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Amelioration of Diabetes-Induced Nephropathy by *Loranthus regularis*: Implication of Oxidative Stress, Inflammation and Hyperlipidaemia

Ahmed Z. Alanazi [1], Mohamed Mohany [1], Fawaz Alasmari [1], Ramzi A. A. Mothana [2], Abdulaziz O. A. Alshehri [1], Khalid Alhazzani [1], Mohammed M. Ahmed [1] and Salim S. Al-Rejaie [1,*]

[1] Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, P.O. Box 55760, Riyadh 1145, Saudi Arabia; azalanazi@KSU.EDU.SA (A.Z.A.); mmohany@ksu.edu.sa (M.M.); ffalasmari@KSU.EDU.SA (F.A.); 439105704@student.ksu.edu.sa (A.O.A.A.); Kalhazzani@ksu.edu.sa (K.A.); mmahmed114@yahoo.com (M.M.A.)

[2] Department of Pharmacognosy, College of Pharmacy, King Saud University, P.O. Box 55760, Riyadh 1145, Saudi Arabia; rmothana@KSU.EDU.SA

* Correspondence: rejaie@ksu.edu.sa

Abstract: In traditional Yemeni medicine, various preparations of *Loranthus regularis* (*L. regularis*), such as powder, decoctions and infusions are commonly used to treat diabetes, kidney stone formations and inflammation. In the present study, we evaluated the antinephrotoxic effects of *L. regularis* extract in experimentally-induced diabetes in male Wistar rats. A single dose (60 mg/kg/day) of Streptozotocin (STZ) was used to induce type 1 diabetes. Animals were then treated for four weeks with *L. regularis* extract (150 or 300 mg/kg/day) by oral gavage. Renal and blood samples were subsequently harvested. Several biochemical indices, oxidative stress and inflammatory markers were assessed. Additionally, histological alterations in the renal tissue were examined. Serum glucose levels were significantly ($p < 0.01$) lowered while insulin levels were enhanced in *L. regularis*-treated diabetic animals. The increased renal markers in diabetic rats were decreased by *L. regularis* treatment. Serum elevated lipid profiles were markedly decreased by the plant extract. The serum and renal cytokines that were significantly increased ($p < 0.001$) by STZ were diminished by *L. regularis* treatment. Finally, renal tissue antioxidant enzymatic activity was enhanced with *L. regularis* treatment. Taken together, the data here indicate that *L. regularis* possesses therapeutic ability to reduce the development of diabetic nephropathy (DN) by minimizing oxidative injury and inflammation.

Keywords: antioxidants; diabetic complications; *Loranthus regularis*; phenolic compound

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1. Introduction

Diabetes represents a massive health issue worldwide whose prevalence has only increased over the last decade, particularly in developing countries [1]. Chronic diabetes results in numerous complications, including nephropathy, retinopathy, and neuropathy [2–4]. The incidence of diabetic nephropathy (DN) in type 1 diabetes was about 15–25%, contributing to complications and a high mortality rate [5]. One important pathophysiological feature in DN is oxidative stress, which can be caused by hyperglycemia and involves the increased production of reactive oxygen species (ROS) [6,7].

Previous studies have shown that diabetes is accompanied by a decrease in enzymatic antioxidant activity, increased lipid peroxidation (LPO), and changes in glutathione redox state [2,8]. Oxidative stress is associated with increased proinflammatory cytokines, such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α), which contribute to DN development [9,10]. DN is characterized by decreased thickness of basement membrane, mesangial growth, damage to glomerular podocytes, and microalbuminuria [11] as well as extracellular matrix (ECM) and protein aggregation [12,13].

For decades, herbologists have used plants to cure various diseases [14,15]. Herbal medicine can be used to treat various metabolic disorders via different types of phytochemicals, such as flavonoids, tannins, alkaloids, polysaccharides, and hormones [16,17]. The search for new herbal medicines is gaining popularity, as these may typically lack the side effects of chemical medicines [18].

Loranthus regularis Steud. (Loranthaceae), is a shrub that is widely distributed throughout Yemen, Southern Saudi Arabia and several African countries. The Loranthaceae family members are commonly known as mistletoes. Many mistletoe species are extensively used in traditional medicine to treat hypertension, diabetes, inflammatory disorders and gastrointestinal problems [19]. They are also used for general health and cancer therapy [20–23].

L. regularis is used in the treatment of nephrolithiasis, diabetes and inflammation in various types of preparations such as decoctions, infusions and powders [24]. The antioxidant, anti-inflammatory, antinociceptive, antipyretic and antimicrobial activities in *L. regularis* were demonstrated previously [25,26]. Despite the aforementioned studies that support the use of *L. regularis* in different diseases, their impact on the alleviation of DN has not been addressed to date. Therefore, the purpose of this study was to investigate the potential protective effects of *L. regularis* extract against DN using an animal model of diabetes, with a focus on its antioxidant, anti-inflammatory and antihyperlipidemic effects.

