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The effect of Astaxanthin on Experimentally Induced Diabetic Kidney Disease

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Authors' contributions

This work was carried out in collaboration among all authors. Author YSA designed the protocol, managed the literature searches, conducted the experimental study, performed the statistical analysis, and wrote the first draft of the manuscript. Authors HMA and AE contributed in the experimental design supervised the practical work, revised results and statistical analyses. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To identify the potential renal protective effect produced by astaxanthin on streptozotocin-produced diabetic kidney disease in rats.
Study Design: Male Wistar rats (n=60) were separated into six groups, control, diabetic (streptozotocin 45 mg/kg single intraperitoneal injection), diabetic + ramipril (1 mg/kg oral gavage), diabetic + astaxanthin (10mg/kg oral gavage), diabetic + astaxanthin (50 mg/kg oral gavage), and astaxanthin-alone (50 mg/kg oral gavage) group.
Place and Duration of Treatment: Department of Pharmacology, College of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia. Duration of treatment was eight weeks.
Methodology: Fasting blood glucose, and symptoms of diabetes were evaluated weekly. Kidneys were evaluated by measuring serum creatinine level, kidney index, and hematoxylin and eosin staining.
Results: Eight-week astaxanthin treatment (50 mg/kg) in streptozotocin-produced diabetic kidney

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disease in rats significantly alleviated the diabetic symptoms (p = 0.05), and the decrease in body weight (P = .05) compared to nontreated diabetic rats. Nonetheless, the same dose produced a nonsignificant decline in fasting blood glucose level contrasted to diabetic rats (P = .45). Kidney index and serum creatinine of diabetic rats were significantly attenuated by both 10 and 50 mg/kg astaxanthin doses (P = .05). Additionally, renal architecture was preserved by high-dose astaxanthin treatment compared to nontreated diabetic rats.

Conclusion: Astaxanthin could protect against kidney damage associated with diabetes. Nevertheless, the impact of astaxanthin on biological markers of kidney damage in diabetes and the molecular pathways implicated in diabetic kidney disease requires additional investigation.

Keywords: Astaxanthin; diabetes; kidney; rats; diabetic kidney disease.

1. INTRODUCTION

Diabetes mellitus is (DM) is an endocrine disorder represented by a chronic rise in blood glucose level, due to imperfection in insulin release or function [1]. The worldwide prevalence of DM and its associated complications have greatly increased. In 2019, around 463 million adults were diabetic, and the cases are projected to reach 700 million by 2025 globally [2]. Additionally, 1 in 2 people worldwide have DM, but are not diagnosed. Diabetic Kidney disease (DKD), formerly known as diabetic nephropathy (DN), is an incremental complication of the microvasculature in DM of all types occurring at a ratio of 20-40% in diabetic patients [3-5]. Albuminuria and/or a stepwise decrease in estimated glomerular filtration rate (eGFR) are the main two parameters for the diagnosis of DKD, with the exclusion of other primary causes of kidney damage [6]. Nonetheless, decreased eGFR in the absence of albuminuria has been identified in both type1 and type2 DM patients underscoring that albuminuria is not an accurate parameter for the diagnosis of DKD [7-9]. DKD is the prominent element in the advancement to end-stage renal disease (ESRD) worldwide [4,10]. It was found that DKD along with hypertension were the reasons for around 80% of ESRD in the world raising a question regarding the current preventive and therapeutic measures [11]. Eventually, DKD patients require dialysis or kidney and/or pancreas transplantation [12]. Furthermore, DKD patients are more vulnerable to develop other diseases, such as cardiovascular diseases and infections [11,13]. Overall, quality of life and economy will be affected [11].

Currently, there is no drug used for the impediment of kidney disease in diabetic people. Controlling glycemia, blood pressure, and lifestyle measures, along with the periodic screening of renal function are the key preventive and treatment approaches to reduce the risk of DKD [6,14]. For the treatment of DKD, three classes of medications are currently in use, where two classes are not intended to be used for type 1 DM patients. An inhibitor of the angiotensin-converting enzyme, or the angiotensin receptor is prescribed for diabetic patients with hypertension who are not pregnant and have urinary albumin-to-creatinine (UA/Cr) ≥30 mg/gram and/or eGFR <60 ml/minute. A sodium-glucose cotransporter 2 inhibitor (SGLT2i) is recommended for type 2 DM patients with eGFR ≥30 ml/minutes and UA/Cr > 30mg/gram. Glucagon-like peptide 1 agonist (GLP1 agonist) if used in DKD patients who are at high risk of cardiovascular morbidity could lessen albuminuria and cardiovascular events [6].

Astaxanthin (ASTA) is a natural product discovered in aquatic animals and microorganisms [15,16]. It gives the distinctive red color of salmon, shrimp, and other crustaceans [15]. Synthetic forms are also existing [17]. ASTA is a powerful antioxidant with 100 times and 6000 times more antioxidant activity than vitamin E and C, respectively [18,19]. Regarding its safety, the oral median lethal dose (LD50) of ASTA in rats is >12 g/kg body weight [20]. In addition, it has no reported toxicity in humans [15,21,22].

