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The Potential Anti-Obesity, Anti-Diabetic and Anti-Oxidant Effects of Vitamin a in Streptozotocin-Induced Diabetic Mice

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Authors' contributions

This work was carried out in collaboration among all authors. Authors YA and SE designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AB, AB2, MS, NB, RA, SA, RA2, LA, AF and SE managed the analyses of the study. Author AF and SE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Evidence suggests that there is a link between diabetes mellitus and Vitamin A. Moreover, it has been reported that diabetes induces oxidative stress. Lately, a wide attention has been developed to the protective biochemical function of natural antioxidants contained vitamins, which can reduce the oxidative damage caused by free radical species.
Objective: To investigate the anti-obesity, anti-diabetic and anti-oxidative effects of vitamin A in streptozotocin (STZ)-induced diabetic mice.
Methods: Male mice were randomly divided into three groups: Control- nondiabetic, received a normal diet and water; Control-diabetic, received STZ 45mg/kg once intraperitoneally; and Treated-diabetic, received both STZ as before plus Vitamin A (4-IU/day) orally daily for 16 weeks. Food

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intake, body weight, fat mass, fasting blood glucose, serum insulin, and lipid profile were estimated. Also, superoxide dismutase (SOD), glutathione peroxidase (GPO), catalase (CAT), and malonaldehyde (MDA) were measured.

Results: Treated diabetic mice with Vitamin A showed a significant improvement in their body weight, fat mass, lipid profile as well as SOD, GPO and CAT compared to Control-diabetic mice. However, Vitamin A caused no significant change on fasting blood glucose and insulin levels. Furthermore, plasma level of MDA was significantly elevated in diabetic mice compared to normal mice. Diabetic mice treated with vitamin A had a significantly reduced level of MDA, suggesting that vitamin A might have a vital role in the protection of tissues from damage by free radicals.

Conclusion: Supplementation with vitamin A may be a useful treatment strategy for diabetic patients to reduce/prevent the pathological complications of diabetes.

Keywords: Vitamin A; antidiabetic; antioxidant; oxidative stress; diabetes mellitus; STZ-induced diabetic mice; catalase; superoxide dismutase.

1. INTRODUCTION

Diabetes mellitus is a major health problem. Epidemiologically, individuals with diabetes are expected to double to more than 350 million by 2025 [1]. There is an increasing rate of obesity worldwide that is significantly correlated with diabetes, which can be due to consuming high-calorie foods and low physical activity [2]. Hyperglycemia in prediabetes can lead to oxidative stress and the up regulation of proinflammatory factors, which ultimately lead to vascular dysfunction. To prevent the development of co morbidities, it is fundamental to determine the mechanisms involved in the progression from prediabetes to diabetes. Oxidative stress leads to impaired glucose uptake in muscle and fat cells and decreases insulin secretion from pancreatic β-cell [3].

Vitamin A is one of the vital nutrients that should be included in the diet in order to maintain health. It is essential for multiple life mechanisms such as the reproductive system, immune system, vision and differentiation at the cellular levels [4]. Moreover, Vitamin A is a very powerful antioxidant that helps maintain the functions of the heart, lungs, and kidneys, along with various other organs in the human body [5].

Recently, multiple studies started to investigate the effect of Vitamin A and its derivatives in multiple conditions. An evidence has shown that there is a link between diabetes and vitamin A. This previous study elucidated that Vitamin A can enhance insulin levels that are produced by pancreatic β-cell. In Addition, researchers discovered receptor for vitamin A on the cell surfaces of the beta cells [4]. By reviewing the literature, it is evident that Vitamin A deficiency predisposes individuals to type I and type II diabetes. Vitamin A deficiency has been shown to decrease the release of insulin and its synthesis and secretion in both humans and in diabetic animal models. Furthermore, multiple epidemiological studies have demonstrated that there is a link between vitamin A deficiency in both early onset and delayed onset of type I diabetes [6].

Lately, a wide attention has been developed to the protective biochemical function of natural antioxidants contained vitamins, which can prevent of the oxidative damage caused by free radical species [7]. Little information is available regarding the ant diabetic an antioxidant effect of vitamin A in diabetes mellitus. As diabetes has been shown to trigger oxidative damage, we hypothesized that Vitamin A might have an improvement effects on glycemic control as well as oxidative stress in diabetes mellitus.

2. MATERIALS AND METHODS

2.1 Reagents

Reagents and chemicals including (Vitamin A) were purchased from Sigma-Aldrich (MO, USA), unless otherwise stated.

2.2 Animals

All procedures concerning animal care and treatment were approved by the Ethical committee for the use of experimental animals at Umm Al-Qura University. For this study males of C57BL/6J (B6) mice were purchased from Harlan (Charles River Laboratories, Wilmington, Massachusetts, USA). Mice were maintained in a temperature-controlled room (21 ± 1 °C) under a 12-h to 12-h light to dark cycle. Mice were individually housed in standard cages with ad

libitum water and standard chow (CRM pellets, SDS diets, U.K.). Male of B6 strains were studied (total n = 21).