2. Materials and Methods

2.1. Animals

Male Wistar albino rats (n = 24) weighing 250–270 g were obtained from the Experimental Animal Care Centre, Pharmacy College, University of King Saud, Riyadh. Animals were acclimated to the laboratory conditions for one week prior to the commencement of the experiment. Free access to food and water was provided. The norms of the National Institute of Health Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research (NIH Publications NO. 80-23; 1996) were strictly followed throughout the experiment. This study was approved by the Research Ethics Committee of King Saud University (SE-19-146).

2.2. Induction of Diabetes

A single dose of Streptozotocin (STZ) (65 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) prepared in 0.1 M citrate buffer (pH 4.5) was used to induce type-1 diabetes. The equivalent volume of citrate buffer was injected into control rats. Subsequently, diabetes was confirmed through the measurement of fasting blood glucose levels using Accu-Chek Compact-Plus glucose meter system (Roche Diagnostics, Meylan, France) 48 h after the injection of STZ. A reading > 13.9 mmol/L was considered diabetic, and these animals were included in the study.

2.3. Collection and Preparation of Plant Extract

In June 2015, leaves, flowers and twigs of *L. regularis* were collected from Al-Mahuit governate (Yemen). These were identified at the Pharmacognosy Department, Faculty of Pharmacy, Sana'a University. Voucher specimens (Mo-M05) were deposited at the Pharmacognosy Department, Faculty of Pharmacy, Sana'a University. A crude methanol extract of *L. regularis* was obtained through grinding one kg of air-dried plant material followed by extraction with 3 L of methanol for 5 h using a Soxhlet apparatus. A rotary evaporator was used to filter and evaporate the extract to obtain 114 g of crude methanol extract.

2.4. Experimental Design

Experimental design included 4 groups (n = 6 per group) treated as follows: (i) control (vehicle-treated), (ii) diabetic (STZ-treated), (iii) diabetic plus *L. regularis* 150 mg/kg/day (Lr150 + STZ − treated) and (iv) diabetic plus *L. regularis* 300 mg/kg/day (Lr300 + STZ − treated). *L. regularis* extract was suspended in 0.25% carboxymethyl cellulose sodium

(CMC) solution and given once per day orally by gavage for 4 weeks, beginning one week after STZ injection. An equal volume of CMC solution was used as a vehicle for control and STZ-treated animals. Animals were anesthetized through intraperitoneal injection of a ketamine (92 mg/kg, Hikma Pharmaceuticals, Amman, Jordan) and xylazine (10 mg/kg, Bayer, Turkey) mixture followed by the collection of blood samples through cardiac puncture. The collected samples were centrifuged at 1800 g for 10 min. The obtained serum samples were separated and stored at −20 °C. Animals were euthanized, and the kidneys were dissected. A cross-section of the harvested kidneys was fixed in 10% neutral buffer formalin (NBF) (pH 7.4) for histopathological analysis, and the remaining harvest was dipped in liquid nitrogen for one minute and stored at −80 °C.

2.5. Blood Biochemistry

Using commercially available diagnostic kits (Human, Wiesbaden, Germany), lipid profile (total cholesterol (TC), TG, LDL-C, and LHD-C, renal function (SCr and urea), blood glucose, and insulin levels were measured. Proinflammatory biomarkers (TNF-α, IL-1β and IL-6) levels were estimated using rat ELISA kits (R&D systems Inc., Minneapolis, MN, USA).

2.6. Tissue Analysis

Kidney tissues were homogenized in physiological buffer (1:10, *w*/*v*), and TBARS and GSH levels were subsequently measured using ELISA kits (Cayman Chemical Co., Ann Arbor, MI, USA) according to the manufacturer's protocol. Kidney postmitochondrial supernatants were collected, and enzymatic activities of SOD, CAT and GPx were measured using ELISA kits (R&D systems Inc., Minneapolis, MN, USA). Furthermore, proinflammatory markers such as TNF-α, IL-1β and IL-6 levels were also measured using ELISA kits for rats (R&D systems Inc., Minneapolis, MN, USA) in kidney homogenate.

2.7. Histopathological Analysis

Fixed kidney specimens were immersed in paraffin for blocking, and 5-µm sections were obtained by microtomy. Sections were then stained with Hematoxylin and Eosin for microscopic examination. The histopathological changes were examined by a trained pathologist who was blinded to the treatment groups.

2.8. Statistical Analysis

Results were presented as the mean and standard error of the mean (mean ± SEM). To determine significant differences between the study groups, One-way ANOVA was used, followed by Newman–Keuls multiple comparisons as a post hoc analysis (Graph Pad Prism version 8). *p*-values less than 0.05 were considered significant (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

3. Results

Blood glucose levels were significantly elevated ($p < 0.001$) in STZ-treated rats in comparison with control rats. Treatment of these type 1-diabetic rats with of *L. regularis* (150 or 300 mg/kg/day) for four consecutive weeks significantly lowered blood glucose levels ($p < 0.01$ and $p < 0.001$, respectively) as compared to STZ-treated animals (Figure 1a). Serum insulin levels were also decreased in STZ-injected rats compared to normal control animals ($p < 0.05$). Furthermore, high taken dose (300 mg/kg/day) of *L. regularis* treatment significantly ($p < 0.01$) enhanced the serum insulin levels compared to STZ group.