Ample evidence showed that ASTA has promising health benefits in various diseases including DM mainly through its antioxidant, anti-inflammatory, antiapoptotic, and antifibrotic effects [23-26]. Oxidative stress, inflammation, apoptosis, and fibrosis are pathologic factors in DKD. However, a few studies have examined the role of ASTA and the molecular mechanisms by which astaxanthin attenuated DKD [18,21,27]. In DKD models, ASTA prevented renal damage through its antioxidant and antifibrotic effects, while it produced antiapoptotic and anti-

inflammatory effects in acute kidney injury and metabolic syndrome models, respectively [18,21,28,29]. Taken together, ASTA could be a promising nutraceutical for DKD contributing to the utilization of marine resources of ASTA and the prosperity of its industry worldwide. Therefore, it was tempting to choose ASTA and investigate its potential effect on kidney damage due to DM, where the central objective of the current study was to determine the potential renal protective effect of astaxanthin in streptozotocin-produced diabetic kidney disease in rats.

2. MATERIALS AND METHODS

2.1 Chemicals

Streptozotocin (lot # WXBD2372V) was obtained from Merck Co. (St Louis, MO, USA). Natural astaxanthin powder from Haematococcus pluvialis (AstaZine®, lot # SDHP-05) was obtained from the Beijing Gingko Group (Beijing, China). Ramipril Sandoz® (lot # KB4060) was purchased from Novartis (Barleben, Germany).

2.2 Animals

The animal experiment was approved in advance by the Research Ethics Committee, King Abdulaziz University (KAU), Jeddah, Saudi Arabia (Reference No "PH-1442-43"). The study was conducted on six-week-old male Wistar rats weighing 150-200 g, procured from the animal house of College of Pharmacy at KAU, where the animals were housed in an air-conditioned atmosphere at 22 ± 2°C, under a 12h light/dark cycle and provided with rodent chow and water ad libitum.

2.3 Experimental Protocol

After one week of acclimatization, 60 male Wistar rats were randomly separated into six groups (n= 10/group). Group 1 (control), group 2 (diabetic), group 3 (diabetic + ramipril 1mg/kg body weight), group 4 (diabetic + 10 mg/kg body weight astaxanthin), group 5 (diabetic + 50 mg/kg body weight astaxanthin), and group 6 (50 mg/kg body weight astaxanthin alone). Diabetic groups were intraperitoneally (IP) injected with freshly prepared streptozotocin (STZ) in 0.1 mol/L citrate buffer (pH 4.5), at 45 mg/kg after fasting for 18 hours. Group 1 and group 6 received an equal amount of citrate buffer only [26]. The dose of STZ was selected based on the literature, where starting from 40 mg/kg, STZ produced spontaneous DKD four to eight weeks after IP injection [30]. Following STZ injection, rats were given 10% glucose solution for three days to avoid early STZ-produced hypoglycemia [30]. Then, fasting blood glucose (FBG) level was measured for one week before treatment, to maintain a stable level between 200 and 300 mg/dl [30-32]. After the establishment of the STZ-produced DKD model, treatment was started for eight weeks. ASTA powder was dissolved in corn oil just before administration by oral gavage (OG) [33,34]. Similarly, the preparation of ramipril was just before OG administration, but in normal saline [35,36]. Control and diabetic groups received an equal amount of corn oil only.

After eight weeks, the rats were anesthetized by ketamine-xylazine mixture [37], and blood samples were drawn from the retro-orbital plexus in 5 ml gel and clot activator tubes and sera were separated by centrifugation at 3000g for 15 min and stored at -80 °C for biochemical analysis. After blood collection, rats were sacrificed by cervical dislocation and kidney tissues were dissected, washed with ice-cold saline, kept on dry ice, and then stored at −80°C. Representative kidney tissues were fixed in 10% neutral buffered formalin for histopathological analysis.

2.4 Assessment of Diabetes Mellitus

2.4.1 Determination of fasting blood glucose level

ACCU-CHECK Performa® glucose meter and ACCU-CHECK Performa® test strips (Roche, Germany) were used to determine FBG level from the blood obtained from the tail vein during DKD model induction and then, once/week during the treatment duration.

2.4.2 Determination of water intake, food intake, and urine volume

Measurement of water and food intake was done once/week (over 24 hours) during treatment duration, using scaled drinking water bottle for water (500 mL), and ADAM® AQT–2600 scale (Adam Equipment Inc, Oxford, CT, USA) for food measurement. On the other hand, urine collection was done at the last week of treatment (over 24 hours) using the Tecniplast™ metabolic cage system obtained from Fisher Scientific (Waltham, MA, USA), where each rat was placed

individually with unrestricted entry to water and food.

2.4.3 Determination of percentage change in body weight

Calculation of the percentage change in body weight (%CBW) was done using the subsequent formula: %CBW = (Initial weight – Final weight (g)) /Initial weight (g)*100 [38].

2.5 Assessment of Diabetic Kidney Disease

2.5.1 Determination of relative kidney weight

Relative kidney weight (RKW) or (kidney index) was computed implementing the subsequent equation: RKW = Organ weight (g)/Body weight (g)*100 [18].

2.5.2 Determination of serum creatinine

Serum creatinine (Scr) was measured using rat creatinine ELISA kit (Cat # MBS749827), obtained from My BioSource (San Diego, CA, USA).