We calculated the samples based on one-way ANOVA, the between-subject error DF (that is, the within-subject degree of freedom (DF)) is calculated as: DF = N – k = kn – k = k (n – 1), where N = total number of subjects, k = number of groups, and n = number of subjects per group. By rearranging the formula, n is given as: n = DF/k + 1 Based on the acceptable range of the DF, the DF in the formulas are replaced with the minimum (10) and maximum (20) DFs to obtain the minimum and maximum numbers of animals per group: Minimum n = 10/k + 1 Maximum n = 20/k + 1 In total, the minimum and maximum numbers of animals required are: Minimum N = Minimum n x k Maximum N = Maximum n x k as suggested from [8].

In our study Minimum n = 10/3 + 1 = 4.3 = rounded up to 5 animals/group Maximum n = 20/3 + 1 = 7.7 = rounded down to 7 animals/group. Please note that the minimum and maximum numbers of animals per group are rounded up and down, respectively, to keep the DF for each sample size/group within the limit (e.g., DF = 12 for n = 5, and DF = 18 for n = 7). The total sample sizes are: Minimum N = Minimum n x 3 = 5 x 3 = 15 animals Maximum N = Maximum n x 3 = 7 x 3 = 21 animals. In conclusion, for the proposed study, between 5 and 7 animals per group are required. In other words, a total of 15 to 21 animals are required to keep the DF within the range of 10 to 20.

Measurements then started at the age of 10 weeks and were taken over a period of 16 weeks. Body weight, food intake and fat mass were monitored, just prior to lights off, 3 times a week (Mondays, Wednesdays and Fridays) throughout the experimental protocol. After 16 weeks mice were fasted for 4 h and euthanized by CO2 and blood samples were taken by cardiac puncture and liver tissues were preserved.

2.3 Induction and Assessment of Diabetes

Diabetes was induced in overnight-fasted, mice by a single dose of streptozotocin 45mg/kg, intraperitoneally [9], dissolved in cold citrate buffer (0.1M, pH 4.5). This dose was selected to cause incomplete destruction of pancreatic beta cells, as it has been reported that streptozotocin is capable of producing mild-to-severe types of diabetes according to the dosage used, when administered to adult mice by either a single intravenous or intraperitoneal injection [10]. After the injection, the mice had free access to food and water and were given a 5% glucose solution to drink overnight to counter hypoglycemic shock. Age-matched and weight-matched control mice received citrate buffer at the identical amount and pH. Diabetes was confirmed by measuring the fasting tail blood glucose concentration using a glucose meter device (Accu-chek Go, Roche Diagnostics GmbH, Mannheim, Germany) 96 hours after injection with streptozotocin. Ninety-nine percent of the animals had blood glucose above 235mg/dl and they were considered diabetic; mice with blood glucose below 235mg/dl were excluded from the present study.

2.4 Experimental Design

Mice were randomly classified into 3 groups (n=7 each) one group served as a control non diabetic group and two groups were injected with streptozotocin (45mg/kg, intraperitoneally) to induce diabetes, then the three groups received their respective drug treatments daily for 16 consecutive weeks according to the following design:

- Group 1: control non-diabetic mice treated with distilled water orally and citrate buffer intraperitoneally.
- Group 2: nontreated induced diabetic mice.
- Group 3: induced diabetic mice treated with vitamin A (4- IU/ day orally) as suggested by (Beck and Spiegelberg, 1989). At 2^{nd} and 16^{th} weeks, the levels of fasting blood glucose, fasting insulin levels, lipid profile were examined. After 16 weeks, free radicals and antioxidants were examined.

2.5 Assessment

2.5.1 Fasting blood glucose and fasting insulin measurements

At week 2 and 16 all mice were fasted overnight and blood were taken from their tails in order to measure their fasting blood glucose and insulin levels [11]. Fasting blood glucose was measured with a One Touch II glucose meter (Lifescan, Inc., Johnson & Johnson, Milpitas, CA). Fasting insulin concentrations were measured in duplicates using the enzyme-linked

immunosorbent assay (insulin ELISA, # nr 10-1247-01, Mercodia, Sweden) and the spectrophotometric plate reader (Synergy HT Multi-Mode Microplate Reader, BioTek, UK).

2.5.2 Plasma lipids

Blood samples were taken from mice tails and heart. After heart puncture blood was collected into 2 ml containers and centrifuged (5702/R, Eppendorf, Hauppauge, USA) at 1500 g at 4◦ C for 10 min. Total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL) levels were analyzed (using kits supplied by AMP diagnostics, Austria,) according to the manufacturer's instructions. Low density lipoprotein cholesterol (LDL) was calculated [12].

2.5.3 Body composition

Fat mass (FM) of mice was measured using dual energy X-ray absorptiometry (DEXA; PIXImus2 Series Densitometers with software version 1.46.007; GE Medical Systems Ultrasound and BMD, Bedford, UK). Mice were anesthetized by inhalation of a mixture of isoflurane and oxygen for the duration of the scan (∼3 min). The head was excluded from the analysis of body fat (Hambly et al. 2012).