(Figure 1b). Nephrotoxicity markers such as creatinine and urea levels were significantly increased in the STZ-injected group compared to control rats ($p < 0.01$ and $p < 0.001$, respectively) (Figure 1c,d). These increased serum creatinine levels were markedly reduced by treatment with *L. regularis* (150 or 300 mg/kg/day) in diabetic rats compared to the vehicle- and STZ-treated group ($p < 0.05$). Similar reductions in serum urea levels

were observed in *L. regularis*-treated (150 mg/kg/day and 300 mg/kg/day) groups when compared to the STZ group ($p < 0.05$ and $p < 0.01$, respectively) (Figure 1c,d).

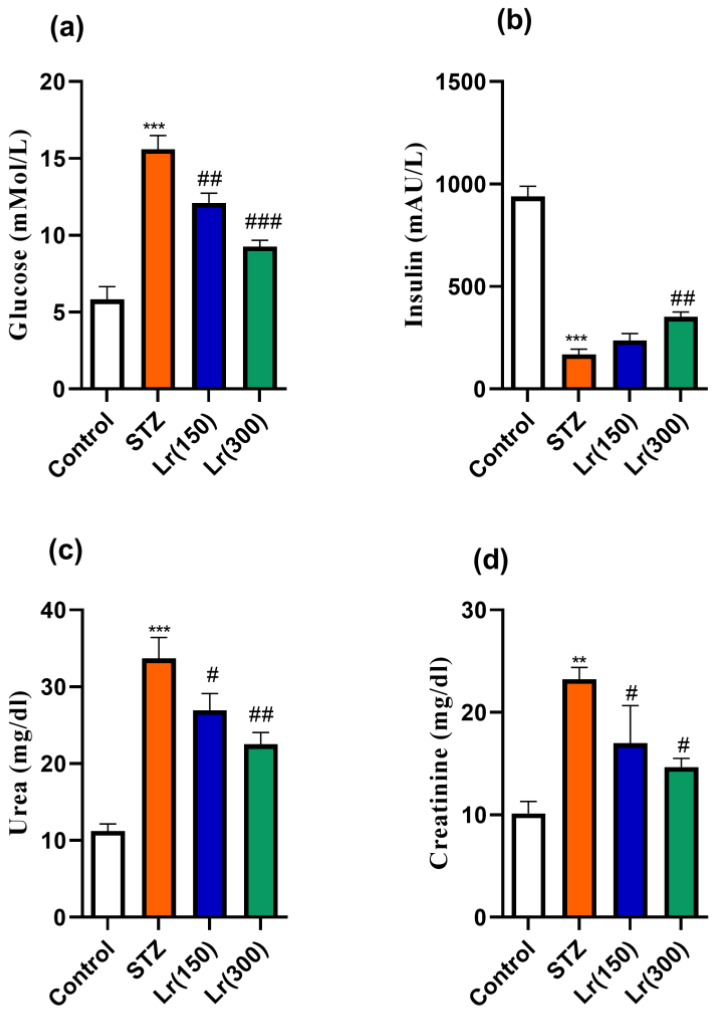

Figure 1. Effect of *L. regularis* (150 or 300 mg/kg for 4 consecutive weeks) on diabetic-induced changes in serum glucose (**a**), insulin (**b**), urea (**c**) and creatinine (**d**) levels. Data are expressed as means ± SE ($n = 6$) and were analyzed using one-way ANOVA followed by Student Newman–Keuls as post hoc test. * Control vs. STZ group; # STZ vs. *L. regularis* (150) or *L. regularis* (300). # $p < 0.05$, ** or ## $p < 0.01$, *** or ### $p < 0.001$.

Serum lipid parameters, including TC, triglycerides (TG) and LDL-cholesterol levels were significantly increased in STZ-injected rats compared to control rats ($p < 0.001$) (Figure 2a–c). The serum HDL-cholesterol levels were not significantly affected (Figure 2d). Serum TC and TG levels were significantly reduced in *L. regularis* (150 or 300 mg/kg/day)-treated groups compared to STZ- and vehicle-treated group ($p < 0.05$ and $p < 0.05$, respectively) (Figure 2a,b). Similarly, LDL-cholesterol levels were also significantly reduced in *L. regularis* (150 or 300 mg/kg/day)-treated groups compared to STZ- and vehicle-treated group ($p < 0.01$ and $p < 0.001$, respectively) (Figure 2c). However, serum HDL-cholesterol levels were not significantly altered in *L. regularis* (150 or 300 mg/kg/day)-treated groups compared to the STZ- and vehicle-treated group (Figure 2d).