2.5.3 Histopathological examination

The representative tissues of the kidney were kept in 10% neutral-buffered formalin for 24 hours at room temperature. After that, tissues were dehydrated in a tissue processor for 24 hours and embedded in paraffin blocks, where they were kept on ice before sectioning at 4 µm thickness. Subsequently, mounting on glass slides, staining with hematoxylin and eosin (H & E), and examination under a microscope for glomerular and tubular changes were performed.

2.6 Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's as a post-*hoc* test. Data are presented as mean ± S.D and the level of significance was P = .05. However, repeated measures two-way ANOVA followed by Bonferroni's post-*hoc* test was utilized to evaluate FBG level, water intake, and food intake from week zero to week eight in different groups. Data are presented as mean ± S.D and the level of significance was P = .05. The analysis of statistical results was done through GraphPad Prism® software (version 8.0.1).

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Treatment with astaxanthin produced a non-significant decrease in hyperglycemia in STZ-produced diabetic kidney disease in rats

Weekly fasting blood glucose levels significantly elevated around two to three folds in DM and DM-treated groups compared to the control (P =.05) (Fig. 1). In contrast, FBG levels in ramipril and low-dose ASTA treated DM rats did not differ significantly from DM rats. In high-dose ASTA treated DM rats, FBG level insignificantly decreased by 8.8% (P = .33) and 16% (P = .45) compared to DM, at week four and eight of treatment, respectively. Contrariwise, normal rats were given high-dose ASTA had a statistically significant lower FBG level compared to DM (P = .05), which is almost equivalent to the level in the control.

3.1.2 Treatment with astaxanthin decreased polydipsia and polyphagia in STZ-produced diabetic kidney disease in rats

Weekly 24-Hr water intake was significantly higher in DM and DM rats treated with ramipril or low-dose ASTA in comparison to the control (P = .05) (Fig. 2, A). Only high-dose ASTA was able to significantly reduce weekly 24-Hr water intake compared to DM rats (P = .05). Regarding weekly 24-Hr food intake, a significant increase was detected in DM rats compared to the control, where at week eight, it was almost double that of the control (P = .05) (Fig. 2, B). Treatment of DM rats with high-dose ASTA significantly decreased 24-Hr food intake compared to DM rats approaching the level of control and ASTA alone rats (P = .05). Likewise, treatment of DM rats with low-dose ASTA or ramipril also showed a decline in 24-Hr food intake, but the significance level compared to DM was observed only in some weeks of treatment. 24-Hr food intake was significantly lower than DM rats in week one, two, seven, and eight for the low-dose ASTA group (P = .05), while the significant decline for the ramipril group was observed in week four, seven, and eight of treatment (P = .05).

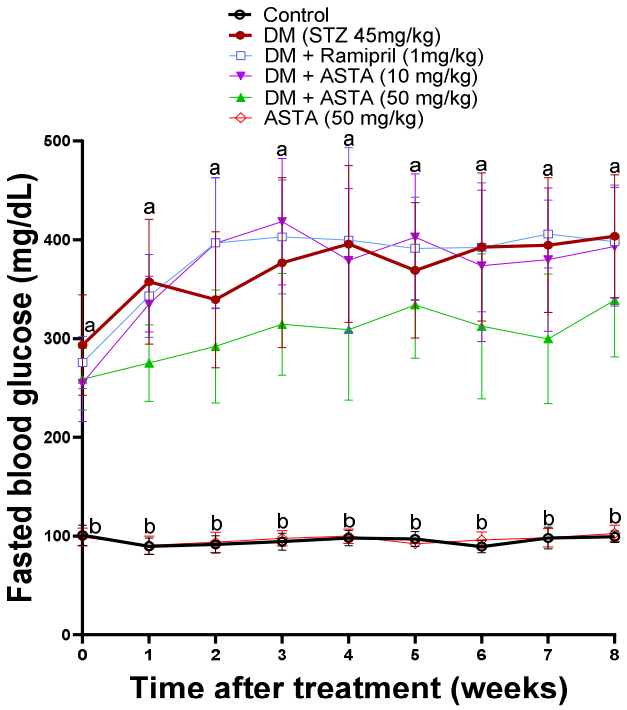

Fig. 1. Effect of astaxanthin on hyperglycemia in STZ- induced diabetic kidney disease in rats

Statistical analysis was performed using repeated measures two-way ANOVA followed by Bonferroni's post-hoc test. a: statistically significant difference from the control group, P = .05. b: statistically significant difference from the diabetic group, P = .05. Data are represented as mean ± SD. n = 7. DM: diabetes mellitus; STZ: streptozotocin; ASTA: astaxanthin

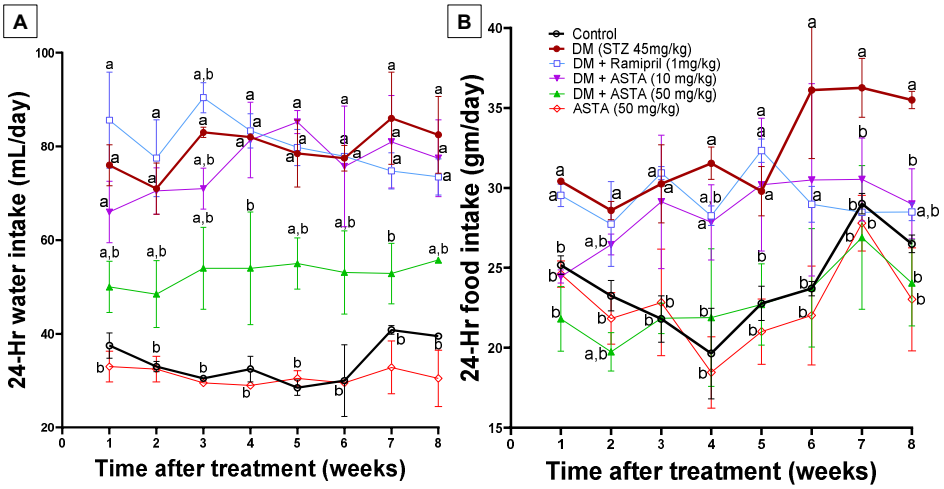

Fig. 2. Effect of Astaxanthin on polydipsia (A) and polyphagia (B) in STZ-induced diabetic kidney disease in rats