2.5.4 Preparation of tissue homogenate

The liver tissues were accurately weighed (0.5 g m from each liver) and homogenized in 5 ml phosphate buffer (pH 7.4) using an electrical homogenizer to prepare 10% (w/v) clear tissue homogenate for determination of the glutathione peroxidase, catalase and SOD activities.

2.5.5 Evaluation of antioxidant enzymes

SOD activity in the supernatant was measured according to the method suggested by [13]. Results were defined as units per milligram protein (U/mg protein).

CAT activity in the supernatant was determined according to the method described by Aebi [14] Results were reported as the rate constant per second per milligram protein (k/mg protein). Measurements of the decomposition of hydrogen peroxide (H_2O_2) were performed spectrophotometrically according to the method suggested by Aebi [14] in the UV area of the light spectrum (240 nm, reading every 0.1 sec for 65 sec) using Lambda Software for data computation.

GPO activity in the supernatant was measured according to the method described by Griffith [15]. Results were defined as the units per milligram protein (U/mg protein).

2.5.6 MDA determination

Estimation of MDA was performed in the plasma using a thiobarbituric acid-reactive substance assay according to the method described by Ohkawa et al. [16] (nmol/g).

2.6 Statistical Analysis

All results are expressed as group means ± SEM. Results were analyzed by one-way analysis of variance, followed by Tukey's post-hoc test to assess significance, using a criterion of *P* value of less than 0.05. The statistical analysis was carried out using Graph Pad Prism version 5 (Graph Pad Software Inc., California, USA).

3. RESULTS

Fasting blood glucose levels were examined at week 2 and week 16 post-injection. Elevated fasting blood glucose levels (8.6 ± 4.3 mM) were observed at the third day post-injection. At two weeks both untreated and treated diabetic mice showed a significant increase in fasting glucose levels (9.7 ± 1.7 mM, 9.6 ± 1.5 mM, $P < 0.05$, respectively) compared with that of normal mice. At 16 weeks both untreated and treated diabetic mice still showed a significant increase in their fasting glucose levels (9.7 mM ± 1.7, 8.6± 1.3 mM, $P < 0.05$, respectively) compared with that of normal mice (Fig. 1).

Fasting blood insulin levels were examined at week 2 and week 16 post-injection. Elevated blood insulin levels (9.3 ± 7.1 mM) were observed at the third day post-injection. At two weeks both untreated and treated diabetic mice showed a significant increase in fasting insulin levels (9.8 ± 8.7 mM, 9.7 ± 7.5 mM, $P < 0.05$, respectively) compared with that of normal mice. At 16 weeks both untreated and treated diabetic mice still showed a significant increase in their fasting insulin levels (9.7 mM ± 7.7, 8.9± 8.3 mM, $P < 0.05$, respectively) compared with that of normal mice (Fig.2).

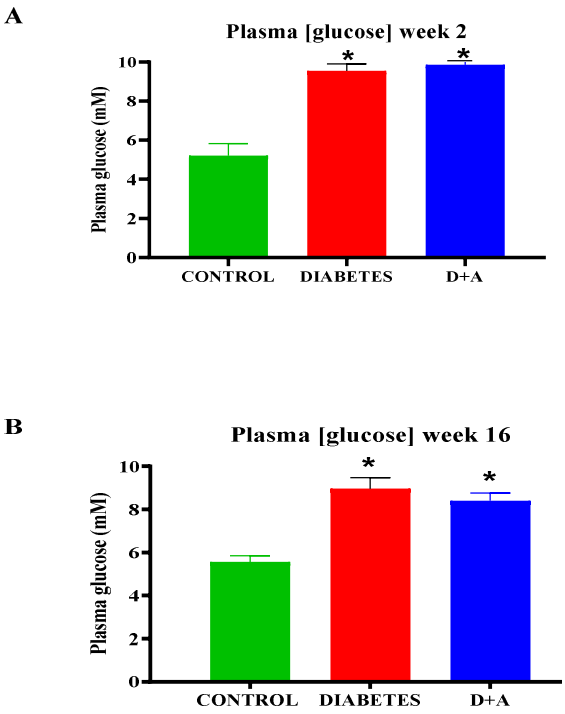

Fig. 1. Fasting plasma glucose levels at 2 and 16 weeks of treatment. D+A; diabetic mice treated with vitamin A. *P<0.05 compared to control nondiabetic mice