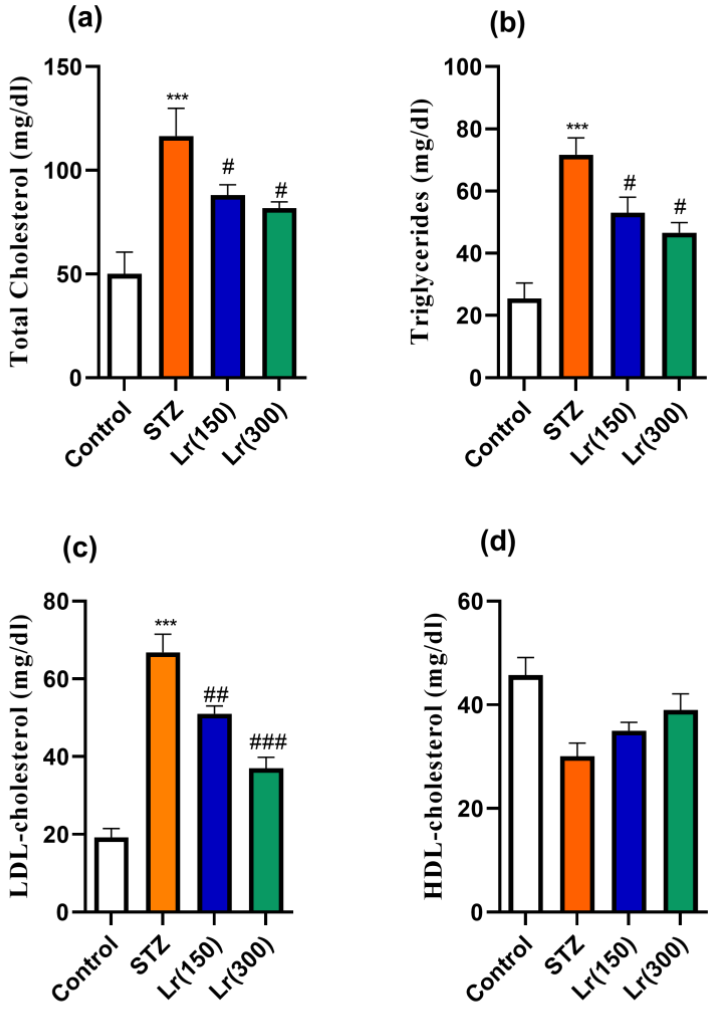

Figure 2. Effect of *L. regularis* (150 or 300 mg/kg for 4 consecutive weeks) on diabetic-induced changes in serum total cholesterol (**a**), triglycerides (**b**), low density lipoprotein cholesterol (LDL-cholesterol) (**c**) and high-density lipoprotein cholesterol (HDL-cholesterol levels) (**d**). Data are expressed as means ± SE (n = 6) and were analyzed using one-way ANOVA followed by Student Newman–Keuls as post hoc test. * Control vs. STZ group; # STZ vs. *L. regularis* (150) or *L. regularis* (300). # $p < 0.05$, ## $p < 0.01$, *** or ### $p < 0.001$.

Serum proinflammatory cytokines including TNF-α, IL-1β and IL-6 levels were significantly increased in STZ-treated rats compared to control rats ($p < 0.001$). *L. regularis* (150 or 300 mg/kg/day) treatment for 4 weeks significantly reduced the TNF-α, IL-1β and IL-6 levels compared to the STZ- and vehicle-treated group ($p < 0.001$) (Figure 3).

Proinflammatory cytokines were also estimated in renal tissue homogenates (Figure 4). IL-1β levels in renal tissue were significantly elevated in STZ-treated group compared to the control group ($p < 0.01$). *L. regularis* (300 mg/kg/day) significantly reduced IL-1β expression in renal tissue compared to STZ- and vehicle-treated rats ($p < 0.05$) (Figure 4a). Similarly, renal tissue IL-6 levels were significantly increased in STZ-treated rats compared to control rats ($p < 0.01$), and *L. regularis* (150 or 300 mg/kg/day) treatment in diabetic rats significantly reduced IL-6 levels compared to the STZ- and vehicle-treated group ($p < 0.05$) (Figure 4b). TNF-α renal tissue levels were also significantly elevated in STZ-treated rats compared to the control group ($p < 0.001$). The higher dose of *L. regularis* extract (300 mg/kg/day) significantly reduced renal TNF-α levels compared to STZ- and vehicle-treated rats ($p < 0.05$) (Figure 4c).