Statistical analysis was performed using repeated measures two-way ANOVA followed by Bonferroni's post-hoc test. a: statistically significant difference from the control group, P = .05. b: statistically significant difference from the diabetic group, P = .05. Data are represented as mean ± SD. n = 7. DM: diabetes mellitus; STZ: streptozotocin; ASTA: astaxanthin

3.1.3 Treatment with astaxanthin attenuated polyuria in STZ-produced diabetic kidney disease in rats

As illustrated in (Fig. 3), there was a significant elevation in 24-hour urine output (UOP) of all the four diabetic groups compared to the control (P = .05). However, the UOP of the ASTA alone group was just as equal as that of the control. Compared to the diabetic group, ramipril and high-dose astaxanthin exhibited a significant decline in UOP by 15% and 60%, respectively (P = .05). ASTA alone group exhibited a significant decline in UOP by 90% compared to the diabetic group (P = .05).

3.1.4 Treatment with astaxanthin normalized the decrease in body weight in STZ-produced diabetic kidney disease in rats

As revealed in (Fig. 4), there was substantial variability in the %CBW of different study groups between week zero and week eight of treatment. By the end of the treatment duration, the mean body weight of DM rats and DM rats treated with ramipril decreased, while that of the control and ASTA treated rats increased. Compared to the control (20%), diabetic and ramipril-treated rats had a significant decline in body weight by around 3% and 6%, respectively (P = .05). Only high-dose ASTA treated diabetic (23%) and nondiabetic rats (26%) had a significant uprise in body weight in comparison to diabetic rats (P = .05), slightly exceeding the % increase in control (20%). In contrast, diabetic rats treated with low-dose ASTA had an increase in body weight that is approaching the significance level compared to the DM (P = .07).

3.1.5 Treatment with astaxanthin normalized kidney hypertrophy and the elevated serum creatinine level in STZ-produced diabetic kidney disease in rats

As shown in (Fig. 5, A), RKW in DM group significantly augmented compared to the control (P = .05). Remarkably, only high-dose ASTA was able to significantly reduce RKW in diabetic rats compared to nontreated diabetic rats (P = .05). Similarly, high-dose ASTA reduced RKW in nondiabetic rats compared to diabetic rats (P = .05), where RKW in both high-dose ASTA treated groups was comparable to the control. On the contrary, ramipril (P = .49) and low-dose ASTA (P = .07) treated rats demonstrated a nonsignificant decline in RKW compared to diabetic rats. Regarding Scr level illustrated in (Fig. 5, B), diabetic rats and diabetic rats given ramipril or low-dose ASTA had a significant upsurge in Scr compared to the control (P = .05), where the diabetic group showed the greatest upsurge in Scr by more than two-third the level of the control. Interestingly, all treatments given to DM rats were able to significantly downregulate the elevated Scr level observed in DM rats (P = .05). However, only high-dose ASTA was able to normalize serum creatinine.

3.1.6 Attenuation of renal injury after eight weeks of treatment with astaxanthin in STZ-produced diabetic kidney disease in Rats

Histopathological investigation of renal tissues of the control group revealed an intact structure of the glomerulus and epithelial lining of the tubules, an average-sized Bowman's space, podocytes, and mesangial cells, and explicit normal parietal layer of Bowman's capsule formed of a single layer of squamous epithelium (Fig. 6, A). In contrast, the distorted structure of the glomerulus was observed in the DM group (Fig. 6, B), with the destruction of the parietal layer and disarrangement of podocytes. In addition, dilatation of glomerular and tubular matrix, thickening of glomerular basement membrane with cell infiltration, and hyaline degeneration were observed in the same group. The previous histological findings in DM rats were improved after treatment with ramipril or ASTA in two doses, whereas the improvement differed in different treatments. Ramipril and high-dose ASTA groups showed restoration of the structure of Bowman's capsule and improvement in glomerular and tubular alterations compared to the DM group, but hyaline degeneration was still observed in the ramipril group (figure 6, C and E). Whilst low-dose ASTA group displayed a partial restoration of the structure of Bowman's capsule, attenuation of tubular dilation, with little improvement in glomerular basement membrane thickness and mesangial dilation compared to DM group (Fig. 6. D). Notably, hyaline degeneration was markedly observed in the low-dose ASTA group. On the other hand, kidney tissues of the normal rats treated with high-dose ASTA exhibited glomerular and tubular structures comparable to the control, with increased vascularity in some interstitial spaces (Fig. 6. F).