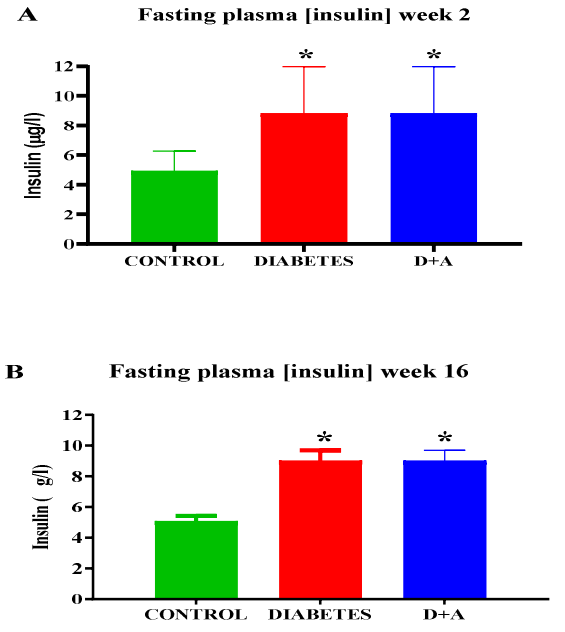

Fig. 2. Fasting plasma insulin levels at 2 and 16 weeks of treatment. D+A; diabetic mice treated with vitamin A. *P<0.05 compared to control nondiabetic mice

LDL levels were examined at week 2 and week 16 post-injection. At two weeks both untreated and treated diabetic mice showed a significant increase in their LDL levels (589 ± 27.9 mg/dl, 566 ± 24.4 mg/dl, $P < 0.05$, respectively) compared with that of normal mice. At 16 weeks treated diabetic mice showed a significant decrease in their LDL levels (369 ± 32.9 mg/dl, $P < 0.05$) compared with that of untreated mice (Fig. 3).

HDL were examined at week 2 and week 16 post-injection. At two weeks both untreated and treated diabetic mice showed a significant decrease in their HDL levels (4.7 ± 0.9 mmol/l, 4.8 ± 0.8 mmol/l, $P < 0.05$, respectively) compared with that of normal mice. At 16 weeks treated diabetic mice showed a significant increase in their HDL levels (12.3 ± 2.5 mmol/l, $P < 0.05$) compared with that of untreated mice (Fig. 4).

Total cholesterol was examined at week 2 and week 16 post-injection. At two weeks both untreated and treated diabetic mice showed a significant increase in their total cholesterol levels (5.8 ± 2.9 mmol/l, 5.9 ± 2.4 mmol/l, $P < 0.05$, respectively) compared with that of normal mice. At 16 week treated diabetic mice showed a significant decrease in their total cholesterol levels (1.9 ± 0.9 mmol/l, $P < 0.05$) compared with that of untreated mice (Fig. 5).

Triglycerides were examined at week 2 and week 16 post-injection. At two weeks both untreated and treated diabetic mice showed a significant increase in their total triglycerides levels (2.56 ± 2.6 mmol/l, 2.61 ± 2.2 mmol/l, $P < 0.05$, respectively) compared with those of normal mice. At 16 weeks treated diabetic mice showed a significant decrease in their triglycerides levels (1.52 ± 0.8 mmol/l, $P < 0.05$) compared with that of untreated mice (Fig. 6).

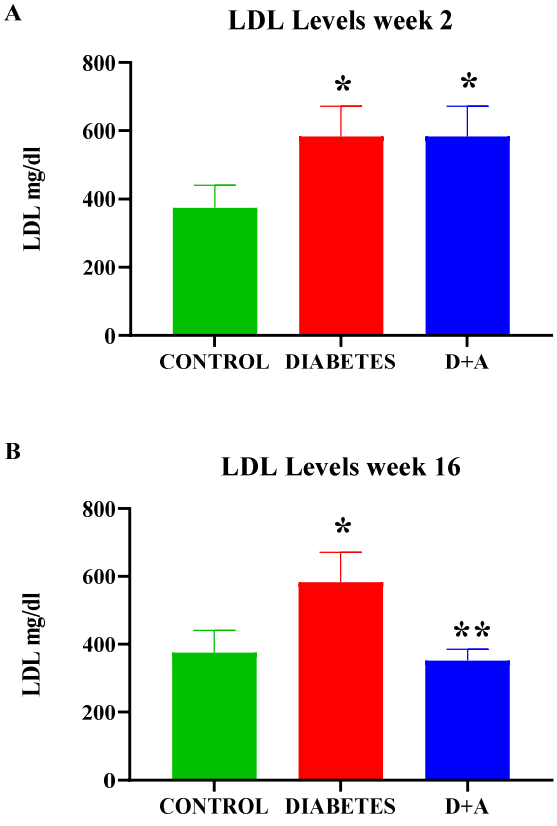

Fig. 3. LDL levels at 2 weeks and 16 weeks of treatment. D+A; diabetic mice treated with vitamin A *P<0.05 compared to control non diabetic mice, **P<0.05 compared to diabetic mice