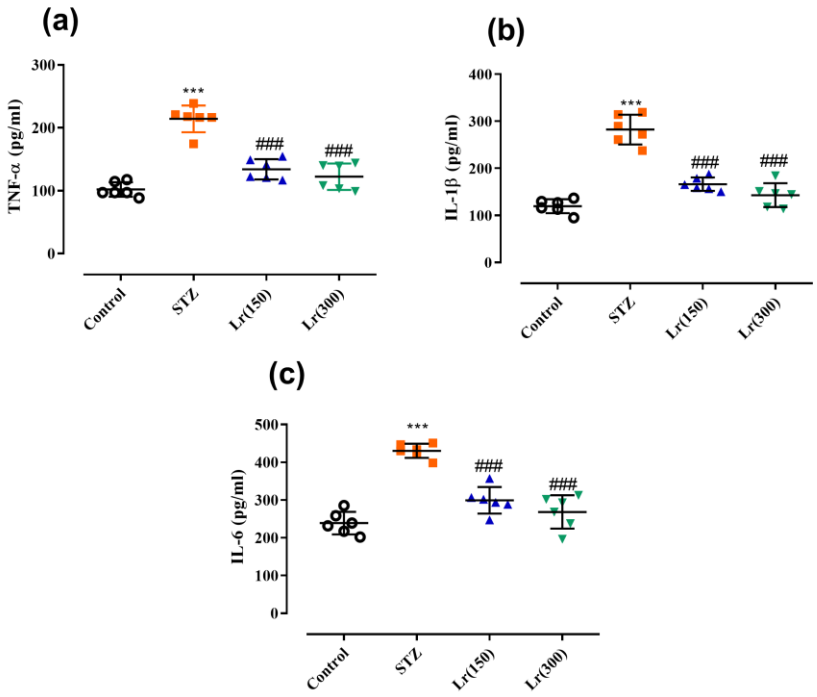

Figure 3. Effect of *L. regularis* (150 or 300 mg/kg for 4 consecutive weeks) on diabetic-induced changes in serum proinflammatory cytokines, including tumor necrosis factor-α (TNF-α) (**a**), interleukin-1β (IL-1β) (**b**) and interleukin-6 (IL-6) (**c**). Data are expressed as means ± SE (n = 6) and were analyzed using one-way ANOVA followed by Student Newman–Keuls as post hoc test. * Control vs. STZ group; # STZ vs. *L. regularis* (150) or *L. regularis* (300). *** or ### $p < 0.001$.

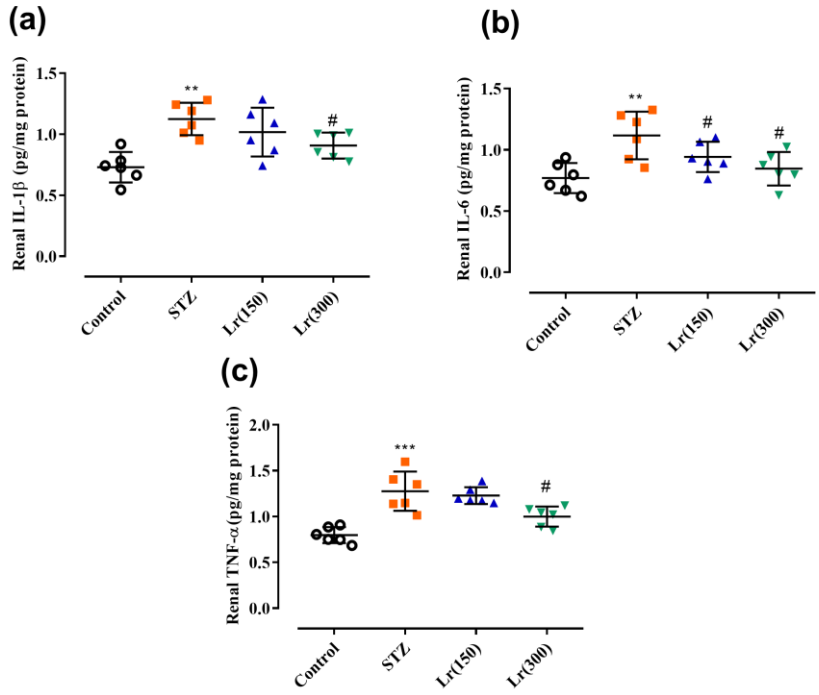

Figure 4. Effect of *L. regularis* (150 or 300 mg/kg for 4 consecutive weeks) on diabetic-induced changes in renal proinflammatory cytokines, including interleukin-1β (IL-1β) (**a**), interleukin-6 (IL-6) (**b**) and tumor necrosis factor-α (TNF-α) (**c**). Data are expressed as means ± SE (n = 6) and were analyzed using one-way ANOVA followed by Student Newman–Keuls as post hoc test. * Control vs. STZ group; # STZ vs. *L. regularis* (150) or *L. regularis* (300). # $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Oxidative stress biomarkers such as thiobarbituric acid reaction substances (TBARS) and glutathione (GSH) levels were estimated in renal tissue homogenate using commercially available ELISA assay kits. TBARS levels were significantly increased ($p < 0.001$), whereas GSH levels were significantly decreased ($p < 0.001$) in STZ-treated rats compared to control animals. Treatment with *L. regularis* (150 or 300 mg/kg/day) significantly reduced TBARS levels ($p < 0.01$), while GSH levels were significantly increased ($p < 0.05$) compared to the STZ- and vehicle-treated group (Figure 5).