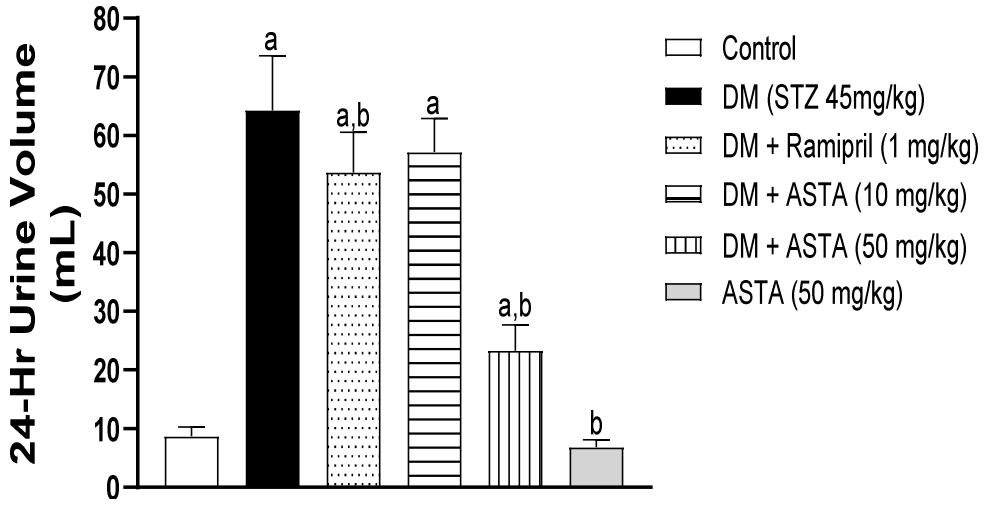

Fig. 3. Effect of astaxanthin on 24-Hr urine volume in STZ-induced diabetic kidney disease in rats

Statistical analysis was performed using one-way ANOVA followed by Tukey's post- hoc test. a: statistically significant difference from the control group, P = .05. b: statistically significant difference from the diabetic group, P = .05. Data are represented as mean ± SD. n = 7. DM: diabetes mellitus; STZ: streptozotocin; ASTA: astaxanthin

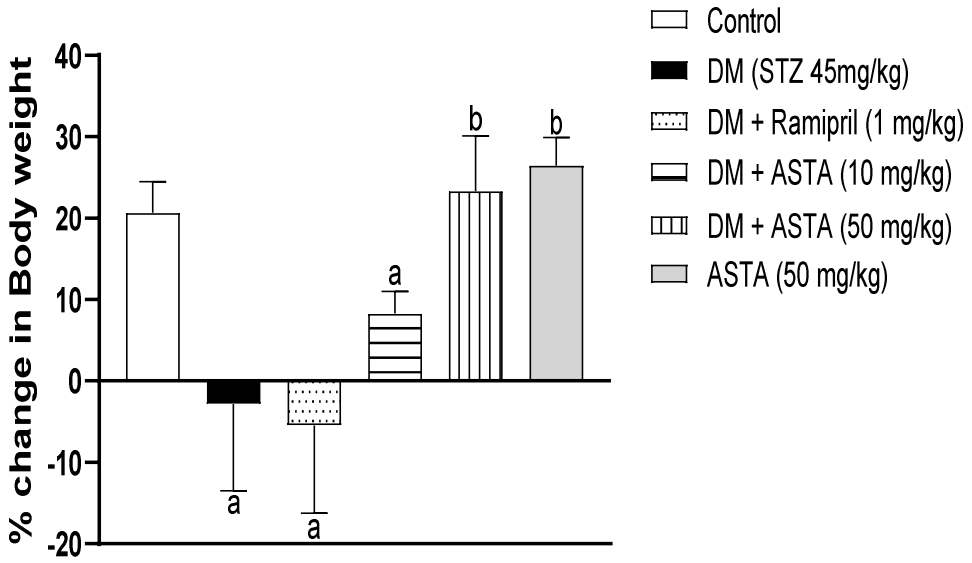

Fig. 4. Effect of astaxanthin on % change in body weight in STZ-induced diabetic kidney disease in rats

Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test. a: statistically significant difference from the control group, (P = .05). b: statistically significant difference from the diabetic group, (P = .05). Data are represented as mean ± SD. n = 7.
DM: diabetes mellitus; STZ: streptozotocin; ASTA: astaxanthin

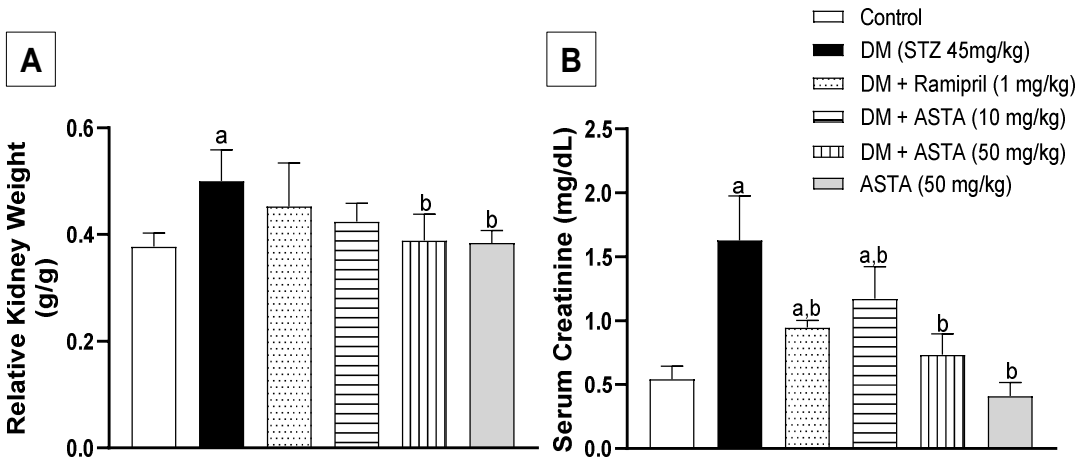

Fig. 5. Effect of Astaxanthin on relative kidney weight (A) and serum creatinine (B) in STZ-induced diabetic kidney disease in rats

Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test. a: statistically significant difference from the control group, (P = .05). b: statistically significant difference from the diabetic group ,(P = .05). Data are represented as mean ± SD. n = 7. DM: diabetes mellitus; STZ: streptozotocin; ASTA: astaxanthin

3.2 Discussion

Parameters related to DM and DKD were measured to verify the establishment of the DKD model, and hence to evaluate the impact of eight weeks of ASTA treatment on STZ-produced DKD in rats. In detail, FBG level and the typical symptoms of DM, polydipsia, polyphagia, and polyuria were evaluated. In this study, only ASTA (50 mg/kg) produced a nonsignificant decline in FBG level in treated versus non-treated DM rats. The same dose of ASTA-alone did not change FBG level from the control. Thus, it could be hypothesized that ASTA has no detrimental effect on blood glucose levels in normal and diabetic rats. In preceding studies, the impact of ASTA on blood glucose level was contradictory. In db/db mice, ASTA (1 mg/day in food) for 12 weeks significantly decreased non-fasting blood glucose level after two and three months of treatment compared to db/db mice. In addition, the intraperitoneal glucose tolerance test (IPGTT) showed a significant decline in blood glucose levels in these mice compared to the diabetic mice [39]. Furthermore, in diabetic rats, ASTA attenuated blood glucose levels at 20mg/kg (OG) or 25 mg/kg intragastric (IG) for three or 12 weeks, respectively [18,21]. However, ASTA (10 or 50 mg/kg in food) for 12 weeks did not affect the level of blood glucose and insulin, as well as Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), an estimate of insulin resistance, in diabetic rats [33]. ASTA (35 mg/kg IG) also did not significantly decrease FBG in treated versus untreated db/db mice [27]. The disagreement in the effect of ASTA on blood glucose level could be explained by the differences in the animal model used, ASTA source, dose, and duration of treatment, which necessitate further studies.

In a clinical trial, 44 type 2 DM patients were randomly given 8 mg/day of ASTA or placebo after lunch for eight weeks. ASTA group showed a borderline decrease in plasma glucose concentration ($P < .057$), and a significant reduction in fructosamine concentration [22]. Fructosamine is a measure of the average blood glucose level in the past 2-3 weeks [40]. However, another study that included 54 Type 2 DM patients found that ASX significantly reduced FBG and HgA1c levels at 2mg and 4 mg three times/day for eight weeks [15]. In a clinical trial that included 29 prediabetic people, ASTA (12mg/day for 3 months) demonstrated a significant decrease in 75g oral glucose tolerance test (OGTT). A significant decrease in HgA1c and improvement in liver insulin resistance and Matsuda index, an index to estimate insulin sensitivity, were also observed [41]. Although ASTA's ability to reduce blood glucose levels in DM and prediabetic people were investigated, larger randomized clinical trials are required to validate these outcomes.

