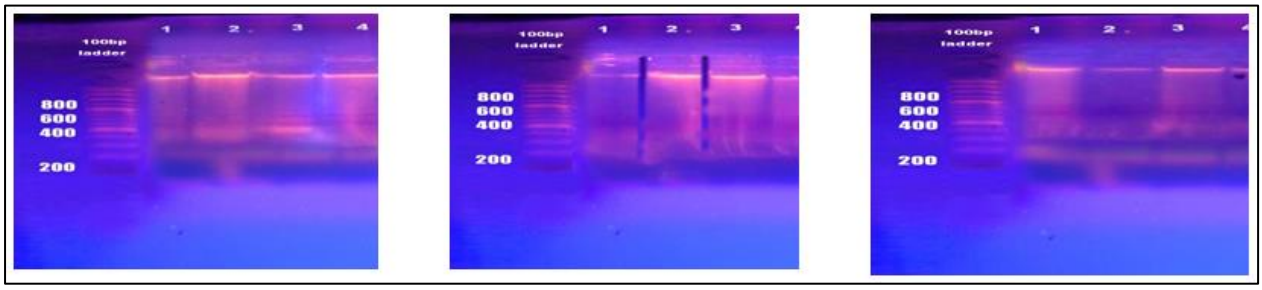

Fig-3. Mean of maximal optical density of apoptotic fragments of DNA of diabetic rats in Liver after a) 24 hours, b) 3 days and c)7 days from treated with strawberry leaves powder(SLP). Lane1: Normal control; Lane 2: diabetic ; Lane 3: diabetic treated with 7.5% (SLP); Lane 4: diabetic treated with10% SLP

4. Conclusion

The present study has demonstrated the potency of the SLP to partially ameliorate hyperglycemia and its complications in diabetic rats. The complications include improved many biological (BWG, FI, FER and relative weight organs), biochemical (liver and kidney functions) and molecular (apoptosis of liver and pancreas, and MODY of RNA) parameters. All of these treated effects could be attributed to the high contents of many bioactive compound categories found in SLP and the resulting different biological effects such as antioxidant activities. Such finding provides a basis for the use of SLP for the prevention and/or treatment of T2D.

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Conflict of Interests

Authors declared no competing of interest whatsoever

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