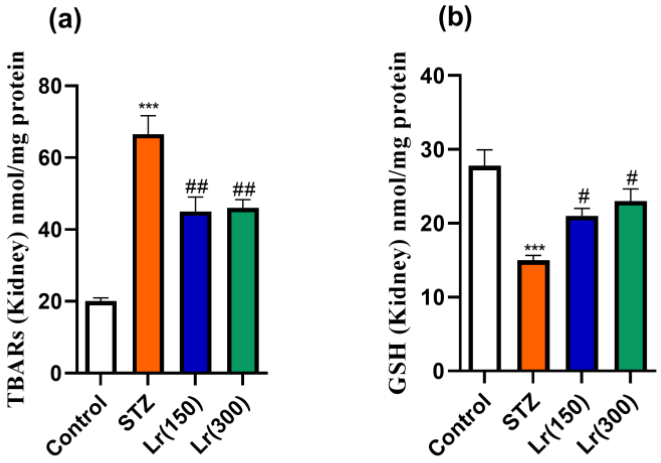

Figure 5. Effect of *L. regularis* (150 or 300 mg/kg for 4 consecutive weeks) on diabetic-induced changes in renal oxidative stress parameters thiobarbituric acid reaction substance (TBARS) (**a**) and glutathione (GSH) (**b**) levels. Data are expressed as means ± SE ($n = 6$) and were analyzed using one-way ANOVA followed by Student Newman–Keuls as post hoc test. * Control vs. STZ group; # STZ vs. *L. regularis* (150) or *L. regularis* (300). # $p < 0.05$, ## $p < 0.01$, *** $p < 0.001$.

Enzymatic activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST) in renal tissues were measured (Figure 6). Antioxidative enzymatic activities were significantly reduced in STZ-treated rats compared to the control group ($p < 0.001$). Treatment with *L. regularis* (150 or 300 mg/kg/day) significantly increased the enzymatic activities of SOD, CAT and GR compared to the STZ- and vehicle-treated group ($p < 0.05$ and $p < 0.01$, respectively) (Figure 6a,b,d). GPx enzymatic activity was significantly increased following treatment with the higher dose of *L. regularis* ($p < 0.05$) (Figure 6c). Both doses of *L. regularis* significantly increased the enzymatic activity of GST compared to the STZ- and vehicle-treated group ($p < 0.01$) (Figure 6e).

Light micrographs of the renal cortex of STZ-induced diabetic rats treated with *L. regularis* (150 or 300 mg/kg/day) are presented in Figure 7. Normal architecture of Bowman's capsules, glomeruli, proximal convoluted tubules and distal convoluted tubules were observed in sections of the renal cortex from control animals (Figure 7A). Vacuolar degeneration, necrosis, narrowed glomeruli and mononuclear cell infiltration were observed in sections of the renal cortex from STZ-treated rats (Figure 7B). However, upon treatment with *L. regularis* (150 mg/kg/day), sections of renal cortex of diabetic rats demonstrated a moderate improvement of pathologies in the kidney glomeruli and renal tubules (Figure 7C). Reversal of STZ-induced abnormalities in kidney histology was observed in sections of the renal cortex of diabetic rats treated with the higher dose of *L. regularis* (Figure 7D).

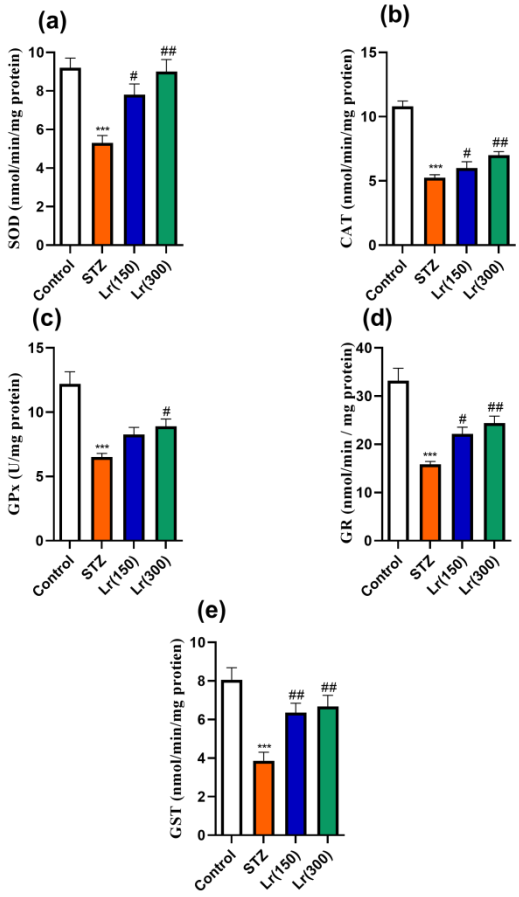

Figure 6. Effect of *L. regularis* (150 or 300 mg/kg for 4 consecutive weeks) on diabetic-induced changes in renal pro-oxidative enzymatic activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST). Data are expressed as means ± SE (n = 6) and were analyzed using one-way ANOVA followed by Student Newman–Keuls as post hoc test. * Control vs. STZ group; # STZ vs. *L. regularis* (150) or *L. regularis* (300). # $p < 0.05$, ## $p < 0.01$, *** $p < 0.001$.