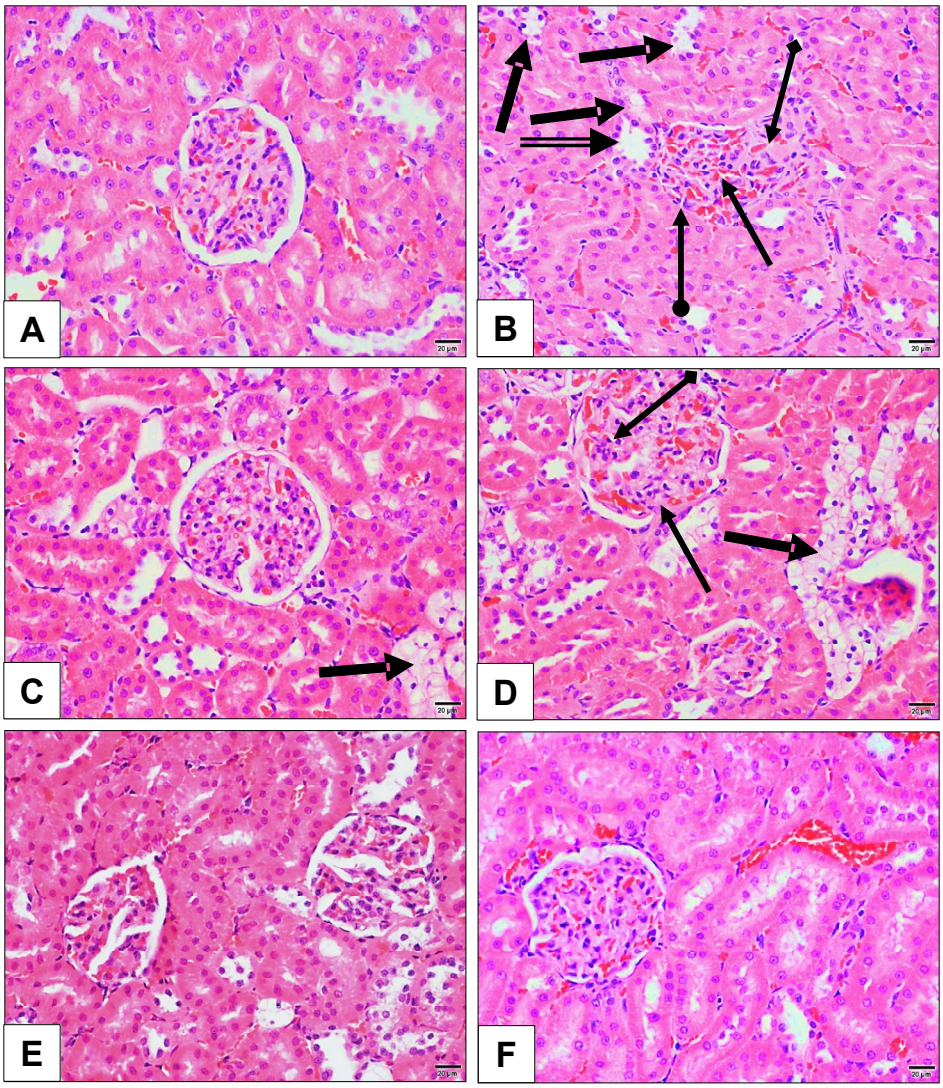

Figure 6. Representative photomicrographs of kidney sections stained by H & E (X200) after eight weeks of treatment with astaxanthin in STZ-induced diabetic kidney disease in rats. A) Control group showed normal renal structure. **B)** DM (STZ 45 mg/kg) group revealed mesangial matrix dilatation with cell infiltration: ⟵, destruction of the parietal layer of the Bowman's capsule and disarrangement of podocytes: ⟵●, thickening of glomerular basement membrane: ⟵◆, dilation of tubular matrix: ⟸, and hyaline degeneration: ⟵■. **C)** DM + ramipril (1 mg/kg) group showed a restoration of the structure of Bowman's capsule and improvement in glomerular and tubular changes compared to DM group, but hyaline degeneration was still obvious **D)** DM + ASTA (10 mg/kg) group showed a partial restoration of the structure of Bowman's capsule, attenuation of tubular dilation, with little improvement in glomerular basement membrane thickness, and mesangial expansion compared to DM. Remarkably, hyaline degeneration was marked compared to DM. **E)** DM + ASTA (50 mg/kg) group showed a restoration of the structure of Bowman's capsule, alleviation of glomerular and tubular changes, and hyaline degeneration compared to DM group. **F)** Normal rats + ASTA (50 mg/kg) exhibited renal structure comparable to the control group, with increased vascularity in some interstitial spaces.

H & E: Hematoxylin and Eosin, DM: diabetes mellitus, STZ: streptozotocin, ASTA: astaxanthin.

Regarding the typical symptoms of DM, the enhanced water and food intake observed in STZ-produced DM rats were significantly attenuated by ASTA. Similar findings were observed when ASTA-s-allyl cysteine (1 mg/kg for 45 days) was given to the same rats [26]. Likewise, the effect of 50mg/kg ASTA (OG) on UOP in our study was similar when ASTA (IG) was used at 25 mg/kg for 12 weeks by Zhu et al, where both had a significant drop in UOP compared to the diabetic rats [18]. The same finding was shown in db/db mice treated with ASTA (35 mg/kg IG for three months) compared to nontreated db/db mice [27]. Therefore, it has been postulated that ASTA reduces the symptoms of hyperglycemia.

Body weight is an important metabolic factor in DM, where obesity is a prominent risk for acquiring type 2 DM and its related complications including DKD [3]. Thus, body weight was measured weekly during the study interval and %CBW was computed. The decline in body weight of STZ-produced diabetic rats in our study was also reported in the literature [18,38,42,43]. It was found that 50 mg/kg ASTA normalized the decrease in body weight detected in diabetic rats versus untreated diabetic rats after eight weeks (P = .05). Advocating our result, Zhu et al found that ASTA (25 mg/kg IG) significantly improved the decrease in body weight of diabetic rats compared to nontreated diabetic rats after 12-weeks [18][18][18]. Therefore, ASTA might prevent weight loss associated with diabetes. Interestingly, the increased body weight of normal rats given ASTA (50 mg/kg) was not significantly different from the control suggesting that the weight gain in 50 mg/kg ASTA treated rats was a normal growth and not due to ASTA. Thus, weight gain is not a possible side effect of ASTA, which could be added to the list of no reported side effects of ASTA [44].

In our study, DKD-related parameters were assessed by measuring RKW, Scr, and histopathological examination. The significant kidney enlargement observed in STZ-produced diabetic rats compared to the control was consistent with previous studies, where the hypertrophy was evaluated by RKW [18, 43,45,46]. Administration of 50 mg/kg (OG) ASTA for two months significantly attenuated kidney hypertrophy in diabetic rats, where a similar result was found when IG ASTA at 25 mg/kg was used for three months [18].