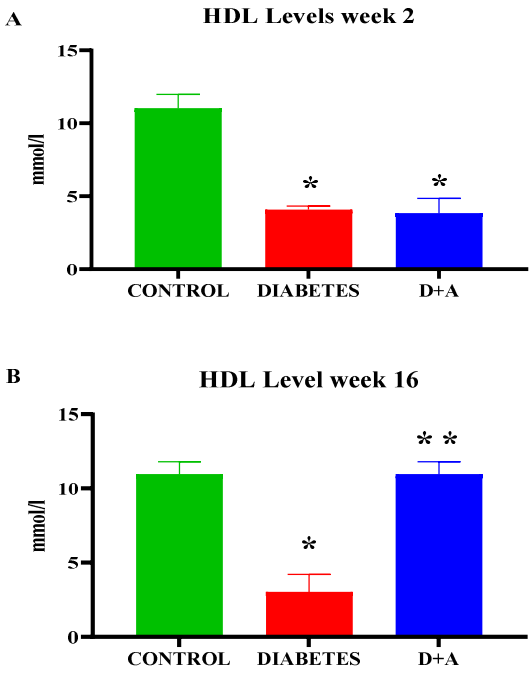

Fig. 4. HDL levels at 2 weeks and 16 weeks of treatment. D+A; diabetic mice treated with Vitamin A *P<0.05 compared to control nondiabetic mice, **P<0.05 compared to diabetic mice

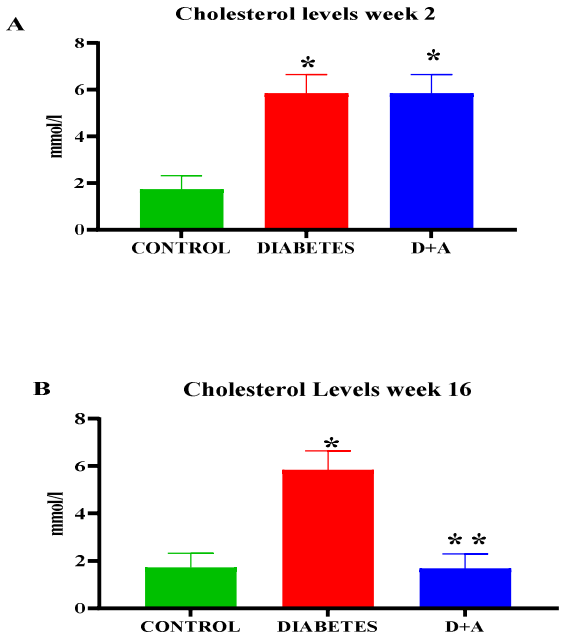

Fig. 5. Total cholesterol levels in mmol//l at 2 weeks and 16 weeks of treatment. D+A; diabetic mice treated with vitamin A *P<0.05 compared to control nondiabetic mice, **P<0.05 compared to diabetic mice

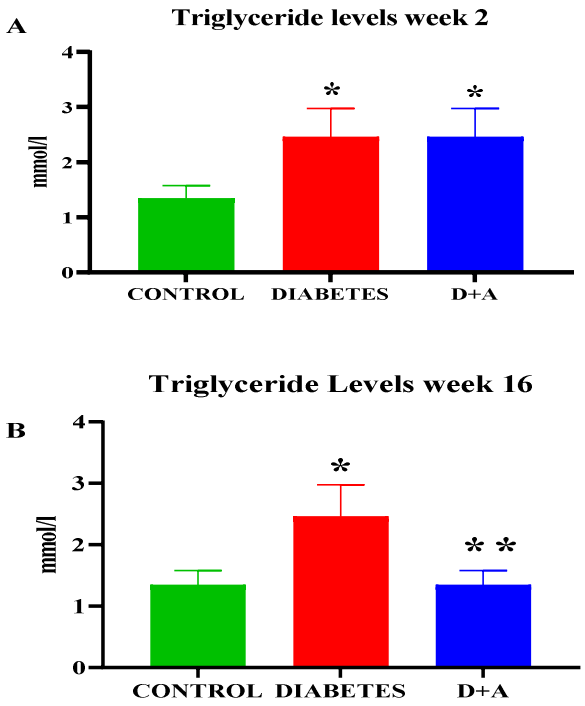

Fig. 6. Triglycerides levels in mmol/l at 2 weeks and 16 weeks of treatment. D+A; diabetic mice treated with vitamin A *P<0.05 compared to control undiabetic mice, **P<0.05 compared to diabetic mice

Body weight and food intake were examined at the beginning of the experiment and then every two weeks until the end of the study. Throughout the study period, the untreated mice showed a significant increase in their body mass ($P < 0.05$) compared with those of normal as well as treated diabetic mice. After 12 weeks, treated diabetic mice showed a significant decrease in their body weight ($P < 0.05$) compared with those of untreated diabetic mice (Fig. 7). By week 8, the untreated diabetic mice showed a significance increase in their food intake ($P < 0.05$) compared with that of normal control mice. Diabetic mice treated with Vitamin A showed a significant decrease in their food intake ($P < 0.05$) compared with that of untreated diabetic mice (Fig. 8).

Body fat was examined at week 4,8,12 and 16. At week 4 all mice groups did not show a significance difference in their fat masses. At weeks 8, 12 and 16 untreated diabetic mice showed a significant increase in their fat mass (16.3±10.8g, 16.4±10.7g, 22.3± 20.4g, P<0.05, respectively) compared with that of normal mice. Meanwhile, at weeks 8, 12, and 16; Vitamin A treated diabetic mice showed a significant decrease in their fat mass (7.8± 6.2 g, 8.3±2.5 g, 5.1± 3.3 g, P<0.05, respectively) compared with that of untreated diabetic mice (Fig. 9).