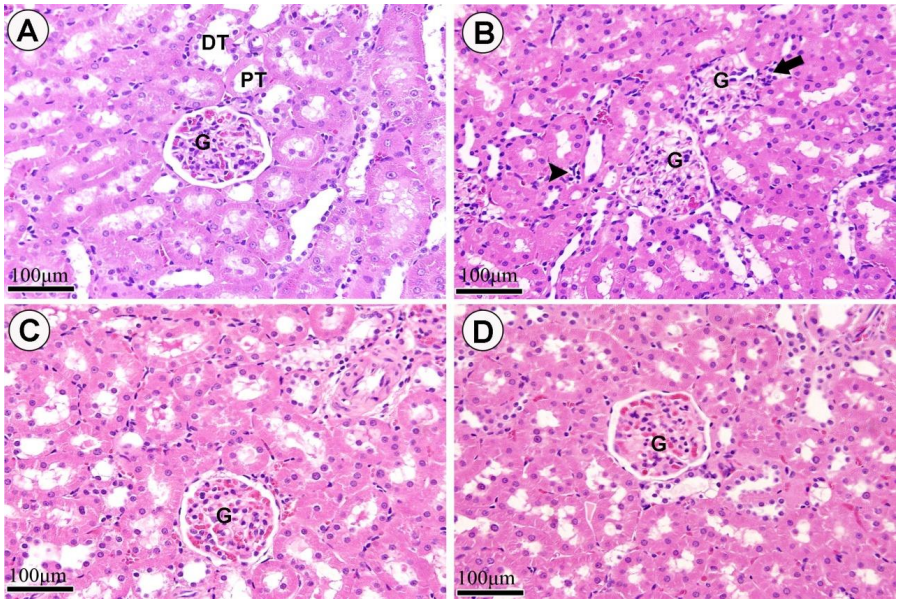

Figure 7. Light micrographs of renal cortex of STZ induced diabetic rats treated with two doses of

L. regularis (150 and 300 mg/kg/day). Section from the renal cortex of the control group shows the normal architecture of the proximal convoluted tubules (PT), distal convoluted tubules (DT), Bowman's capsule and glomerulus (G) (**A**). Renal cortex of untreated diabetic animals showed vacuolar degeneration, signs of necrosis (head arrow), narrowed glomerular (G) and mononuclear cells infiltration were observed (arrow) (**B**). Renal cortex of diabetic animals co-treated with 150 mg/kg/day (**C**) and 300 mg/kg/day (**D**) of *L. regularis* remediated the adverse effects of STZ on kidneys in glomeruli and renal tubules. (H&E, Scale bar, 100 µm).

4. Discussion

Although *L. regularis* has been used in the management of many diseases such as diabetes, nephrolithiasis and inflammatory diseases [24,26], little is known about its impact in DN. Therefore, we evaluated the effects of *L. regularis* extract in STZ-induced DN. Following the treatment by *L. regularis* extract, a dramatic reduction in blood glucose levels, toxic renal markers, proinflammatory cytokines and oxidative stress markers as well as improvement in renal histopathology were observed.

This study found that DN development may be inhibited by *L. regularis* extract administration. We found that *L. regularis* extract could increase the antioxidant ability of diabetic rats which displayed high glucose levels. Treatment with *L. regularis* extract improved the antioxidant potential by increasing SOD and CAT activities, and GSH levels, in diabetic rats. The progression of DN is mainly attributed to oxidative stress generated by hyperglycemia [27,28]. In this context, the generation of ROS results in loss of renal function and elevation of lipid profiles [29,30].

Most medicinal plant-isolated compounds are relatively safe and readily available. The presence of active phenols, flavonoids, and tannins in *L. regularis* extract may explain its hypoglycemic effect as evidenced by our data. Vessal et al. (2003) have shown that supplementation with flavonoids, specifically quercetin, which is one of the main constituents of *L. regularis*, had the ability to regenerate pancreatic islets and increase insulin release in STZ-induced diabetic rats [31]. Quercetin 3-O-b-L-galactopyranoside, quercetin 3-Ob-L-arabinopyranoside and quercetin 3-O-a-L-rhamnopyranoside are three flavonoid glycosides present in *L. regularis*, according to phytochemical analysis performed by Mothana et al. (2013). Such ingredients in the extract may explain the strong hypoglycemic effect in diabetic rats [32]. In addition, Eid and Haddad (2017) attributed the antidiabetic properties of quercetin to various mechanisms including the inhibition of intestinal glucose absorption, insulin secretory and insulin sensitizing activities as well as increased glucose usage in peripheral tissues [33]. The significant increase in serum insulin level after *L. regularis* treatment could be related to the plant extract ability to protect and regenerate damaged pancreatic β cells resulting in enhancing insulin secretion.