Renal function in STZ-produced DKD in rats was assessed by measuring Scr level, where the treatment of STZ-produced diabetic rats with ASTA (10 mg/kg or 50 mg/kg) or ramipril (1mg/kg) significantly improved Scr level. This result was consistent with previous investigations, where ASTA (20 mg/kg OG for 21 days) or ASTA (25 mg/kg IG for 12 weeks) treated diabetic rats revealed a significant upsurge in Scr level compared to the diabetic rats [18, 21]. Additionally, db/db mice treated with 35 mg/kg IG ASTA for 12 weeks revealed a significant decline in Scr compared to db/db mice [47]. The dose-dependent decline of Scr level by ASTA in our DKD model can be supported by the comparable finding in another kidney damage model. It was observed that Scr decreased in a dose-dependent way by administration of ASTA (5, 10, or 20 mg/kg single intravenous injection) in acute kidney injury in rats following severe burn [28].

In type 2 DM group of patients, Scr level was measured after eight-week ASTA treatment (2 or 4 mg capsule three times daily), where it did not significantly differ compared to the placebo group [15]. Indeed, one of the exclusion criteria was type 2 DM patients with renal impairment. Thus, the effect of ASTA on DKD patients from this study cannot be determined. A clinical trial investigated the effect of ASTA in subjects with a history of kidney transplantation, where 33 and 28 patients received ASTA (4 mg three times/day) and placebo, respectively. Measuring Scr level after six and 12 months of treatment did not exhibit a statistically significant difference between the groups (p= .60) [48]. Only 2 patients in the ASTA group had kidney transplantation due to DKD. Thus, the potential preventive effect of ASTA in DKD patients requires future investigation with a higher number of patients and an extended duration of treatment.

Renal structure in STZ-produced DKD in rats was assessed by H & E staining, where the main pathological changes involved in DKD, were observed. Glomerular basement membrane enlargement is one of the initial structural alterations associated with DKD, while glomerular matrix dilation is the subsequent alteration [8]. Both structural alterations were observed in diabetic rats and attenuated by treatment with ramipril or ASTA. Our results were consistent with previous studies. In db/db mice treated with ASTA, glomerular matrix dilation was attenuated compared to nontreated db/db mice [27]. In the same study, glomerular matrix

accumulation was quantified by mesangial matrix index (MMI), which was significantly greater in db/db mice in comparison to the control and ASTA use significantly reduced MMI. Similarly, MMI was reduced by ASTA in STZ-produced diabetic rats, in addition to the improvement in basement membrane enlargement and glomerular matrix dilation [18].

On the other side, alteration in kidney tubules or tubulopathy has been an area of concern in recent DKD research. Within days of hyperglycemia, tubular injury can be detected in patients with albuminuria as well as in patients without albuminuria [4,49]. In the current experiment, tubular matrix dilation was characteristic in the untreated diabetic group compared to ramipril or ASTA treated groups. In alloxan-produced diabetic rats, treatment with ASTA ameliorated both tubular dilation and glomerular hypertrophy supporting our findings [21]. Additionally, hyaline degeneration, a characteristic kidney pathology in DKD, was detected in diabetic rats that was alleviated after treatment with ASTA [8]. Therefore, we can postulate that ASTA improves STZ-produced DKD in rats through normalization of Scr, and attenuation of renal hypertrophy and both tubular and glomerular renal injury.

In the histopathology of the ASTA alone group, foci of blood were observed. This effect could be attributed to the anticoagulant effect of ASTA that was uncovered in previous research [33]. When ASTA was given at 50 mg in the diet of diabetic rats, the activities of antithrombin-III and protein C (anticoagulation factors) were significantly increased, whereas the activities of factor VII and plasminogen activator inhibitor-1 (PAI-1) (coagulation factors) were significantly decreased compared to DM. Moreover, the level of Von 14 Willebrand factor (VWF) (implicated in platelet adhesion and aggregation) in plasma significantly reduced compared to DM [33]. In 2019, the previous parameters were investigated in a double-blind placebo-controlled trial [15]. 54 patients with type 2 DM were separated into three groups, one group received cellulose starch as a placebo, the other group received 2mg ASTA, the third one received 4mg ASTA in oral capsules three times per day for two months. Coagulation factors V and VII, VWF, and PAI-1 (fibrinolysis inhibitor) were found elevated in type 2 diabetic subjects. Significant downregulation of factor VII and VWF level was observed with both ASX doses, while a significant reduction in PAI-1 was observed with the 12mg/day ASX. In contrast, a significant increase in antithrombin-III level was observed with both ASX doses compared to placebo [15].

4. CONCLUSION

In conclusion, the effect of ASTA on diabetes mellitus was observed through amelioration of the symptoms of diabetes mellitus and normalization of weight loss in STZ-produced diabetic kidney disease in rats. Importantly, ASTA produced a renal protective effect in diabetic kidney disease through normalization of kidney hypertrophy, serum creatinine, and attenuation of kidney lesion in STZ-produced diabetic kidney disease in rats. Indeed, identifying the influence of ASTA on the biomarkers of kidney damage and the molecular mechanisms involved in its renal protective effect requires additional investigation. Furthermore, clinical trials studying the impact of ASTA in diabetic kidney disease patients are a future research scope.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The animal experiment was approved by the Research Ethics Committee, King Abdulaziz University (KAU), Jeddah, Saudi Arabia (Reference No “PH-1442-43”).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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