The untreated group of diabetic mice showed a significant decrease ($P < 0.001$) in the activities of CAT (by −80.1% 3.79 ± 0.11), GPO (by – 80%, 2.98 ± 0.04), and SOD (by −76.9%, 127.3 ± 2.1) compared with that of normal mice. On the other hand, diabetic mice treated with Vitamin A revealed a significant increase ($P < 0.001$) in the levels of the previous antioxidant enzymes; CAT, GPO, and SOD (by 206%, 547%, 309% respectively), compared with that of the untreated diabetic mice.

On the contrary, the level of MDA was significantly elevated ($P < 0.001$) in the untreated diabetic induced mice (by 291%, 56.66 ± 1.7) compared with the normal mice (15.22 ± 0.05). Diabetic mice treated with vitamin A showed a significant lowering ($P < 0.001$) in the level of MDA (28.44 ± 0.2) in comparison with untreated diabetic ones (Table 1).

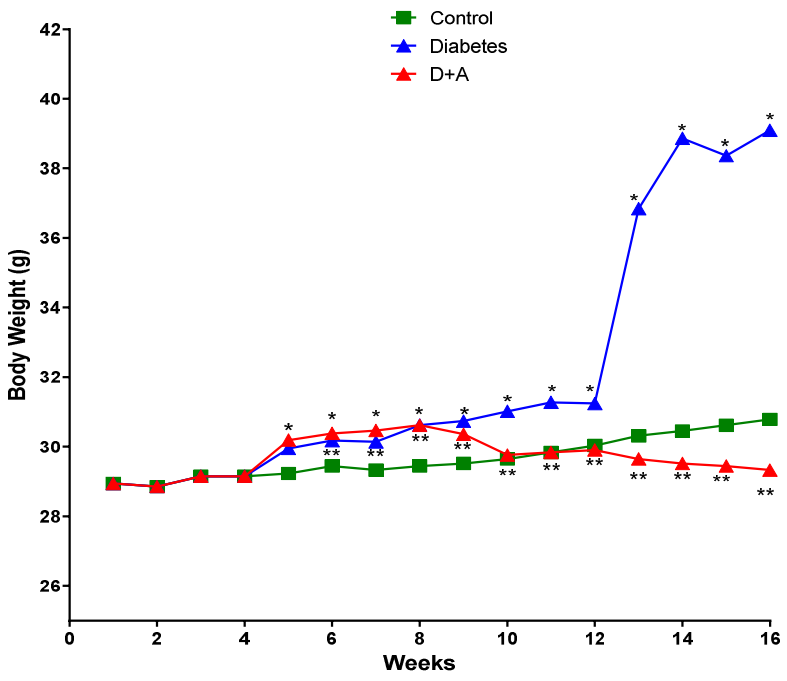

Fig. 7. Body weight in grams throughout the study period. D+A; diabetic mice treated with vitamin A.*P<0.05 compared to control undiabetic mice. **P<0.05 compared to diabetic mice

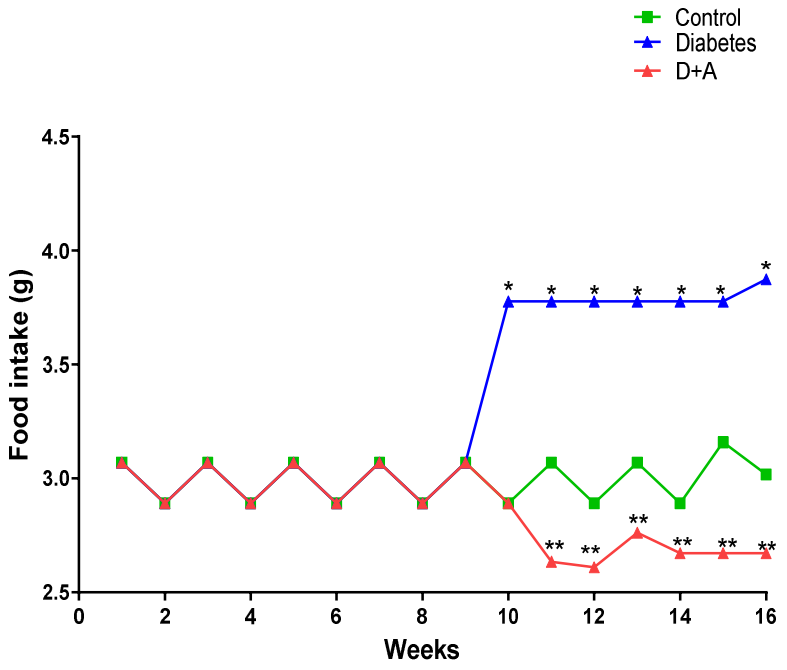

Fig. 8. Food intake in grams throughout the study period. D+A; diabetic mice treated with vitamin A.*P<0.05 compared to control nondiabetic mice. **P<0.05 compared to diabetic mice