Different diabetes-related complications occur with the progression of disease, including DN, which can further aggravate the patient's health condition [34,35]. In this study, elevation in creatinine and urea levels concomitant with structural and histopathological alterations have confirmed the presence of DN. However, serum creatinine and urea levels were decreased and renal histopathology was alleviated following the treatment of diabetic animals with *L. regularis* extract, which can be linked to its active phenolic constituents [24]. Hypercholesterolemia and hypertriglyceridemia are also among the complications caused by diabetes, which are well-recognized causative agents of chronic vascular disease [36]. Consistent with many previous reports, STZ-induced diabetes-induced dyslipidemia, as evidenced by increased serum cholesterol, TG and LDL [37,38]. Interestingly, the extract from *L. regularis* not only lowered TC but also increased HDL. In addition, it reduced TG and LDL in diabetic rats, perhaps due to the presence of quercetin [39,40].

In this study, the most significant finding is that *L. regularis* extract administration could restrict oxidative damage, lipid peroxidation and inflammation in STZ-induced diabetic rats, which together may account for its renoprotective impact. Chronic hyperglycemia-mediated DN induces oxidative damage and inflammation [41]. As a consequence, release of ROS can damage lipids, DNA and other cellular components, leading to cell death. In

addition, the generation of ROS results in the elevation of TNF-α, IL-1β, IL-6 and other inflammatory mediators [42]. Thus, hyperglycemia contributes greatly to kidney injury in diabetes. The ability of ROS to cause renal dysfunction and damage has been confirmed by studies that indicated a reduction in renal injury after ROS suppression [43]. Here, TBARS, a byproduct of lipid peroxidation, was increased, while the antioxidant defenses (SOD, CAT and GSH) were markedly diminished in the diabetic treatment group, indicative of a hyperoxidative state. Decreased cellular antioxidant enzymes (SOD, CAT and GSH) in diabetic animals have been previously shown [44]. In addition, TNF-α, IL-1β and IL-6 in both serum and tissue were increased in diabetic rats. Administration of the diabetic rats with *L. regularis* extract significantly reduced oxidative stress and boosted antioxidant defenses. In addition, *L. regularis* extract suppressed inflammation as evidenced by the decreased expression of these serum and renal proinflammatory cytokines. These findings demonstrate the potent antioxidant and anti-inflammatory effects of *L. regularis* extract. The increased insulin levels and suppression of renal damage and lipid accumulation may be specifically linked to antioxidant and anti-inflammatory treatment by *L. regularis* extract. A previous study by Mothana et al. (2013) showed the in vitro antioxidant and anti-inflammatory activity of crude extract, fractions, and isolated compounds from *L. regularis* [32]. The strong anti-inflammatory and free radical-scavenging activity evidenced by DPPH assays were associated with the high phenolic content of *L. regularis*.

However, few studies have investigated the antidiabetic or renoprotective properties of *L. regularis* extract. Our data present the first evidence that *L. regularis* extract ameliorated hyperglycemia, oxidative stress, inflammation and renal injury in STZ-induced diabetic rats. These beneficial effects may be directly related to the active constituents of *L. regularis* extract, specifically quercetin glucosides, which have well documented anti-inflammatory and antidiabetic effects [33,45,46].

5. Conclusions

Taken together, this work presents strong evidence of the beneficial therapeutic impact of *L. Regularis* against renal complications associated with diabetes. *L. regularis* extract improved renal functions and alleviated cellular damage in diabetic rats. Additionally, the capacity of *L. regularis* to reduce free radical production caused by hyperglycemia and to attenuate inflammation and enhance antioxidative enzyme activities may be underlying mechanisms. Additional clinical trials could support the therapeutic uses of *L. regularis* in diabetic patients.

Author Contributions: All authors participated in the design and interpretation of the study, the analysis of the data and the review of the manuscript. R.A.A.M. collected and authenticated the plant material, carried out phytochemical analysis and prepared the extract. A.Z.A., F.A. and M.M. made substantial contributions in animal treatment, biochemical analysis and interpretation of the data as well as drafted the manuscript. M.M.A., A.O.A.A., K.A. and S.S.A.-R. made substantial contributions to the conception and design of the study, interpretation of the data and final revision of the manuscript. M.M., M.M.A. and A.Z.A. contributed to histopathological preparation and slide screening. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of King Saud University (SE-19-146).

Data Availability Statement: The data used to support the findings of this study are included within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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