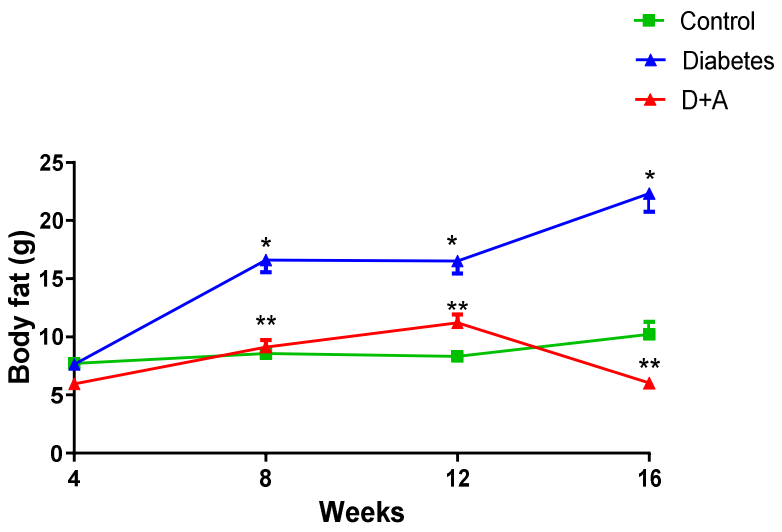

Fig. 9. Body fat mass in grams throughout the study period. D+A; diabetic mice treated with Vitamin A.P*<0.05 compared to control undiabetic mice. ***P*<0.05 compared to diabetic mice**

4. DISCUSSION

Diabetes and the associated comorbidities such as overweight and cardiac diseases are lead causes of human morbidity and mortality. Overweight is a metabolic disorder due to the accumulation of excess dietary calories into visceral fat and the release of high concentrations of free fatty acids into various organs. It represents a state of chronic oxidative stress and low-grade inflammation that may progress to hyperglycemia and type 2 diabetes [17]. The status of the disease diabetes produces vital changes at the intracellular levels [18,19]. An increasing body of evidence has reported that there is a link between vitamin A and diabetes. Moreover, reported the increase in the oxidative stress status in diabetes mellitus [20,21,22]. Few studies used the retinoic acid and suggested that it can have a reno-protective effect in the early stages of diabetic nephropathy through a unique anti□inflammatory pattern [23].

In the present study, streptozotocin induced diabetic mice demonstrated the expected increases in body weight, plasma glucose and total lipids, consisted with the metabolic abnormalities in diabetes patients [24,25]. In the current experiment, treatment with Vitamin A had no significant effects on neither fasting blood glucose nor insulin levels. Nonetheless, previous clinical study proved that Vitamin A caused a transient impairment of glucose metabolism and insulin sensitivity, as indicated by the increases in insulin levels and HOMA-IR indices [26]. Interestingly, Vitamin A in our study showed a significant improvement in body weight, fat mass, and lipid profile. Three weeks after the beginning of the experiment, the untreated diabetic mice significantly gained more weight compared to either the normal control or vitamin A treated group. Subsequently, the rate of weight and fat gain also increased in the diabetic untreated mice while decreased significantly in the treated group especially after week 12 (at the end of experiment). Multiple studies have shown that adipose tissue act on the metabolism and homeostasis of vitamin A. Our results was different than the fact that chronic vitamin A enriched diet feeding can significantly impacted the obesity development both in young and adult obese rats of WNIN/Ob strain, possibly through thermogenic and glucocorticoid pathways without eliciting any toxic symptoms [27]. The exact mechanism of this is unclear and need further investigations. In accordance with our results, previous research indicated that retinoids can reduce body weight, subcutaneous and visceral fat of mice fed high fat diet [28]. A possible mechanism by which vitamin A reduces fat and body mass, as well as increases energy expenditure, is through the activation of uncoupling protein 1 located in the inner mitochondrial membrane of adipocytes [29]. Nevertheless, Ilić and his colleagues

Table 1. Effects of vitamin A on the biomarkers of oxidative stress in diabetic- induced mice after 16 weeks of treatment. data are shown as the mean ± standard error of mean (SEM)

Parameters	Control Mice	Diabetic-induced Mice Groups	
		Untreated	Treated with Vitamin A
Catalase (k/mg protein)			
Mean ± SEM	19.13 ± 0.07	3.79 ± 0.11	11.6 ± 0.4
% Improvement			40.4%,
% Change		- 80.1% a	-39.40% a
			206.00% b
Significance		*P* < 0.001a	*P* < 0.001a
(ANOVA, *P*)			*P* < 0.001b
Glutathione peroxidase (U/mg protein)			
Mean ± SEM	14.90 ± 0.07	2.98 ± 0.04	6.74 ± 0.03
% Improvement			25.30%
% Change		- 80% a	-53.40% a
			574% b
Significance		*P* < 0.001a	*P* < 0.001a
(ANOVA, *P*)			*P* < 0.001b
Superoxide dismutase (U/mg)			
Mean ± SEM	552.42 ± 6.2	127.3 ± 2.1	520.2 ± 2.5
% Improvement			71.10%
% Change		- 76.9% a	-5.30% a
			309% b
Significance		*P* < 0.001a	*P* < 0.001a
(ANOVA, *P*)			*P* < 0.001b
Malonaldehyde (nmol/g)			
Mean ± SEM	15.22 ± 0.05	59.66 ± 1.7	28.44 ± 0.2
% Improvement			-205%
% Change		291%	-52.30% a
			-71.60% b
Significance		*P* < 0.001a	*P* < 0.001a
(ANOVA, *P*)			*P* < 0.001b

%Improvement = mean treated-mean untreated/mean control × 100;%Change (a) = mean treated-mean control/mean control × 100;%Change (b) = mean treated-mean untreated/mean untreated × 100; P (a) = ANOVA significance in comparison with control; P (b) = ANOVA significance in comparison with untreated

,2020, [30] stated that vitamin A caused increase in body weight in rats on high fat diet while high dose of vitamin A lowered body weight in rats fed on standard diet. The discrepancy between their results and our results may be due to the difference in dose and duration of treatment as well as the species and gender of animals.

To evaluate the effects of vitamin A on the antioxidant enzymes activities in diabetic-induced mice, the levels of SOD, CAT, and GPO in the liver tissues of all groups were estimated. These enzymes act as one unit with each other to decrease any abnormalities of the reactive oxygen species (ROS), that can lead to oxidative stress [31,32,33,34].

In the present research, we demonstrated a significant decrease in the activity of SOD in diabetic induced mice compared with control normal group. Meanwhile, vitamin A showed a significant amelioration in the activity of SOD in the treated diabetic mice compared with untreated diabetic ones. It is well known that second to SOD in defense is the CAT and GPO. [31,35]. It has been shown that CAT is essential in a pancreatic β-cells protection from oxidative stress. Any deficiency in this activity will lead to ROS elevation, oxidative stress and finally cellular dysfunction that is seen in diabetes mellitus [36]. GPO works also when the level of oxidative stress is increased, to protects damaging the cells from free radicals [37,35]. In our experiment, we demonstrated that the activities of CAT and GPO were significantly decreased in diabetic induced mice compared with those in control normal mice. However, treatment with vitamin A showed a significant increase in their activities compared with untreated diabetic ones. Coinciding with the

previous results, other studies proved the hypothesis that oxidative stress in diabetes mellitus can be caused by impaired antioxidant system [38,39]. The mechanism of vitamin A on the antioxidant enzymes still understudied. However a possible explanation is that vitamin A can produces protein carriers, such as zinc and copper, that can lead to an increase in their bioavailability inside the cell. These ions can play a role in the activity of multiple enzymes, such as the antioxidant enzymes [40].

To examine lipid peroxidation in our study we measured MDA levels in the serum that serves as a biomarker of lipid damage and oxidative stress [41]. Our results demonstrated a significant elevation in MDA in diabetic induced mice compared with controls. These results are in agreement with previous studies that showed an elevated level of MDA in diabetes mellitus in both human and animal studies [38,39]. On the other hand, treatment with vitamin A in diabetic induced mice demonstrated a significant decrease in MDA levels compared with that in untreated diabetic mice. These Results can support the hypothesis that vitamin A can have an antioxidant effect and protection effect on the damage induced by lipid oxidation. Vitamin A's antioxidant properties have been frequently reported in vivo and in vitro [42]. Retinol can react with peroxide radicals (ROO •), thereby it interrupts the chain reaction of lipid peroxidation to form hydroperoxides (ROOH). Furthermore, vitamin A is capable of directly reacting with ROS to form a 5,6-epoxide retinoid [43].

The present research demonstrated that lipid profile was significantly improved after 12 weeks of treatment with Vitamin A in diabetic induced mice. This amelioration can be explained by the relationship of lipids and the oxidative symptom, however, the definitive role of lipid contribution to the oxidative system has yet to be fully elucidated. In consistent with our results, a clinical study proved that patients with cardiovascular diseases have elucidated Low levels of vitamin A. Moreover, these patients had lower HDL-cholesterol levels indicating the possible beneficial effects of vitamin A on serum lipids as well as cardiovascular diseases [44,45].

5. CONCLUSION

In conclusion, the results of the present study demonstrated some beneficial effects of Vitamin A in streptozotocin-induced diabetic mice. It caused significant improvement in body weight, lipid profile, lipid peroxidation and oxidative stress. These data suggest that Vitamin A supplementation might help in decrease the metabolic complications of diabetes mellitus. However, further studies should be done to examine the appropriate dose of Vitamin A to avoid side effects or toxicity. Also, more clinical research is needed to determine how Vitamin A status affects humans.

DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

CONSENT

It is not applicable

ETHICAL APPROVAL

Ethical approval No. (HAPO-02-K-012-2020-12-344) was obtained from Umm Al-Qura university's Biomedical and research Ethics Committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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